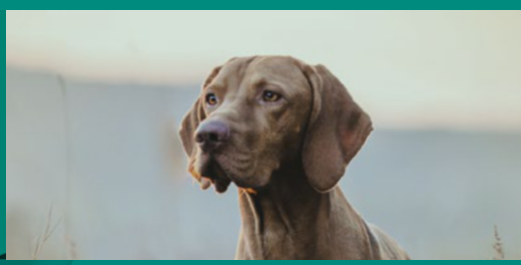
Ramesh C. Gupta
Ajay Srivastava
Rajiv Lall *Editors*





Nutraceuticals in Veterinary Medicine

Springer

Nutraceuticals in Veterinary Medicine

Ramesh C. Gupta • Ajay Srivastava • Rajiv Lall
Editors

Nutraceuticals in Veterinary Medicine

Springer

Editors
Ramesh C. Gupta
Department of Toxicology, Breathitt Veterinary Center
Murray State University
Hopkinsville, KY, USA

Rajiv Lall
Vets Plus Inc.
Menomonie, WI, USA

Ajay Srivastava
Vets Plus Inc.
University of Wisconsin–Stout
Menonomie, WI, USA

ISBN 978-3-030-04623-1 ISBN 978-3-030-04624-8 (eBook)
https://doi.org/10.1007/978-3-030-04624-8

Dedicated to my wife Denise, daughter Rekha, and parents the late Chandra and Triveni Gupta.

Ramesh C. Gupta

Dedicated to my wife Garima, son Sankalp, daughter Ayana, my mom, my brother Sanjay, and my friends.

Ajay Srivastava

Dedicated to my wife Swati Lall, sons Dr. Rohan Lall and Dr. Rishi Lall, mother Kanak Lata Lall, and late father Professor R.B. Lall.

Rajiv Lall

Introduction

According to the North American Veterinary Nutraceutical Council, a veterinary nutraceutical is defined as "a substance which is produced in a purified or extracted form and administered orally to patients to provide agents for normal body structure and function and administered with the intent of improving the health and well-being of animals." This definition differs slightly from country to country. It seems that nutraceuticals fall somewhere between food/feed nutrients and drugs. Although the herbal medicines have been used for thousands of years in Ayurvedic, Homeopathic, Siddha, Unani, Chinese, Tibetan, Egyptian, Russian, African, Amazonian, and many other systems, by the turn of the twenty-first century, their use became even more popular throughout the world because of their easy access, affordability, and greater tolerability with a wide margin of safety. Outside the USA and Europe, more than 80% of the population relies upon herbal medicines because they are available over the counter and have fewer side effects. Currently, the nutraceutical industry totals more than $250 billion per year, and the use of nutraceuticals in animal health and diseases is more popular than in humans. Due to overriding factors, such as low cost and safety, today's veterinarians may prefer nutraceuticals over modern medicines.

Unlike synthetic pharmaceuticals, nutraceuticals often consist of many bioactive compounds that hit multi-targets and pathways. As a result, nutraceuticals may exert multiple activities, such as free radical scavenging and antioxidative, anti-inflammatory, immunomodulatory, adaptogenic, sedative, antimicrobial, etc., with diverse pharmacological effects (Gupta 2016; Attiq et al. 2018). By having such biological and pharmacological activities, nutraceuticals appear to have wide applications in many human and animal diseases, such as diabetes, hypertension, periodontitis, cognition dysfunction, arthritis, allergies, gastrointestinal, hepatic, renal, cardiovascular, respiratory, genitourinary, and other body organ/system-related dysfunctions, and cancer. A large number of nutraceuticals in the form of prebiotics, probiotics, and synbiotics are used to promote animals' gut health, but appear to favorably influence the functionality of other vital organs as well. By having multiple bioactive constituents, nutraceuticals often provide synergistic effects and impede drug resistance to antibiotics, which is a global health issue (Lillehoj et al. 2018).

In the present world situation, due to the healthcare crisis and dwindling financial resources, the nutraceutical industry faces many challenges. For a large number of nutraceuticals, no data are available on safety and toxicity due to lack of pharmacokinetics, pharmacodynamics, pharmacological, and toxicological studies (Gupta 2016). While some nutraceuticals enjoy the GRAS (generally recognized as safe) status from the US FDA, others pose a toxic threat to human and animal health (Gupta et al. 2018). Due to inadequate quality control, contamination of nutraceuticals with metals, mycotoxins, and inherently toxic plant alkaloids and adulteration with drugs of abuse not only compromise their quality but raise serious health concerns, in addition to giving a bad image to the nutraceutical industry. Patients receiving nutraceuticals also consume therapeutic drug(s), and with the increasing polytherapy trend, adverse interactions and outcomes ensue due to metabolic perturbances from food–nutraceutical–drug interactions (Doorman et al. 2016; Gupta et al. 2018). Furthermore, in many cases, claims for nutraceutical use are either exaggerated or asserted without sound scientific merit.

Nutraceuticals are primarily derived from biological (plants, invertebrate and vertebrate animals, fish, and birds) sources and are well characterized for chemical constituents and biological and pharmacological properties. But, unlike pharmaceuticals, nutraceuticals lack unremitting efforts for the basic core scaffold, mechanism of action (essential and adverse pathways), bioinformatics, pharmacovigilance, structure–activity, dose–response, temporal relationships, and clinical studies.

Unlike modern medicines, nutraceuticals are not strictly regulated in any country. In the USA, the only major regulation related to nutraceuticals is the 1994 passage by the US Congress of the Dietary Supplement Health Education Act. Based on this loosely regulated act, dietary supplements are classified as foods, not dugs, allowing them to be sold without proof of safety and effectiveness (US FDA 1994). However, unlike food, nutraceuticals are not generally recognized as safe, nor can one assume that they are all safe (Gupta et al. 2018). As of now, the FDA's position is clearly asserted that this act does not apply to animals, and the American Veterinary Medical Association (AVMA) does not believe that the act should be modified to include animals (Burns 2017). In the European Union, current regulations require evidence that herbal medicinal products meet acceptable standards of quality, safety, and efficacy before a product license can be issued. Quality control and regulatory guidelines for nutraceuticals, from production, distribution, and national and international trade up to end-user level, appear to vary widely from country to country, and currently they are not strictly adhered to as for pharmaceuticals.

At recent national and international conferences (American Veterinary Medical Association, International Veterinary Congress, World Veterinary Association Congress, European nutraceuticals, and many others), a large number of veterinarians, nutritionists, food scientists, and animal health professionals recognized the importance of nutraceuticals for animal health and diseases. Accordingly, *Nutraceuticals in Veterinary Medicine* has been prepared to meet the challenges of today's veterinarians, pet lovers, animal health professionals, farm animal producers, and the veterinary nutraceutical industry. The book contains more than sixty chapters, arranged under seven sections. Each chapter is prepared using a very user-friendly format to provide scientific insight for academicians and veterinary practitioners with an interest in animal nutrition, complementary veterinary medicine, and nutraceutical product development and research.

The factual statements are substantiated with pertinent references for further reading. Some chapters are prepared from the one health perspective, encompassing animal and human health and experimental studies.

Following a brief introduction, the book begins with Section I on common nutraceuticals that are used in the formulations of hundreds of nutraceutical products. This follows Section II on prebiotics, probiotics, synbiotics, enzymes, antibacterial alternatives, and feed additives. The bulk of this book (20 chapters) lies in Section III that deals with nutraceuticals in organ/system-related diseases and disorders. Section IV covers chapters devoted to nutraceuticals for specific species including cattle, equine, camelids, and poultry. This follows Section V on safety and toxicity evaluation of nutraceuticals and functional foods using *in vitro*, *in vivo*, and other models, biomarkers for selected foods and nutraceuticals, and toxic interaction of nutraceuticals with foods and pharmaceuticals. Section VI deals with newer trends in nutraceutical research and product development covering chapters on proteomics and foodomics, nanoparticle-based bioavailability of nutraceutical ingredients and nanosupplements, and veterinary nutraceuticals stability testing. Lastly, Section VII extensively covers chapters on regulatory aspects of nutraceuticals in different continents and countries, including North America, the European Union, India, China, Australia, New Zealand, Turkey, the Philippines, and South Africa. *Nutraceuticals in Veterinary Medicine* is the most comprehensive book in the field of veterinary nutraceuticals, and it offers many chapters on novel topics that are not

covered in any previously published book. This book will serve academia, industry, and government sectors alike.

The editors remain grateful to the contributors of this book from many countries (the USA, Australia, Canada, China, India, Philippines, Russia, Saudi Arabia, South Africa, Turkey, and the UK) for their hard work and dedication. These authors are highly qualified and trained in diverse disciplines (veterinary medicine, nutrition, food science, animal science, pharmacology, toxicology, molecular biology and technology, omics, chemistry, biochemistry, and others), who shaped this book using a framework of integrative approach. The editors would like to thank Ms. Annette Klaus, associate editor, Mr. Bibhuti Sharma, project coordinator (Springer Nature), and Ms. Krithika Shivakumar, project manager, for their untiring support in the production of this book. Last but not least, the editors would also like to thank Ms. Robin B. Doss for critically checking the text and references.

Department of Toxicology, Breathitt Veterinary Center, Murray State University, Hopkinsville, KY, USA — Ramesh C. Gupta

Vets Plus Inc., University of Wisconsin–Stout, Menomonie, WI, USA — Ajay Srivastava

Vets Plus Inc., Menomonie, WI, USA — Rajiv Lall

References

Attiq A, Jalil J, Husain K, et al (2018) Raging the war against inflammation with natural products. Front Pharmacol 9:976

Burns K (2017) Assessing pet supplements. Use widespread in dogs and cats, evidence and regulation lacking. J Am Vet Med Assoc 250(2):117–121

Doorman G, Flachner B, Hajdu I, András CD (2016) Target identification and polypharmacology of nutraceuticals. In: Gupta RC (ed) Nutraceuticals: efficacy, safety and toxicity, Academic Press/Elsevier, Amsterdam, pp 263–286

Gupta RC (2016) Nutraceuticals: efficacy, safety and toxicity. In: Gupta RC (ed) Academic Press/Elsevier, Amsterdam, p 1022

Gupta RC, Srivastava A, Lall R (2018) Toxicity potential of nutraceuticals. In: Nicolotti O (ed) Computational toxicology: methods and protocols. Springer, New York, NY, pp 367–394

Lillehoj H, Liu Y, Calsamiglia S, et al (2018) Phytochemicals as antibiotic alternatives to promote growth and enhance host health. Vet Res 49(1):76

US FDA (1994) Dietary supplement health and education act of 1994. Congress, Pub. L. www.fda.gov/DietarySupplement/default.htm

Contents

List of Contributors

Pratik Adhya Department of Pharmacology and Toxicology, National Institute of Pharmaceutical Education and Research (NIPER), Mohali, Punjab, India

Anitha Alex Salt Lake City, UT, USA

Mohamed Ali-Seyed Faculty of Medicine, Department of Clinical Biochemistry, University of Tabuk, Tabuk, Saudi Arabia

Arturo Anadón Universidad Complutense de Madrid, Madrid, Spain

Irma Ares Universidad Complutense de Madrid, Madrid, Spain

Nikee Awasthee Laboratory for Translational Cancer Research, Department of Biochemistry, Institute of Science, Banaras Hindu University, Varanasi, India

Alessia Bertero Department of Veterinary Medicine, Università degli Studi di Milano, Milan, Italy

Bilwa Bhanap Dr. Rath Research Institute, Santa Clara, CA, USA

Vijay K. Bharti DRDO-Defence Institute of High Altitude Research, Leh-Ladakh, India

Karyn Bischoff New York State Animal Health Diagnostic Center, Cornell University, Ithaca, NY, USA

Kyle Boyar Medicinal Genomics, Woburn, MA, USA

Petra Cagnardi Department of Health, Animal Science and Food Safety, Università degli studi di Milano, Milano, Italy

Francesca Caloni Department of Veterinary Medicine, Università degli Studi di Milano, Milan, Italy

Naresh Chand ADRx Synergy, North Potomac, MD, USA

Soumen Choudhury Department of Pharmacology and Toxicology, College of Veterinary Science and Animal Husbandry, U.P. Pandit Deen Dayal Upadhyaya Veterinary University (DUVASU), Mathura, Uttar Pradesh, India

Teresa Coccini Laboratory of Clinical and Experimental Toxicology, Toxicology Unit, ICS Maugeri SpA-BC, IRCCS, Pavia, Italy

Rhian B. Cope Australian Pesticides and Veterinary Medicines Authority, Armidale, NSW, Australia

Robert W. Coppock DVM, Toxicologist and Assoc. Ltd, Vegreville, AB, Canada

Clinton D'Souza Division of Environmental Health and Toxicology, Nitte University Centre for Science Education and Research (NUCSER), Mangaluru, KA, India

Leena Dhruw Department of Chemistry, Center for Basic Sciences, Pandit Ravishankar Shukla University, Raipur (C.G.), India

Robin B. Doss Toxicology Department, Breathitt Veterinary Center, Murray State University, Hopkinsville, KY, USA

Dan DuBourdieu Vets Plus Inc., Menomonie, WI, USA

Ayhan Filazi Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, Ankara University, Ankara, Turkey

Tarun K. Gahlot Rajasthan University of Veterinary and Animal Sciences, Bikaner, Rajasthan, India

Satish Kumar Garg Department of Pharmacology and Toxicology, College of Veterinary Science and Animal Husbandry, U.P. Pandit Deen Dayal Upadhyaya Veterinary University (DUVASU), Mathura, Uttar Pradesh, India

Arvind Kumar Geda Department of Plant Physiology, Agril. Biochemistry, Medicinal and Aromatic Plants, Indira Gandhi Agricultural University, Raipur, Chhattisgarh, India

Kallol K. Ghosh School of Studies in Chemistry, Pandit Ravishankar Shukla University, Raipur (C.G.), India

Arup Giri Arni University, Kathgarh, Himachal Pradesh, India

Nikolay Goncharov Research Institute of Hygiene, Occupational Pathology and Human Ecology, Leningrad Region, Russia

Sechenov Institute of Evolutionary Physiology and Biochemistry, St. Petersburg, Russia

Likun Gong Academy of Sciences, Shanghai Institute of Materia Medica, Center for Drug Safety Evaluation and Research, Pudong, Shanghai, P.R. China

Ramesh C. Gupta Toxicology Department, Breathitt Veterinary Center, Murray State University, Hopkinsville, KY, USA

Bhanushree Gupta Department of Chemistry, Center for Basic Sciences, Pt. Ravishankar Shukla University, Raipur, India

Denise M. Gupta Hopkinsville, KY, USA

Subash Chandra Gupta Laboratory for Translational Cancer Research, Department of Biochemistry, Institute of Science, Banaras Hindu University, Varanasi, India

P. K. Gupta Toxicology Consulting Group, Bareilly, Uttar Pradesh, India

Academy of Sciences for Animal Welfare, Bareilly, Uttar Pradesh, India

Society of Toxicology of India, Ludhiana, Punjab, India

Division of Pharmacology and Toxicology, (IVRI), Bareilly, Uttar Pradesh, India

World Health Organization, Geneva, Switzerland

International Union of Toxicology, Reston, VA, USA

Sharon M. Gwaltney-Brant Veterinary Information Network, Mahomet, IL, USA

Joshua A. Hartsel Delta-9 Technologies, LLC, Lake Forest, CA, USA

Amul Jain Department of Chemistry, Center for Basic Sciences, Pt. Ravishankar Shukla University, Raipur, India

Richard Jenkins School of Allied Health Sciences, De Montfort University, Leicester, UK

Zhongqi Jiang Department of Veterinary Medicine, College of Animal Science, Zhejiang University, Hangzhou, Zhejiang, P.R. China

Sahil Kalia DRDO-Defence Institute of High Altitude Research, Leh-Ladakh, India

Dharmendra Khokhar Department of Plant Physiology, Agril. Biochemistry, Medicinal and Aromatic Plants, Indira Gandhi Agricultural University, Raipur, Chhattisgarh, India

Ekaterina Korf Sechenov Institute of Evolutionary Physiology and Biochemistry, St. Petersburg, Russia

Dinesh Kumar Division of Pharmacology & Toxicology, Indian Veterinary Research Institute, Bareilly, Uttar Pradesh, India

Bhuvnesh Kumar DRDO-Defence Institute of Physiology and Allied Sciences, Timarpur, Delhi, India

Vivek Kumar Department of Chemistry, Center for Basic Sciences, Pt. Ravishankar Shukla University, Raipur, India

Prafulla Kumar Ayurvet, Ghaziabad, UP, India

Bhupesh Kumar Center for Basic Sciences, Pt. Ravishankar Shukla University, Raipur, Chhattisgarh, India

Rajiv Lall Vets Plus Inc., Menomonie, WI, USA

Alexandros Makriyannis Northeastern University, Boston, MA, USA

Jitendra K. Malik IVRI, Dehradun, Uttarakhand, India

Maria Aŕanzazu Martínez Universidad Complutense de Madrid, Madrid, Spain

Maria Rosa Martínez-Larrañaga Universidad Complutense de Madrid, Madrid, Spain

Saumya Mehta School of Pharmaceutical Sciences, Jamia Hamdard University, New Delhi, India

Camillo La Mesa Department of Chemistry "Stanislao Cannizzaro", Sapienza University of Rome, Rome, Italy

Neha Meshram Department of Chemistry, Center for Basic Sciences, Pandit Ravishankar Shukla University, Raipur (C.G.), India

Dejan Milatovic Alexandria, VA, USA

Shruti Mishra Laboratory for Translational Cancer Research, Department of Biochemistry, Institute of Science, Banaras Hindu University, Varanasi, India

E. Mokantla Department of Agriculture, Forestry and Fisheries, Pretoria, South Africa

V. Naidoo Faculty of Veterinary Science, Department of Paraclinical Science, University of Pretoria, Pretoria, South Africa

Aleksandra Niedzwiecki Dr. Rath Research Institute, Santa Clara, CA, USA

Amit Kumar Pandey Ayurvet, Ghaziabad, UP, India

Andrew Pham BelCosta Labs, Long Beach, CA, USA

Atul Prakash Department of Pharmacology and Toxicology, College of Veterinary Science and Animal Husbandry, UP Pandit Deen Dayal Upadhyay Pashu Chikitsa Vigyan Vishwavidyalaya Evam Go-Anusandhan Sansthan, Mathura, UP, India

Anu Rahal Central Institute for Research on Goat (CIRG), Mathura, Uttar Pradesh, India

Vipin Rai Department of Biochemistry, Laboratory for Translational Cancer Research, Institute of Science, Banaras Hindu University, Varanasi, UP, India

Matthias Rath Dr. Rath Research Institute, Santa Clara, CA, USA

Gianfranco Risuleo Department of Biology and Biotechnologies "Charles Darwin", Sapienza University of Rome, Rome, Italy

Pushpkant Sahu Department of Chemistry, Center for Basic Sciences, Pt. Ravishankar Shukla University, Raipur, India

Jacob Anderson C. Sanchez College of Arts and Sciences (CAS), Pampanga State Agricultural University, Magalang, Pampanga, Philippines

Nutraceutical Research Laboratory, Pampanga State Agricultural University (PSAU), Magalang, Pampanga, Philippines

Geraldine C. Sanchez Nutraceutical Research Laboratory, Pampanga State Agricultural University (PSAU), Magalang, Pampanga, Philippines

College of Veterinary Medicine (CVM), Pampanga State Agricultural University (PSAU), Magalang, Pampanga, Philippines

A. Sankaranarayanan Vivo Bio Tech Ltd, Hyderabad, Telangana, India

M. J. Saxena Ayurvet, Ghaziabad, UP, India

Shyam Sunder Sharma Department of Pharmacology and Toxicology, National Institute of Pharmaceutical Education and Research (NIPER), Mohali, Punjab, India

Rahul Sharma Department of Plant Physiology, Agril. Biochemistry, Medicinal and Aromatic Plants, Indira Gandhi Agricultural University, Raipur, Chhattisgarh, India

Anshuman Sharma Center for Basic Sciences, Pt. Ravishankar Shukla University, Raipur, Chhattisgarh, India

Anurag Sharma Division of Environmental Health and Toxicology, Nitte University Centre for Science Education and Research (NUCSER), Mangaluru, India

Amit Shukla Department of Pharmacology and Toxicology, College of Veterinary Science and Animal Husbandry, U.P. Pandit Deen Dayal Upadhyaya Veterinary University (DUVASU), Mathura, Uttar Pradesh, India

Robert J. Silver RX Vitamins, Elmsford, NY, USA

Vidya Rani Singh Department of Chemistry, Center for Basic Sciences, Pandit Ravishankar Shukla University, Raipur (C.G.), India

Anita Sinha Vets Plus Inc., Menomonie, WI, USA

Vladislav Sobolev Research Institute of Hygiene, Occupational Pathology and Human Ecology, Leningrad Region, Russia

Sechenov Institute of Evolutionary Physiology and Biochemistry, St. Petersburg, Russia

Deeksha Sori Center for Basic Sciences, Pt. Ravishankar Shukla University, Raipur, Chhattisgarh, India

Leon J. Spicer Department of Animal and Food Sciences, Oklahoma State University, Stillwater, OK, USA

Krishnamoorthy Srinivasan Department of Pharmacology and Toxicology, National Institute of Pharmaceutical Education and Research (NIPER), Mohali, Punjab, India

Ajay Srivastava Vets Plus Inc., Menomonie, WI, USA

Anil K. Srivastava Agricultural Scientists Recruitment Board, Indian Council of Agricultural Research, New Delhi, India

Szabina A. Stice Rockville, MD, USA

Jianhua Sun Academy of Sciences, Shanghai Institute of Materia Medica, Center for Drug Safety Evaluation and Research, Pudong, Shanghai, P.R. China

Jamil Talukder Vets Plus, Inc., Menomonie, WI, USA

Doriana Eurosia Angela Tedesco Department of Environmental Science and Policy, Università degli Studi di Milano, Milano, Italy

Maxim Terpilowski Sechenov Institute of Evolutionary Physiology and Biochemistry, St. Petersburg, Russia

Surabhi Verma Department of Chemistry, Center for Basic Sciences, Pandit Ravishankar Shukla University, Raipur (C.G.), India

Kavitha Vijayaraghavan Department of Chemical Engineering, Agni College of Technology, Chennai, Tamil Nadu, India

Daljit Vudathala PADLS New Bolton Center Toxicology Laboratory, Department of Pathobiology, School of Veterinary Medicine, University of Pennsylvania, Kennett Square, PA, USA

M. Waheed Roomi Dr. Rath Research Institute, Santa Clara, CA, USA

Feng Wang Amlan Trading (Shenzhen) Company, Ltd., Shenzhen, Guangdong, P.R. China

Ramdas Singh Wangkheirakpam Department of Pharmacology & Toxicology, College of Veterinary Sciences & Animal Husbandry, R.K. Nagar, West Tripura, India

Christina Wilson-Frank Department of Comparative Pathobiology, Animal Disease Diagnostic Laboratory, College of Veterinary Medicine, Purdue University, West Lafayette, IN, USA

Moges Woldemeskel Tifton Veterinary Diagnostic and Investigational Laboratory, Department of Pathology, College of Veterinary Medicine, University of Georgia, Tifton, GA, USA

Begüm Yurdakok-Dikmen Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, Ankara University, Ankara, Turkey

Csaba K. Zoltani Emeritus US Army Research Lab, Aberdeen Proving Ground, Aberdeen, MD, USA

Part I

Common Nutraceuticals

Standardized Turmeric and Curcumin

Naresh Chand

Abstract

Turmeric root is an ancient Ayurvedic herb, and it is used as a spice, and in very low doses, it may modulate immune-inflammatory diseases of the gut, joints, brain, and body in turmeric-consuming part of the world. Turmeric contains more than 235 active ingredients including essential oils, curcuminoids (>89), and turmerosaccharides as well as curcuminoid-free ingredients and fiber. These phytochemicals and fiber as well as their metabolites and products of microbial degradation may act in additive or synergistic fashion as a modulator of persistent dysregulated chronic immune inflammation and pain in horses, pets, and people. The limited preclinical data support that low doses of turmeric or its active ingredient (curcumin/curcuminoids) may have modulatory applications in preventing or treating immune-inflammatory diseases of the eyes, brain, joints, and gut in pets and people. The standardized turmeric (ST) is a novel concept; it is based on a recently filled patent. ST may reduce the need for analgesics (opiates), antidepressants, steroids, and anticancer medications. Using the latest drug-targeted delivery and reliable clinical trial strategies, ST may be considered for R&D for the prevention and treatment of OA, dementia, and other age-related diseases of the eyes, brain, gut, and joints in pets and humans. The consumers need to be aware of the adulterations of turmeric and its extracts.

Keywords

Standardized turmeric · Curcumin · Modulator of persistent dysregulated chronic immune inflammation and pain · Pets

N. Chand (✉)
ADRx Synergy, North Potomac, MD, USA

1 Introduction

Turmeric is the dried rhizome (root) from three major varieties of the *Curcuma longa* plant—*Curcuma aromatica* (wild turmeric, South Asia), *Curcuma wenyujin* (China), and *Curcuma domestica* (Thailand). It is an herbaceous perennial plant which belongs to the ginger family, *Zingiberaceae*. Turmeric is native to Southeast Asia particularly to the Indian subcontinent. It is cultivated in India, China, Thailand, Indonesia, Japan, and other tropical regions including Africa (Gopinath and Karthikeyan 2018). More than 133 different species of turmeric have been identified (Prasad and Aggarwal 2011). The composition of turmeric has been summarized in Table 1. Turmeric root contains curcuminoids, curcumin-free ingredients, essential oils, water-soluble turmerosaccharides, and fiber. The amount of medicinal ingredients such as curcumin/curcuminoids in turmeric powder may vary considerably from region to region (Ashraf et al. 2015). This may depend on the species, phylogenetic and epigenetics of the turmeric plant, cultivation practices, soil nutrition, rainfall, and sun exposure, as well as different extraction methods. Native turmeric's more than 235 complex phytochemicals naturally assemble in the roots and may exert additive or synergistic health beneficial effects (Aggarwal et al. 2013; Javeri and Chand 2016; Li et al. 2011). Turmeric may be considered the "poor man's aspirin" as it is available in every Indian home and is affordable as food (spice).

Pulverized turmeric root is an ancient Ayurvedic herb (spice) often used for cosmetic, religious, and spiritual festivities as well as for flavoring and coloring foods in traditional cooking. It is also used as a traditional herbal medicine since ancient times in India and other turmeric-consuming parts of the world. In addition, small quantities of turmeric are used two or three times a day as a flavoring spice in the Indian subcontinent and Southeast Asia. Turmeric alone, or in combination with other herbs, is seasoned by frying in cooking oil or ghee (clarified butter) before

R. C. Gupta et al. (eds.), *Nutraceuticals in Veterinary Medicine*, https://doi.org/10.1007/978-3-030-04624-8_1

Table 1 Composition of turmeric and major curcuminoids

Turmeric constituents		Curcuminoids	
	% (w/w)		%
Curcuminoids	1.4–5[a]	Curcumin	60–70
Essential oils	3–7	Demethoxycurcumin	20–27
Fiber	2–7	Bismethoxycurcumin	10–15
Minerals	3–7		
Fat	5–10		
Protein	6–8	Turmerosaccharide	10
Carbohydrate	60–70		
Moisture	6–13		

Modified after Nelson et al. The essential medicinal chemistry of curcumin. Mini-perspective. J Med Chem 60, 1620–1637
[a]Ashraf et al. (2015)

adding beans, grains, rice, and vegetables. This method is part of the lipidation (solubilization), activation, and stabilization of turmeric's active ingredients. It is used as food, and it may offer some health benefits in early stages of chronic diseases. This ancient formulation method (may resemble to nanotechnology of the modern era) is used in traditional Indian kitchens. It may improve the bioavailability of phytochemicals in turmeric. The traditional Indian kitchen is a living and functional polypharmacy. Turmeric use has been described to prevent food and lung allergies, aches, pain, flu, common cold, skin wounds, and digestive and other disorders; it has been used to overcome the effects of concussions (TBI) and has been used in many herbal formulations (>800) in dietary supplements for prevention of a wide variety of diseases (Gopinath and Karthikeyan 2018). The safety and efficacy of these combinations (nutraceuticals or dietary supplements on the market) have not undergone rigorous testing in animals and people suffering with immune-inflammatory diseases of the joints, gut, and brain.

The health benefit of turmeric or its active ingredients, such as curcumin, may be more pronounced during early disease states (Javeri and Chand 2016; Sundaram et al. 2017; Kumar et al. 2018). Therefore, turmeric or curcuminoids and other ingredients are likely to be part of the preventive strategies rather than a cure. Standardized turmeric (meaning containing 1.4, 3, 5% curcumin, Chand 2018) and other ingredients at low doses may be used as an adjunct therapy in the management of dysregulated persistent chronic immune-inflammatory diseases of the musculoskeletal, gastrointestinal (digestive), pulmonary, cardiovascular, and nervous systems.

Ayurveda is an ancient art of restoring "homeostasis" under early disease states. It proposes that the amount of spice or its active ingredient(s) does not follow a perfect relationship to effectiveness (pharmacokinetics/pharmacodynamics (PK/PD) modeling)—meaning that more is not better. Preclinical studies in Alzheimer's mouse models support this concept (Lim et al. 2001; Begum et al. 2008). In fact, the opposite situation may occur at higher doses, meaning higher dose may negate its own health benefits (Dr. Frautschy UCLA; personal communication). One of the Ayurvedic medicine's basic principles is that efficacy cannot be related to plasma levels of a major ingredient in a spice (herb), and the whole herb is often more efficacious than its individual ingredients. In most preclinical studies, the distribution of active ingredient(s) of turmeric to site of action (inflammation or disease states) is not carefully investigated. The active ingredients of turmeric seem to be preferentially delivered to the site of inflammation (personal observation) and the brain of mice with progressive Alzheimer's disease state (Begum et al. 2008).

Curcumin, chemically known as (1*E*, 6*E*)-1,7-bis (4-hydroxy-3-methoxyphenyl) hepta-1,6-diene-3,5-dione, is a highly pleiotropic natural polyphenolic chemical (Fig. 1). Recently, Kumar et al. (2018) summarized the potential use of curcumin in aging-related diseases. Curcumin is one of the active ingredients in the spice turmeric—a routinely used spice in traditional Indian cuisine. Curcumin is known to exert numerous in vitro and in vivo pharmacological activities in experimental animals often at relatively high doses (concentrations). The broad pharmacological profile of curcumin in experimental animals tends to suggest that it may exert health beneficial effects; however, clear clinical evidence of efficacy in any disease state is still lacking.

Over the past decade, the field has made enormous progress in improving the bioavailability of curcumin (Gopi et al. 2017). In addition to improvement of curcumin's bioavailability (pharmacokinetics), high doses of curcumin were utilized in most clinical trials conducted so far. Neither improvement of bioavailability nor increased dose improved curcumin's efficacy in Alzheimer's disease in humans (Mazzanti and Di Giacomo 2016). This is in agreement with the Ayurvedic principle—more of one active ingredient is not better (Begum et al. 2008; Chand 2018).

The chemical structure of major curcuminoids—curcumin, demethoxycurcumin, and bisdemethoxycurcumin—and major metabolites of curcumin is shown in Fig. 1.

Curcumin is an unstable, reactive, and non-bioavailable physiochemical, and therefore it is not a lead candidate for R&D. This concept is likely to be true when high "astronomical" doses of curcumin are utilized in animals or patients, but low doses of standardized turmeric or curcumin may present R&D opportunities which should be explored. In this book chapter, I (as a lifelong Ayurvedic scholar and an R&D pharmacologist since 1981) take a different perspective on this subject matter of great economic and healthcare importance for aging populations. Standardized turmeric (containing 3, 9, 27, or 81 mg curcuminoids) taken with

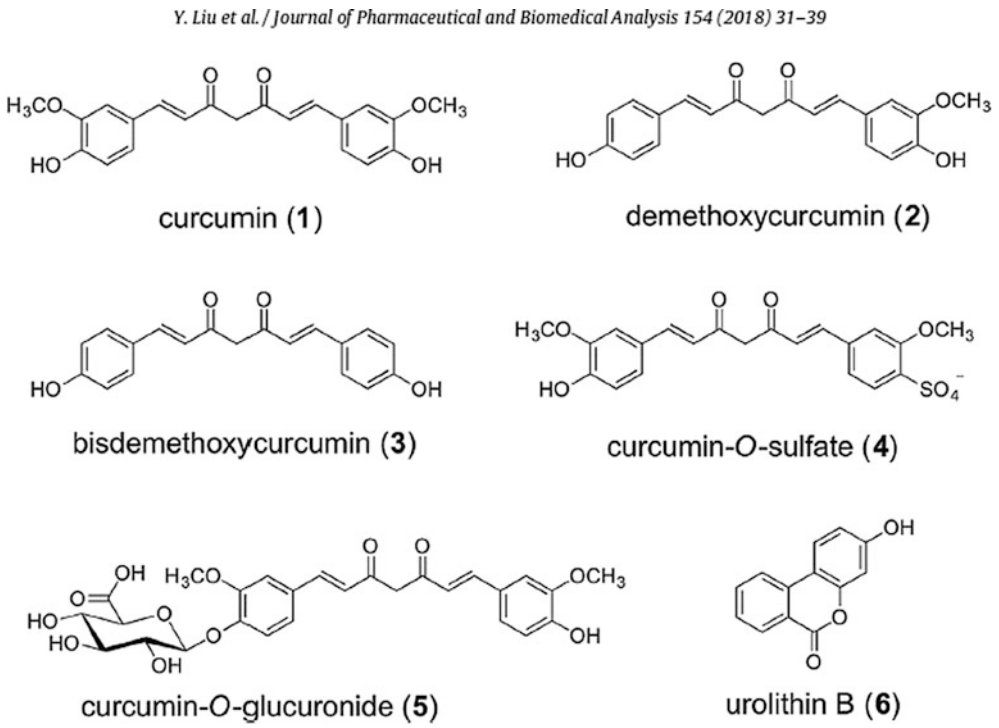


Fig. 1 Chemical structures of curcumin (**1**), demethoxycurcumin (**2**), bisdemethoxycurcumin (**3**), curcumin-*O*-sulfate (**4**), curcumin-*O*-glucuronide (**5**), and the internal standard, urolithin B (**6**)

food two to three times a day for life may have some long-term potential for slowing the age-related progression of chronic diseases in pets, including horses, and people. This chapter described the effects of turmeric or low doses of curcumin in animals, which may have some clinical relevance in the prevention of chronic illnesses of the joints, digestive system, eyes, brain, etc.

Curcumin may act as an immune modulator (Mollazadeh et al. 2017). Low doses of standardized turmeric or curcumin (curcuminoids) taken as a dietary supplement (as food/spice) for long duration (lifelong exposure) may exert modulation of immune inflammation in many chronic disease states including chronic pain, inflammatory bowel disease (IBD), osteoarthritis, and Alzheimer's disease (Javeri and Chand 2016; Lim et al. 2001; Begum et al. 2008; Sundaram et al. 2017). Furthermore, turmeric, which contains diverse and complex phytochemicals (>235; 69 curcuminoids), may serve as a preventive (modulatory) agent or as an adjunct therapy. It may reduce the need for medications such as opiates, NSAIDs, steroids, antiarthritic, or anti-gout, as well as anticancer agents. In other words, low doses of ST, especially when taken as a spice (herb in low doses) added to foods on regular basis—rather than in a pill (capsule)—may act as an adjunct therapy in many diseases with underlying dysregulated persistent chronic immune inflammation in aging pets, horses, and people.

The products of bioactive degradation and microbial metabolism of curcuminoids, polysaccharides, curcumin-free phytochemicals, and fiber in the digestive system may exert regulatory effects on genetics, epigenetics, protostomes, and function of microbiota in the digestive system. The photochemical metabolites from the liver and microbiota processing may exert additive or synergistic activity in modulating the gut-immune-brain axis, the colon and its surroundings—permeability and barrier functions of epithelium in immune-inflammatory diseases of the gastrointestinal tract and infections, constipation, and diarrhea. In Ayurvedic medicine, mild laxative effects of turmeric or ST and its interaction with microbiota in the digestive system have been suggested to improve gut-brain functioning (Shen et al. 2017).

Low doses of turmeric (~200–300 mg, once a day, BID) and curcumin (~3–30 mg once a day, BID, in foods) are often considered safe. In addition, many people in non-turmeric-consuming nations may be using too much of these dietary supplements—curcumin (curcuminoids) and turmeric alone or in combination with other dietary supplements and medications. They may not be benefiting from using these higher doses, and in fact, some may face adverse consequences, especially among seniors taking three to ten medications. Such drug-drug interactions remain to be evaluated. The efficacy and safety of standardized turmeric (ST) in disease states in pets and people taking various other medications or supplements is warranted. The level of various ingredients at the inflammatory site or brain or in cancer tissues in the disease states in dogs, cats, or horses may

offer better understanding of the absorption, distribution, metabolism, and excretion (ADME; pharmacokinetics—PK) and drug delivery issues in using herbs (nutraceutical "dietary supplements") in foods as well as adjunct medicine. The long-term use of low doses of ST as an oral supplement or as nanoparticles in disease states in pets and people may help in lowering the doses of medications such as NSAIDs, opiates, steroids, anticancer, anti-gout, anti-RA, and CNS-acting drugs. Thus, ST and other formulations of curcumin (curcuminoids, turmerosaccharides, curcumin-free phytochemicals) may exert sparing effects on morphine, steroids, and other medications.

2 Chemical Composition

Jia et al. (2017) reviewed and offered details about the chemical constituents of turmeric. It contains curcuminoids, steroids, terpenoids, flavonoids, and phenylpropene derivatives and alkaloids. The major curcuminoid (curcumin) has been extensively studied in preclinical animal models. In addition to turmeric's three major curcuminoids, there are several minor curcuminoids, which may exert significant bioactivities. They identified 89 curcuminoids in the turmeric samples using ultrahigh-performance liquid chromatography—quadrupole time-of-flight tandem mass spectrometry. Ashraf et al. (2015) demonstrated that content of curcuminoids in turmeric varies significantly from region to region of India (1.4–5.0%).

Commercially available curcumin contains at least three curcumin compounds including curcumin, demethoxycurcumin (DMC), and bisdemethoxycurcumin (BDMC) in a ratio of 66, 23, 11, respectively.

3 Turmeric Consumption

Prasad and Aggarwal (2011) summarized multiple uses of turmeric in Asian cuisine and its consumption. It is used as a preservative and antimicrobial agent and is used in making pickles (mango, limes, lemons, and others) and savory and sweet dishes and is widely used in Eastern cooking specialties. The consumption of turmeric in Asian countries in humans is in the range of 200–1000 mg/day (160–440 g/person/year, often costing less than $1 dollar). Intake in urban areas is lower (200 mg/day/person) than in rural areas (600 mg/day/person). This information may be used in translational sciences, meaning the consumption of curcumin from turmeric in Asian countries in humans is in the range of 2.8–30 mg/day (average of 15 mg/day; Chand 2018). In the USA, most people (healthy enthusiasts) are using 1–5 g of turmeric every day which is >5–25 times of that used in India. High doses of herbs often act as prooxidant and pro-inflammatory. Because of high consumption and demand for turmeric in Western countries, the adulteration with lead and other products has become a common practice.

3.1 Safety

Turmeric use (low dose) as a spice in food is safe for human consumption. However, high doses may alter taste and disturb gastrointestinal functions leading to nausea, diarrhea, and vomiting. Gupta et al. (2013) summarized that curcumin is safe in rodents, primates, horses, rabbits, cats, and humans. Curcumin inhibits the activity of drug-metabolizing enzymes such as cytochrome P450, GST, and UDP-glucuronosyltransferase in vitro and in animal models. Therefore, the possibility exists that drug-drug interaction in patients taking medications such as acetaminophen, digoxin, and morphine may increase the plasma concentrations after curcumin dosing. This may lead to potential drug safety concerns. Curcumin is an active iron chelator and induces anemia in mice fed iron-poor diets. These possibilities such as GI effects—nausea, diarrhea, and vomiting—anemia, and bleeding, under some circumstances, need to be kept in mind while advancing the R&D on standardized turmeric and curcumin (Gupta et al. 2013; Chand 2018).

It remains to be explored if standardized turmeric (containing >235 phytochemicals, essential oils, and fiber) offers better efficacy and safety than curcumin alone.

4 Pharmacokinetics

Despite a vast amount of publications on curcumin, detailed oral pharmacokinetic studies using turmeric and curcumin/curcuminoids (many formulations and brands) are still lacking. Following the latest PK/PD modeling and analytical techniques, detailed oral pharmacokinetic studies using turmeric or curcumin/curcuminoids (many formulations and brands) in the blood (cells) and tissues of cats, dogs, and horses living with chronic immune-inflammatory diseases are warranted. Such investigations would help in finding safe, effective, preventive, and therapeutic nutraceuticals for many diseases of pets and people.

Curcumin is insoluble in aqueous media; is unstable under conditions of ambient light, room temperature, and basic pH; and is readily metabolized or degraded upon oral dosing (Matabudul et al. 2012). Limited pharmacokinetic studies in mice (Begum et al. 2008), rats (Suresh and Srinivasan 2010), dogs (Bolger et al. 2017, 2018; Matabudul et al. 2012), and human (Schiborr et al. 2014; Small et al. 2017; Bolger et al. 2018) have been reported. Matabudul et al. (2012) studied

PK profiles following prolonged intravenous infusion of curcumin (10 mg/kg lipocurc™, either over 2 h or over 8 h) in dogs. The ratio of tetrahydrocurcumin (THC)/curcumin was highest in hippocampus > brainstem > striatum > spleen. Based on the data obtained in this study, they raised the possibility that this formulation may facilitate distribution into tissues via a transporter-dependent mechanism and that elevated tissue concentrations of curcumin may inhibit or saturate a putative reductase enzyme converting curcumin to THC in the body.

Bolger et al. (2017) investigated the distribution of curcumin (Lipocurc™) and its major metabolite tetrahydrocurcumin (THC) in dog (Beagles) and human red blood cells, peripheral blood mononuclear cells (PBMC), and hepatocytes. They observed a good correlation between the species differences of red blood cell metabolism of curcumin to THC and in vivo plasma levels of curcumin and THC from clinical studies. They found that curcumin's distribution into, and metabolism by, red blood cells significantly impacts the ADME (pharmacokinetics) of curcumin. They reported many species-related differences in distribution of curcumin and THC in dogs and humans. The metabolism of curcumin to THC was similar. Curcumin distribution into PBMC from patients with chronic lymphocytic leukemia (cancer) was higher compared to PBMC from healthy individuals. The greater distribution of curcumin into PBMC in patients with cancer may have therapeutic advantage (Bolger et al. 2018).

Suresh and Srinivasan (2010) studied pharmacokinetics following oral administration of piperine (170 mg/kg) and curcumin (500 mg/kg) in rats. The tissue concentrations of curcumin and piperine were determined by HPLC. Curcumin's bioavailability was 63.5% with a C_{max} at 1 h (intestine) and 6 h (blood) and remained at significantly higher level even at 24 h. Only a small portion of curcumin (0.2%) was excreted in urine. Concomitantly oral administration with piperine improved curcumin's intestinal absorption and stayed significantly longer in the body tissues. Curcumin was detected in the brain at 24, 48, and 96 h with a maximum at 48 h. They concluded that curcumin could be traced in the brain following its oral administration, and its bioavailability can be improved by co-administration with piperine. The long-term clinical studies using turmeric or curcumin at low doses are needed in pets, horses, and people.

Schiborr et al. (2014) conducted a randomized small crossover study in healthy subjects (13 women, 10 men). A single oral dose of 500 mg curcuminoids as native powder, micronized powder, or liquid micelles was utilized. Blood and urine samples were collected for 24 h, and total curcuminoids and safety parameters were quantified. In the area under the plasma concentration-time curve (AUC), micronized curcumin indicated 14-, 5-, and 9-fold and micellar curcumin 277-, 114-, and 185-fold better bioavailable than native curcumin in women, men, and all subjects, respectively. Curcumin was better absorbed in women than men. Both the micronized powder and, in particular, the liquid micellar formulation of curcumin significantly improved its oral bioavailability without altering safety parameters. The liquid micellar formulation of curcumin or nanoparticle may be well suited to deliver curcumin in human intervention trials. All safety parameters remained within the reference ranges following the consumption of these formulations. The observed differences in curcumin absorption (pharmacokinetics) warrant further ADME investigation in pets and horses of both genders.

5 Mechanism of Action

The precise mode of action for turmeric and curcumin (curcuminoids) remains unknown. Javeri and Chand (2016) and Kumar et al. (2018) summarized that curcumin may act via multiple modes of action. It may act as modulator of dysregulated immune inflammation. Curcumin is well known to influence many genes, epigenetic steps, enzymes, and pathways. This profile may be relevant to its broad spectrum of pharmacology (Javeri and Chand 2016; Cavaleri 2018; Kumar et al. 2018; McCubrey et al. 2017).

Colitti et al. (2012) evaluated the effects of dietary curcumin (CurcuVet, 4 mg/kg BID for 20 days, $n = 6$) and compared it with NSAID (firocoxib, 5 mg/kg BID for 20 days, $n = 6$) in dogs suffering with osteoarthritis (OA). This small clinical trial was designed to study the effects of NSAID or dietary administration of curcumin on canine transcriptome using circulating leukocytes. This study highlights the complexities of mode of action of curcumin on gene level using a chronic disease model. At the end of the treatment on day 20, a reduction of pain and a partial recovery of articular function were observed by the veterinarians. On day 20, these investigators discovered that curcumin treatment reduced 228 downregulated genes to 110 and reduced 271 upregulated genes to 31. Treatment with curcumin (CurcuVet, 4 mg/kg BID for 20 days, $n = 6$) altered gene expression, inhibited macrophage proliferation, downregulated genes involved in inflammatory response (TNFα, TLR4, IL8, IL18, and MAPK14), and upregulated Ikβ in the TNRF1 signaling pathway (improving communication between immune cells), as well as activated genes involved in fibrinolysis. However, NSAID upregulated genes (TNFα, TLR4, IL8) but did not influence genes (IL18 and Ikβ) in the TNRF1 signaling pathway. These findings show differential modulation of genes by curcumin and NSAID. A long-term large clinical trial is warranted in aging cats, dogs, horses, and people suffering with OA and other dysregulated chronic immune-inflammatory diseases of the joints, brain, eyes, and digestive system. In this study, the effect size was highly variable. These investigators

concluded that due to the small number of dogs (six) in the study and highly variable clinical effect size, the clear proof of clinical efficacy could not be established.

In the brain, diverse mechanisms of action may involve modulation of transcription pathways, protostomes, neurogenesis, and the hypothalamic-pituitary-adrenal axis as well as immune inflammatory pathways (Seo et al. 2015). The potential antiarthritic effects of turmeric or its extracts may be related to the establishment of equilibrium between catabolism and anabolism of joint cartilage as well as its well-known broad spectrum of anti-inflammatory activities. In 2016, de Oliveira et al. provided evidence that curcumin improves mitochondrial dynamics—mitochondrial biogenesis and mitophagy (a key step in keeping the cell healthy). They also elegantly summarized curcumin biosynthesis, source, bioavailability, and metabolism.

The long-term effect of turmeric- or curcumin-containing dietary supplements (nutraceuticals) and medications using wide dose ranges and longer duration of treatment on gene transcription (expression and function in circulatory leukocytes or at the site of inflammation such as the joints or the brain) in horses, pets, and patients living with specific diseases may offer reliable, reproducible, and viable biomarkers of clinical efficacy and safety. This knowledge may help the R&D experts in discovering and formulating safer and effective combination(s) of herbs or their active ingredients.

6 Digestive System

Inflammatory bowel disease (IBD), a chronic immune disorder of the digestive system, is very common in cats and humans. IBD can be divided into two subgroups: Crohn's disease (CD) and ulcerative colitis (UC). The current pharmacological approaches for treating IBD are generally not curative and are often associated with serious side effects. The disease-altering medications, such as thiopurines, methotrexate, tacrolimus, thalidomide, cyclosporine, and infliximab, are expensive. Brumatti et al. (2014) and Neto et al. (2018) have reviewed and summarized the pathogenesis of IBD and its current therapeutic approaches and potential therapeutic utility of curcumin. Often there is a strong relationship between nutrition and IBD pathogenesis. Therefore, developing new dietary strategies using turmeric or its active phytochemicals such as curcumin may open a door for finding an affordable adjunct therapy and prevention approach for the early stages of IBD. They concluded that it is necessary to find the suitable dose of curcumin and optimal duration of treatment for preventing or treating the recurrence of IBD. Turmeric or its active ingredients may act by decreasing the mucosal immune inflammation and dysbiosis in acute and chronic IBD.

Turmeric has been used in Ayurvedic and traditional folk medicine in the management of inflammatory disorders including IBD. Therapeutic concentration of turmeric and its active ingredients such as curcumin may be achieved in the gastrointestinal tract after oral dosing. This may make it a good candidate for the prevention and treatment of IBD (Hanai et al. 2006; Brumatti et al. 2014; Lang et al. 2015; Neto et al. 2018; Bastaki et al. 2016; Yang et al. 2018). Poor aqueous solubility, poor absorption, bio-distribution, rapid metabolism, and fast elimination of curcumin may cause limitations in its clinical development (Brumatti et al. 2014; Javeri and Chand 2016). However, this profile may be desirable for R&D for IBD. It remains to be discovered if standardized turmeric may overcome such challenges in treating digestive diseases (IBD and diarrhea) in cats, dogs, and people.

Bastaki et al. (2016) evaluated the effect of turmeric on colon histology, body weight, ulcer, IL_{23}, MPO, and glutathione in acetic acid-induced IBD in rats. Turmeric powder (1, 10, and 100 mg/kg/day) was administered orally for 3 days before or 30 min after the induction of IBD. This treatment was found to reduce macroscopic and microscopic ulcers, IL_{23}, myeloperoxidase, and GSH (reduced glutathione peroxidase). The lowest dose of turmeric (1 mg/kg) caused a significant decrease in mean macroscopic ulcer and score after day 7, when compared to untreated groups. Interestingly a high dose (100 mg/kg) also caused a significant (~50%) reduction after 2 days. High dose had no significant effect on mean macroscopic ulcer and score after 4 and 7 days of IBD. They also observed that this treatment increased body weight and reduced colitis-related oxidative stress. The 10 mg/kg dose appeared to be the ideal dose in rat IBD model. These investigators suggested a possibility of developing *C. longa* (turmeric) as a safe and potent anti-inflammatory and antioxidant herbal remedy in the management of IBD.

Hanai et al. (2006) studied the effect of curcumin on ulcerative colitis (UC). Patients suffering with UC were given sulfasalazine (1.0–3.0 g/day) or mesalamine (1.5–3.0 g/day) plus 2 g curcumin (1 g taken after breakfast and 1 g after the evening meal), or placebo, for 6 months. Patients were then followed for an additional 6 months, during which either SZ or mesalamine was continued. All medications except SZ or mesalamine were discontinued 4 weeks before starting this study. Eight of 39 patients in the placebo group relapsed, whereas 2 out of 43 patients on curcumin relapsed during the 6 months of therapy. These authors concluded that curcumin may be a safe and effective medication for maintaining remission in patients with Crohn's disease or UC. In another study, IBD patients were given curcumin (360 mg/dose) three to four times a day for 3 months. This treatment significantly reduced clinical relapse in patients with quiescent IBD. The inhibitory effects of curcumin on inflammatory mechanisms like NF-κB, COX_2, LOX, and TNFα and its safety profile suggest that

curcumin or turmeric may have some prospects in the treatment of IBD. They recommended that randomized controlled clinical investigations in large cohorts of patients are warranted to fully evaluate the clinical potential of curcumin (Hanai and Sugimoto 2009). Similar well-designed long-term clinical studies using turmeric or its active ingredients need to be conducted in cats and dogs suffering from digestive diseases such as diarrhea and IBD.

Later, Lang et al. (2015) demonstrated that the addition of curcumin to mesalamine was superior to the combination of placebo and mesalamine in inducing clinical and endoscopic remission in patients with mild-to-moderate active UC. Patients received 1 month of add-on therapy of 3 g oral capsules of curcumin or an identical placebo in two divided doses daily (consisting of three capsules twice a day before meals). This addition did not produce any apparent adverse effects. They concluded that curcumin may be a safe and effective agent for the management and treatment of UC.

Shen et al. (2017) evaluated the effects of oral curcumin administration on the gut microbiota of C57BL/6 mice. Curcumin significantly affected the abundance of several representative families in gut microbial communities including Prevotellaceae, Bacteroidaceae, and Rikenellaceae. Dou et al. (2018) studied the effect of curcumin (100 mg/kg/day PO for 14 days) on collagen-induced arthritis (CIA) in rats. They demonstrated that curcumin attenuates CIA through modulating the function of the cholinergic system in the gut-brain axis.

Yang et al. (2018) administered curcumin or tetrahydrocurcumin orally (0.1 or 0.25 mmol/kg daily) for 7 days before and together with dextran sulfate sodium (DSS administration, 3% in tap water) in mice. Oral dosing of curcumin significantly reduced the severity of DSS-induced colitis. This treatment also reduced the activation of NF-ϰB and STAT3 as well as expression of COX-2 and inducible nitric oxide synthase. Tetrahydrocurcumin exerted weak inhibitory effects. This group of scientists concluded that oral administration of curcumin inhibits experimentally induced murine colitis. This effect was associated with inhibition of pro-inflammatory signaling mediated by NF-ϰB and STAT3.

Ohno et al. (2017) studied the effect of nanoparticle curcumin on the development of DSS-induced colitis in mice. The rodent diet was mixed with nanoparticle curcumin (0.2%). The administration of nanoparticle curcumin was started 7 days before DSS administration. This treatment significantly improved mucosal permeability and reduced body weight loss, disease activity index, and histological colitis score. This treatment significantly reduced NF-κB activation in colonic epithelial cells and mucosal mRNA expression of inflammatory mediators and increased the abundance of butyrate-producing bacteria and fecal butyrate level. This was accompanied by increased expansion of CD4 + Foxp3+ regulatory T cells and CD103+ CD8α− regulatory dendritic cells in the colonic mucosa. They concluded that nanoparticle curcumin may be a promising candidate as a therapeutic option for the prevention and treatment of IBD.

McCann et al. (2014) demonstrated that turmeric, partly due to its curcumin content, exerts a beneficial effect on two gene variants linked to IBD severity. Turmeric reduces the abnormal transport function of the SLC22A4 503F variant (authenticated cell lines Flp-In™ 293 (Flp293) and 293/TLR4-MD2-CD14). It also increases the activity of the IL_{10} promoter variant, which was reduced in IBD. They suggested that IBD sufferers with the defective gene variants may benefit from turmeric consumption. These in vitro observations suggest a need for conducting long-term clinical studies using standardized turmeric and/or other curcumin formulations in pets, horses, and people suffering with IBD.

Bland et al. (2017) studied the effects of liposomal curcumin on five opportunistic bacterial strains in the equine hindgut. Horses often suffer gastrointestinal (GI) tract illnesses such as colic, enterocolitis, diarrhea, and inflammatory bowel disease. The intestinal tract in horse is sensitive and contains a highly complex microbial population. Infections, immune inflammation, and colic may occur as a result of a shift in the microbial population, or dysbiosis. The use of nutraceuticals in the equine industry is on the rise, and curcumin possesses antimicrobial properties that may help in minimizing the proliferation of opportunistic bacteria. *C. perfringens*, *C. difficile*, *E. coli* in general and K-12, and *Streptococcus bovis/equinus* complex (SBEC) are common opportunistic bacteria found in the hindgut of horses. Liposomal curcumin at higher doses has the potential to increase the concentration of opportunistic bacteria, which would contribute to microbial dysbiosis rather than mitigate it. The use of standardized turmeric or its active ingredients as nanoparticles, and a wide range of low doses with a longer treatment period, may restore homeostasis in the gastrointestinal system during disease states such as enterocolitis, diarrhea, and inflammatory bowel disease and dysbiosis. It may exert antimicrobial properties without adversely affecting cecal characteristics.

Turmeric, or its curcuminoids (curcumin) and other active ingredients, may slow the progression of dysregulated persistent chronic immune inflammation in the wall of the intestine if the treatment is started in the early stages of IBD. The regular use of low doses of turmeric or its extracts as dietary supplement or as spice (as food) may also reduce the need for disease-altering medications in the gastrointestinal tract. Clinical long-term studies using a wide dose range of turmeric or curcumin in pets, horses, and people living with mild-to-moderate IBD are warranted.

In conclusion, curcumin, or its novel nano-formulations, or standardized turmeric (containing >235 phytochemicals, fiber, and their degradation products by the gut microbiota)

may alter permeability and epithelial barrier function of the gastrointestinal (GI) tract by altering the macro- and micro-environment in IBD (UC and CD) in cats, dogs, and people. Turmeric, or its active ingredients' broad spectrum of the mechanism of action, especially under immune inflammatory states in the gastrointestinal tract, may restore homeostasis and may slow the progression of IBD, if treatment is started in the early stages.

7 Common Cold and Infections

Cats and dogs often suffer from ear, skin, urinary, and bladder infections and common cold. The anti-inflammatory, antibacterial, and antiviral activities of turmeric and curcumin may help in preventing or treating infection in pets and people. Kennel cough (common cold) is common in cats and dogs. There is a need for finding natural products or extracts to deal with the emergence of drug-resistant influenza viruses and the threat of pandemics in pets and people. Turmeric and curcumin may act in additive or synergistic fashion with anti-infective agents.

The three new chemical entities and ten known curcuminoids isolated from a methanol extract of turmeric strongly inhibited neuraminidases from two influenza viral strains, H1N1 and H9N2. This inhibition was noncompetitive with IC_{50} values ranging from 6.18 ± 0.64 to 40.17 ± 0.79 μg/ml and 3.77 ± 0.75 to 31.82 ± 1.33 μg/ml, respectively. Three compounds (4, 5, and 13) also exhibited significant inhibitory activity against the neuraminidases from novel influenza H1N1 (WT) and oseltamivir-resistant novel H1N1 (H274Y mutant) expressed in 293T cells. These findings suggest that turmeric or its curcuminoids may have preventive and therapeutic potential in the prevention and treatment of diseases caused by influenza viruses.

Recently, Han and his team (2018) demonstrated that daily oral dose of curcumin (100 mg/kg for 7 days) inhibited influenza A virus (IAV) in vitro and reduced the severity of the disease in mice. Curcumin was found to trigger expression of heme oxygenase-1 in vivo and attenuate IAV-induced lung injury. Furthermore, curcumin regulated immune response following IAV infection through inhibiting production of local inflammatory cytokines and NF-κB signaling in macrophages and by enhancing IκBα and AMPK. These data suggest that turmeric or its extract may have promising efficacy in viral pneumonia.

Nonsurgical traumatic wounds lead to bacterial infections. These infections can be a life-threatening medical situation, especially those caused by multidrug-resistant (MDR) bacteria with limited therapeutic options. The antimicrobial activity of polymyxin B and curcumin, alone and in combination, was determined to be effective against MDR bacterial isolates associated with traumatic wound infections. In the presence of curcumin, the minimum inhibitory concentrations of polymyxin B were significantly reduced by a factor of 3- to 10-fold, and it reduced the cytotoxicity of the antibiotic. These findings demonstrate that curcumin exerts antibiotic-sparing effects and this combination acts in a synergistic fashion (Betts et al. 2016). These studies suggest the developing combination formulations containing turmeric or curcumin with antibiotics. This approach may help to reduce the prevalence of multidrug-resistant (MDR) bacteria in hospital settings.

8 Osteoarthritis (OA, Degenerative Joint Disease)

The most common aging-associated diseases in pets and people include dysregulated and persistent chronic immune inflammation, osteoarthritis, rheumatoid arthritis, diabetes, obesity, atherosclerosis, neurodegenerative diseases, hypertension, ocular diseases, osteoporosis, cancer, cardiovascular diseases, and chronic kidney diseases, as well as infections. A vast body of literature on turmeric and its active ingredient, curcumin, shows that they have potential for preventive medicine in aging-associated diseases. Dende et al. (2017) demonstrated that nano-formulated curcumin has a better therapeutic index than the native form of curcumin. Kumar et al. (2018) reviewed the potential role of curcumin and nanocurcumin with improved stability and oral bioavailability and its putative mechanism of action and recent advances in the management and treatment of aging-associated diseases.

Aging horses exhibit chronic, low-grade inflammation, which is often associated with many afflictions including laminitis and osteoarthritis. Nonsteroidal anti-inflammatory drugs (NSAIDs including flunixin, meglumine, and phenylbutazone) are effective in treating acute inflammatory conditions. The chronic long-term treatment with NSAIDs may result in negative side effects. Curcumin (20 μg/ml) was found to inhibit lymphocyte pro-inflammatory cytokine production in aging horse in vitro (Siard et al. 2016). The long-term preventive and therapeutic effect of standardized turmeric, curcumin, and nanocurcumins (targeted drug delivery technologies) on aging-associated diseases in aging pets, horses, and people are warranted.

In dogs, osteoarthritis (OA) is one of the most common causes of lameness. OA is caused by a deterioration of cartilage in the joints. It leads to inflammation, loss of range of motion of the joint, and pain. More than ten million dogs suffer from OA in the USA alone. Repeated traumatic insults to the joints (hip or elbow), joint dysplasia, aging, obesity, and excessive jumping or running or playing or hunting or

other genetic risk factors may result in osteoarthritis in dogs. It is a multifactorial disease of the joints. Akuri and his associates (2017) summarized the pathophysiology of OA. A series of biomechanical and pathophysiological events perpetuate structural degenerative changes in the joint, which often leads to crippling pain, long-term disability, and poor quality of life. Good nutrition, functional foods, caloric restriction, exercise, and herbs (dietary supplements as food) may reduce the need for pain medications (opiates) and anti-inflammatory agents (NSAIDs and cortisone) in pets and people suffering with OA. For preventive strategies, early diagnosis and intervention of OA and comorbidities is absolutely necessary. Besides preventive strategies (proper nutrition, exercise, weight loss, caloric restriction), Ayurvedic herbal supplements in oils (emu oil, coconut oil, hemp oil, or fish oil, omega-3) may offer promise in managing chronic OA.

Analgesics (opiates), NSAIDs, and cortisone are often used in managing OA in aging pets and people. Their clinical utility is limited by adverse effects, low systemic absorption, and high costs. It remains to be discovered if curcumin or turmeric would reduce the need for NSAIDs, cortisone, and opiates in managing OA.

The pathogenesis of osteoarthritis involves processes such as inflammation, osteoclastogenesis, and proteolytic degradation of cartilage. Turmeric and its extracts (curcumin or curcuminoids and non-curcumin turmeric ingredients) have a broad-range pharmacological profile that can modify many aspects of OA and may slow the progression of OA by reducing inflammation and cartilage and bone destruction, especially in the early stages of OA. The vast literature summarized in this review suggest that curcuminoids in turmeric may have potential to benefit patients suffering with osteoarthritis (Akuri et al. 2017). Canine natural osteoarthritis is a realistic model for finding safe and effective doses of dietary supplements as nutraceuticals and/or prescription-grade nutraceuticals for veterinary and human use.

Innes et al. (2003) studied the effect of P54FP (an extract of Indian and Javanese turmeric). Each capsule contained 20 mg curcuminoids (curcumin and desmethoxycurcumin, 50 mg *C. xanthorrhiza* volatile oil, and 150 mg *C. domestica* essential oil), and dogs were treated twice daily for 8 weeks. They reported that this treatment produced a remittance of pain and a recovery of articular movement in dogs.

Zhang et al. (2016) investigated the effect of curcumin using destabilization of medial meniscus (DMM) osteoarthritis mouse model. Immediately after DMM, mice were treated orally with 50 mg/kg curcumin dissolved in corn oil or vehicle (corn oil only) for 8 weeks. The topical application of curcumin nanoparticles or vehicle control (coconut oil) on the skin, within a 5 mm^2 area directly above the DMM operated knee, once daily for 8 weeks. The data obtained in this study demonstrated that oral and topical curcumin administration slows the progression of OA in this post-traumatic osteoarthritis mouse model. This may be translated into an oral human equivalent dose of curcumin of ~4 mg/kg/day for 8 weeks or longer. The delivery of low doses of turmeric or curcuminoids using nanotechnology or target-specific drug delivery may offer affordable, safe, and effective strategies or adjunct therapy for the long-term management and treatment of OA and other chronic inflammatory diseases of the joints, gut, brain, and body.

Jeengar et al. (2016) reported that topical application of curcumin with emu oil inhibited carrageenan-induced rat paw edema and Freund's complete adjuvant-induced arthritis in rats. This combination was effective in bringing significant alterations in the functional, biochemical, histopathologic, and radiologic parameters in rat paw. Their outstanding findings suggest that that topical application of curcumin with emu oil may offer noninvasive intervention for the treatment of inflammatory arthritis in pets and people.

A polar extract of turmeric produced a dose-dependent decrease in monosodium iodoacetate-induced osteoarthritis in rats. This activity was correlated by upregulating type II collagen gene (COL2A1) as well as downregulating MMP-3 and MMP-7. The beneficial effects of polar extract of turmeric may be related to the establishment of equilibrium between catabolism and anabolism of joint cartilage (Murugan et al. 2017; Velusami et al. 2018).

Curcumin (CurcuVetR containing 20% curcuminoids) was found to reduce PMA-induced stimulation of sheep neutrophils and increased spontaneous apoptosis and inhibited both IL8 and Bcl2A1 expression cultured cells within 22 h (Farinacci et al. 2009). They suggested that curcumin may limit the early phases of neutrophil infiltrations, and such an effect may have potential clinical application in the management of ruminant inflammatory disorders. In addition, Colitti et al. (2012) studied the effect of dietary curcumin (CurcuVetR at 4 mg/kg BID for 20 days) on the gene expression of peripheral white blood cells in dogs suffering with OA. They used a 44K oligo microarray technique. This treatment was found to alter the molecular target of inflammatory response. Specific molecular targets of curcumin were inhibition of macrophages proliferation and downregulation of TNFα, TLR4, IL8, IL18, and MAPK14, as well as activation of fibrinolysis. From a mechanistic point of view, these findings suggest good support for OA treatment with low dose of curcumin. They suggest that for drawing a definitive conclusion from this study, a large number of patients (pets and people) are required to validate the use of curcumin for the treatment of OA in dogs.

Recently, Liu et al. (2018a, b) reviewed the vast literature on the efficacy and safety of dietary supplements for patients with osteoarthritis. Seven supplements (pycnogenol, passion fruit peel extract, *Curcuma longa* extract, L-carnitine, *Boswellia serrata* extract, curcumin, and MSM) were found to exert large and clinically significant effects on physical function in the short term in patients living with OA. Del

Grossi Moura et al. (2017) concluded that good-quality clinical research is still lacking and it does not support the use of curcumin and herbal medicines in treating OA. Poor patient adherence and compliance make it very difficult in finding the short-term or long-term efficacy of dietary supplements as with pharmaceutical medications (Liu et al. 2018a, b).

Recently, Haroyan et al. (2018) studied the effects of CuraMed® 500 mg capsules (333 mg curcuminoids) and Curamin® 500 mg capsules (350 mg curcuminoids and 150 mg boswellic acid), taken orally three times a day for 12 weeks, in 201 patients living with OA. This combination appeared to exert synergistic efficacy in 40- to 70-year-old patients suffering with OA. These studies suggest that the long-term use of turmeric and/or its major ingredients as dietary supplement or as foods may slow the progression of OA in pets and people.

9 Pain

Several complementary and integrative approaches including physical activity, exercise, herbs (turmeric, hot peppers, etc.) or their ingredients (curcumin, capsaicin, etc.), vitamin D, omega-3 fatty acids, lipoic acid, acupuncture, yoga, aquatic yoga, meditation, and mindfulness meditation may play important roles in managing chronic pain in people. The high interindividual variability between patients is expected in responses to these pain management modalities (Wojcikowski et al. 2018). The herbs or their extracts (ingredients such as curcuminoids), vitamin D, omega-3 fatty acids, emu oil, and lipoic acid as oral or topical application may be utilized as veterinary nutraceuticals and dietary supplements for chronic pain management.

Curcumin has been reported to exert analgesic effect in animal models and in humans (Cheppudira et al. 2013; Lin et al. 2011; Motaghinejad et al. 2015; Gaffey et al. 2015; Zhu et al. 2014). It may be acting as a transient receptor potential vanilloid-1 (TRPV1) receptor antagonist (Nalli et al. 2017; Zhi et al. 2013; Yeon et al. 2010).

Jhi et al. discovered that curcumin (4 mg/kg/min IV for over 3 min) caused a marked and rapidly reversible inhibition of colorectal distension-induced visceromotor responses (VMRs) in anesthetized rats. In the mouse jejunum, the mesenteric afferent nerve response to ramp distension was attenuated by curcumin (3 and 10 μM). In addition, curcumin (1–30 μM) inhibited the afferent responses to capsaicin in a concentration-dependent manner. Trinitrobenzene sulfonic acid-induced hypersensitivity of jejunal afferents was also attenuated by curcumin. Curcumin potently inhibited capsaicin-induced rise in intracellular calcium and inward currents in mouse or rat dorsal root ganglia (DRG) neurons. These studies demonstrate that curcumin inhibits visceral nociception via antagonizing transient receptor potential vanilloid-1 (TRPV1) receptor TRPV1. This suggests that curcumin or turmeric may help in the treatment of gastrointestinal diseases such as hypersensitive esophagus and heartburn.

Cheppudira et al. (2013) reviewed the effects of curcumin on various pain and wound-healing models in preclinical studies. Patients suffering with peripheral neuropathy (PN) frequently experience sharp spontaneous pain, allodynia, and hyperalgesia. Opioids, anticonvulsants, and tricyclic antidepressants are often used to treat neuropathic pain. These medications are often unsatisfactory because of limited efficacy and adverse side effects. There is unmet medical need for finding novel chemical entities or dietary supplements (nutraceuticals) to manage chronic pain and wound healing in pets and people. The preclinical studies summarized below suggest that turmeric or curcumin may have some potential to treat both pain and wounds. The oral use of curcumin, alone, or as an adjunct therapy, may be useful in the management of postoperative pain and neuropathic pain (Cheppudira et al. 2013; Zhu et al. 2014).

Lin et al. (2011) demonstrated that morphine injections (10 mg/kg, sc) for 7 days produce tolerance in mice. Morphine tolerance is attenuated by co-administration of low-dose curcumin (25 mg/kg, ip) for 7 days. On the other hand, morphine tolerance is aggravated by chronic high-dose curcumin (400 mg/kg/day for 7 days). The acute low-dose curcumin did not enhance morphine's analgesic activity. These observations tend to suggest that high doses of curcumin may be pro-inflammatory and may act by negating other beneficial effects such as inhibiting the expression of antiapoptotic cytokines and neuroprotective factors.

Zhu et al. (2014) summarized the results of many previously published preclinical studies. In a chronic constriction injury (CCI) model of neuropathic pain in rats, single dosing with curcumin did not influence mechanical and thermal hyperalgesia, but repeated curcumin treatment progressively and completely reversed CCI-induced hypersensitivity. The daily curcumin dosing reverts streptozotocin-induced diabetic neuropathy. However, acute curcumin treatment reduces formalin-induced defensive behaviors, visceral pain as measured by acetic acid-induced writhing response, capsaicin-induced thermal hyperalgesia, and reserpine-induced fibromyalgia-like behaviors. They also demonstrated that a surgical incision on the right hind paw of rats induces a sustained mechanical hyperalgesia. It lasted for 5 days. Curcumin (10–40 mg/kg administered by the mouth) apparently in dose-dependent fashion reversed mechanical hyperalgesia in rats. In addition, repeated curcumin treatment facilitated the recovery from surgery. The repeated treatment with curcumin before surgery did not impact the postoperative pain threshold and recovery rate. However, the repeated

treatment with curcumin after surgery reduced postoperative pain threshold and improved recovery rate. The oral use of curcumin, alone or as an adjunct therapy, may be useful in the management of postoperative pain (Zhu et al. 2014).

Motaghinejad et al. (2015) demonstrated that curcumin attenuates morphine withdrawal syndrome. The antinociceptive activity of curcumin in a mouse model of visceral pain is mediated by opioidergic and serotonergic systems. They suggested that curcumin may be effective in attenuating pain during the opioid withdrawal period.

Gaffey et al. (2015) reviewed the effects of curcumin on musculoskeletal pain. Curcuminoids found in turmeric were effective in enhancing wound healing and in the treatment of burn pain and diabetic neuropathic pain. The use of curcuminoids to treat pain associated with knee osteoarthritis showed greater reductions of pain as compared with a placebo, and the efficacy was comparable to the use of ibuprofen. A significant efficacy was found with the use of turmeric extract in combination with other nutraceuticals (devil's claw and bromelain) to treat acute and chronic osteoarthritis pain. A proprietary lecithin formulation of curcumin exerted significant reduction of delayed onset muscle soreness, and the efficacy was comparative to a standard dose of acetaminophen in the treatment of acute pain.

The prolonged use of opioids for the treatment of chronic pain induces opioid-induced hyperalgesia (OIH). It is one of the major clinical problems. A newly developed PLGA-curcumin nano-formulation (PLGA-curcumin) administered intrathecally or orally significantly attenuated hyperalgesia in mice with morphine-induced OIH. This was associated with the suppression of chronic morphine-induced CaMKIIα activation in the superficial laminae of the spinal dorsal horn. These data suggest that PLGA-curcumin may reverse OIH possibly by inhibiting CaMKIIα and its downstream signaling (Hu et al. 2016).

Earlier, Agarwal et al. (2011) reported that curcumin (500 mg capsule every 6 h for 3 weeks) improved postoperative pain and fatigue in patients undergoing postoperative recovery. The effect was more pronounced on days 7 and 14. Furthermore, oral administration of curcumin significantly reduced progression of osteoarthritis (OA) in destabilization of the medial meniscus mouse model. However, it did not influence pain. In addition, topical application of nanoparticles (curcumin) not only reduced pathogenesis of OA but also relieved OA-related pain. Nanoparticles reduced tactile hypersensitivity and improved locomotor behavior. Gera et al. (2017) reviewed the field of curcumin's nanoformulations. These novel formulations of turmeric or curcumin may be an emerging paradigm shift for improved remedial applications in nutraceutical and pharmaceutical settings.

The effect of repeated daily oral doses of curcuminoids (*C. longa* extract, CLE, at 5, 20, or 80 mg/kg/day) was evaluated in hot-plate test in mice. On day 11, all animals were subjected to foot-shock stress triggered by a hyperthermia test and day 12 to a tail suspension test for antidepressants. CLE produced dose-dependent analgesic activity. Interestingly, only low doses of CLE were effective in relieving central pain (Verma et al. 2017).

Bethapudi et al. (2017) evaluated the analgesic effect of turmerosaccharides rich fraction (NR-INF-02) on monosodium iodoacetate-induced OA pain in rat model that mimics human OA. The oral administration of turmerosaccharides rich fraction at 45 and 90 mg/kg was found to decrease OA pain at 1, 3, 6, and 24 h posttest administration on day 5. The effect of turmerosaccharides rich fraction on OA pain was superior to turmerosaccharides less fraction.

Dry socket (alveolar osteitis) is a painful dental condition that happens after a permanent adult tooth extraction when the blood clot at the site of the tooth extraction fails to develop or it dislodges or dissolves before the wound has healed. In a recent study, Lone et al. (2018) demonstrated a significant reduction in mouth pain, inflammation, and discomfort after turmeric dressing in 178 patients diagnosed with dry socket syndrome. Wound healing progressed faster than dressing with ZOE dressing. These studies suggest that the effect of curcumin (curcuminoids) may depend on the time of initiation of treatment, duration of treatment, dose of curcumin, etc. Double-blind placebo-controlled clinical studies are warranted in pets and people suffering with NP, OA, and postsurgical pain.

Curcumin (nanoparticles) may represent a novel topical antimicrobial (antibacterial, antiviral, and antifungal) and wound healing adjuvant for infected burn wounds and other cutaneous and muscle injuries and related pain conditions (Krausz et al. 2015; Gera et al. 2017). Long-term clinical studies are needed to establish analgesic activity of turmeric or curcuminoid nanoparticles (low doses and long duration of treatment in pain-related conditions).

10 Sports Medicine

Traumatic muscular injuries (soft tissue trauma, bruises, and contusions) are too common in dogs and horses. Dietary supplementation with ST or curcumin and other strategies to reduce muscle soreness and to improve muscle (physical) recovery are of great interest to athletes and to people caring for horses and dogs. ST or curcumin may be a suitable alternative to nonsteroidal anti-inflammatory medications for the management of diseases of the musculoskeletal systems such as muscle soreness and OA (Heaton et al. 2017).

Tanabe et al. (2015) studied the effect of 150 mg of curcumin (taken by mouth before and 12 h after each exercise session in a randomized, crossover design study in 14 untrained young men). This treatment reduced maximal voluntary contraction (MVC) torque, and muscles recovered

faster (e.g., 4 days postexercise $-31 \pm 13\%$ vs. $-15 \pm 15\%$); peak serum creatine kinase (CK) activity was less for those treated with curcumin than with placebo ($P < 0.05$). The researchers concluded that theracurmin ingestion may reduce some aspects of muscle damage.

McFarlin et al. (2016) evaluated the effect of curcumin supplementation (Longvida®; 400 mg/day). It reduced CK (a biomarker of muscle injury) by 48% after subjects consumed curcumin for 2 days before and 4 days after a high-intensity muscle damage-inducing protocol. They concluded that consumption of curcumin reduced a biomarker of muscle injury, but not quadriceps muscle soreness or inflammatory biomarkers, during recovery after exercise-induced muscle damage. The observed reduced biomarker of muscle injury may translate to faster recovery and improved functional capacity during subsequent exercise sessions. This conclusion needs to be explored using a large number of sports participants.

Heaton et al. (2017) reviewed nutritional strategies for muscle regeneration, muscle fatigue, physical and immune health, and preparation for subsequent training sessions in sports medicine. They concluded that the anti-inflammatory and anti-oxidative activities of turmeric and its active ingredients suggest that these agents at low appropriate doses may have a role in sports medicine, especially in preventing the consequences of concussions and muscle injuries.

Curcumin or turmeric may provide some recovery benefit or reduced muscle damage during the intense sport activities. Therefore, clinical research using wide doses of ST or curcumin is warranted prior to incorporating supplemental dosages into the athlete's diet or into pet supplements.

11 CNS Effects

Sarker and Franks (2018) reviewed published preclinical and clinical studies related to efficacy of curcumin for age-associated cognitive decline. Ramkumar et al. (2018) summarized the antioxidant, anti-inflammatory, neuroprotective, and antiproliferative activities of curcumin, demethoxycurcumin (DMC), and bisdemethoxycurcumin (BDMC). DMC may have better anticancer and anti-inflammatory activity as compared with curcumin. Recently, DMC (5, 10, and 20 mg/kg, i.p., for 7 days) was shown to abrogate rotenone-induced dopamine depletion and motor deficits by its anti-oxidative and anti-inflammatory properties in parkinsonian rat model. They concluded that DMC may be a promising therapeutic lead for the treatment of neurodegenerative diseases including Parkinson's disease.

12 Diabetes

In developing countries, about 80% of people depend on traditional herbal medicine to meet their healthcare needs. Turmeric has been used for the management of diabetes in Ayurvedic and traditional Chinese medicine. Curcumin reduces glycemia and hyperlipidemia in rodent models of diabetes (Zhang et al. 2013). Dietary curcumin relieves stress in metabolic tissue, leading to improvements in diabetes and associated disease complications in rodent models and in clinical studies. New improved methods of curcumin delivery (nanoparticles and lipid/liposome formulations) may help in cell-directed targeting, and it may offer improved therapeutic outcomes in diabetes (Maradana et al. 2013). In addition, curcumin or curcuminoid supplementation has been reported to be effective in lowering the fasting blood glucose concentrations in people with prediabetes, diabetes, or metabolic syndrome. Curcumin produced significant decrease in HbA_{1c} as compared to placebo. de Meloa concluded that curcumin supplementation may be an adjuvant aid in the management of dysglycemic patients.

Recently, Yang et al. (2018) studied the effects of curcumin on retinal damage in STZ-induced diabetic rats. Curcumin (100 and 200 mg/kg, PO, daily for 16 weeks) was found to reduce diabetes-induced body weight loss, blood glucose, and retinopathy. This activity may be attributed to the hypoglycemic, antioxidant, VEGF-downregulating, and neuroprotection properties of curcumin. They suggested that curcumin may have a potential in the treatment of diabetic retinopathy.

Curcumin suppresses activities of gluconeogenic enzymes and increases glycogen storage in the liver and reduces blood glucose in db/db mice (Fujiwara 2000). Wickenberg demonstrated that 6 g of turmeric taken by mouth increased postprandial insulin levels in healthy subjects. The increased insulin response may be due to the stimulation of β-cell function by curcumin. In addition, oral administration of 10 mg of curcumin (twice a day for 28 days) lowered LDL levels and increased HDL levels in patients with atherosclerosis (Ramirez et al. 2000). In this study, the low dose of curcumin seems to be relevant to what people get while taking low dose of ST or turmeric in the turmeric-consuming nations.

Inhibition of enzymes such as α-amylase could play a key role in the control of diabetes by slowing starch digestion. The inhibitors of pancreatic α-amylase may be of great therapeutic importance in treating diabetes mellitus. Bisdemethoxycurcumin (BDMC) inhibits porcine and human pancreatic α-amylase with an IC_{50} value of 26 and 25 μM, respectively. This may impart antidiabetic activity of turmeric and its metabolites in pets and people. BDMC could be a good drug candidate to reduce postprandial hyperglycemia (Ponnusamy et al. 2012).

Arun and Nalini (2002) studied the effect of turmeric and curcumin on diabetes mellitus in alloxan-induced diabetes in rats. Administration of turmeric or curcumin reduced the blood sugar and glycosylated hemoglobin levels as well as the activity of sorbitol dehydrogenase, which catalyzes the conversion of sorbitol to fructose. Curcumin was more effective than turmeric in attenuating diabetes mellitus-related changes. These results suggest that curcumin may be effective in attenuating diabetes mellitus-related changes.

Normal and diabetic rats were treated with curcumin (90 mg/kg/day) incorporated in yogurt. After 15 days of treatment, the glucose tolerance and the insulin sensitivity were significantly improved in diabetic rats. This improvement may be associated with an increase in the AKT phosphorylation levels and GLUT4 translocation in skeletal muscles. They suggested that curcumin metabolite(s) may be responsible for the antidiabetic activity (Gutierres et al. 2015).

ST, curcumin, and other ingredients present in turmeric and their metabolites may exert antioxidant and anti-inflammatory properties, which may assist in alleviating the complications in diabetes (Gutierres et al. 2015). In addition, curcumin exerts retina-protective effects (Xu et al. 2018; Yang et al. 2018).

13 Metabolic Syndrome

Curcumin is well known to exert an anti-inflammatory effect through downregulation of inflammatory cytokines, transcription factors, protein kinases, and enzymes that promote inflammation and participate in the development of chronic diseases. Such a multitude of effects of curcumin on gut permeability and barrier function, gut-brain axis, genes, epigenetic and molecular targets in mitochondria, and disease-specific target tissues may offer some health benefits in chronic disease states including metabolic syndrome (Shehzad et al. 2017; Ghosh et al. 2018).

Di Pierro et al. (2015) showed the ability of curcumin (complexed with phosphatidylserine in phytosome form or with pure phosphatidylserine) to reduce weight and omental adipose tissue in overweight people with metabolic syndrome in a preliminary clinical study. At the end of the first 30 days of lifestyle intervention, overweight participants were randomly assigned to one of the two groups for the 30-day treatment phase. Twenty-two of the participants were supplemented twice a day for 1 month with a nutritional supplement formulated to be enteric-coated and containing 800 mg/dose/day of *Curcuma longa* extract (95% curcumin), complexed with sunflower phospholipids (20% phosphatidylserine) and blended with 8 mg/dose/day of piperine from *Piper nigrum* extract (Bioperine). After 60 days, the collective weight loss was 6.7%, the BMI decrease was 8.4% with the percentage of fat reduced by more than 9%, and more than 6 cm was lost in waistline and 3 cm lost in hip circumference. These interesting preliminary observations by Di Pierro et al. (2015) suggest that long-term large randomized double-blind placebo-controlled clinical trials using a wide range of doses and formulations of turmeric and curcumin in overweight dogs, cats, and people are needed.

Sohrabi et al. (2018) summarized that pro-inflammatory cytokines such as interleukin-17F (IL-17F) has an association with induction of tissue inflammation and obesity. IL-17F is produced by T-helper (Th) 17 cells, natural killer cells, γδ T cells, CD4+, and CD8+ T cells. High-fructose consumption often increases body weight and serum level of IL-17. Cinnamon and curcumin supplementation decreases IL-17F under the standard diet. Feeding with cinnamon and turmeric (water-soluble extract) caused a decline in body weight but, surprisingly, increased IL-17F in rats on a high-fructose diet (Sohrabi et al. 2018).

A high-fat diet leading to postprandial hyperlipidemia and inflammation appears to be the key etiologic factor contributing to the development of atherosclerosis and subsequent coronary artery disease (Alipour et al. 2007). Acute supplementation with resveratrol (200 mg and curcumin 100 mg) did not modify high-fat diet-induced postprandial circulating inflammatory markers (C-reactive protein, IL-6, IL-8, monocyte chemoattractant protein-1), adhesion molecules (soluble E-selectin, soluble vascular cell adhesion molecule-1 (sVCAM-1)), or soluble intercellular adhesion molecule-1 in older adults with abdominal obesity (Vors et al. 2018). This study suggests that as-needed intake of dietary supplements (PRN basis) may not offer the desired efficacy in most clinical setting in pets and people. Long-term clinical studies are warranted to examine the dose response and newer formulations of curcumin and turmeric, alone or in combination, with other phytonutrients.

Intragastric administration of curcumin at 250 mg/kg daily for 8 weeks was found to decrease the level of free fatty acid and TNF-α in serum of type II DM rats. This treatment also improved the metabolic disorder of glucose and lipid, enhanced the sensitivity to the insulin, and ameliorated the resistance to insulin in rats (Su et al. 2017).

Mantzorou et al. (2018) reviewed recent, well-designed clinical studies and showed that curcumin appears to offer some health benefits against obesity, metabolic syndrome, and diabetes. Furthermore, curcumin may exert a heath beneficial effect in people suffering with arthritis, skin diseases, gut inflammation IBD, UC, cancer, fatty liver disease, depression, and symptoms of premenstrual syndrome. The concrete and precise recommendation cannot be made with respect to dose, formulations, and duration of treatment. They suggested that large- prospective studies are needed using well-designed clinical trials with proper considerations with respect to follow-up times, dosage, formulation, and duration of curcumin or ST supplementation, medication adherence, and patient compliance. Furthermore, a careful consideration

is needed for confounders in each specific chronic disease of pets and people.

Recently, Jin (2018) reviewed curcumin's complex mechanistic approach to drug discovery in metabolic disorders. Curcumin was shown to improve insulin signaling.

Dexamethasone injection induces insulin resistance, while concomitant curcumin gavage improves insulin tolerance (Tian et al. 2015). Insulin resistance attenuating effect of curcumin appears to be dissociated from its anti-inflammatory effect. In the long term, this protective effect may be attributed to its anti-inflammatory, anti-oxidation, and body weight-lowering effects (Jin 2018).

14 Retinopathies

Peddada et al. (2018) reviewed the etiology of eye diseases and mechanism of action for curcumin in eye diseases. They reviewed literature on the potential therapeutic of curcumin in major retinal pathologies. The retina has a rich blood supply and numerous mitochondria and is consistently exposed to pollutants and ultraviolet light (sun exposure, photons of light), which strikes its surface making the retina at high risk of developing ocular pathologies, particularly in aging populations. Oxidative stress and immune inflammatory pathways are well known to contribute to retinal pathology. Curcumin is known to possess anti-inflammatory, antitumor, antioxidant, and VEGF inhibition properties through modulation of numerous biochemical and transcription processes. Curcumin has been reported to slow, and in some cases even reverse, age-related macular degeneration, diabetic retinopathy, retinitis pigmentosa, proliferative vitreoretinopathy, and retinal cancers. The authors concluded that curcumin exerts limited efficacy, mostly in experimental animal studies.

The use of standardized turmeric as orally administered dietary supplement may slow the progression of age-related eye illnesses. Targeted drug delivery of novel formulations of turmeric or curcuminoids in the eye may reduce immune inflammation (HKH syndrome, uveitis). Oral or topical application of turmeric or its curcuminoids may reduce the need for steroids in treating ocular inflammatory conditions in pets and people.

15 Allergy

Allergic asthma is a complex, multifactorial, chronic immune-inflammatory disease of the airways. The allergic responses in the lungs are mediated via multiple complex pathways leading to release of a number of inflammatory mediators (histamine, cytokines, and enzymes) by activated mast cells, eosinophils, and T lymphocytes. Subhashini et al. (2013, 2016) briefly summarized the pathophysiology and treatment strategies of allergic asthma. The available medications (steroids and bronchodilators) are associated with limitations such as serious side effects. The studies summarized here suggest that turmeric and curcumin may have potential therapeutic utility in modulating (preventing and treating) lung allergy (asthma).

Kurup and Barrios (2007) demonstrated that orally administered curcumin suppressed latex allergen-induced Th2 response in mice. The suppression of Th2-mediated allergic responses was evident by reduced IL-4 and IL-13 production, as well as reduced infiltration of eosinophils in the lungs and reduced expression of molecules such as matrix metalloproteinase (MMP-9), thymic stromal lymphopoietin (TSLP), and ornithine aminotransferase (OAT). They concluded that curcumin may have potential therapeutic utility in lung allergy (asthma).

Subhashini et al. (2013) conducted an elegant preclinical study using curcumin in a mouse model of allergic airway inflammation (asthma). Curcumin was dissolved in dimethyl sulfoxide (DMSO) and administered an hour before every ovalbumin challenge (days 19–22). They discovered that intranasal curcumin application (2.5 and 5 mg/kg) was readily absorbed from airway mucosa and was effective in suppressing airway inflammation and allergic asthma.

Intranasal curcumin significantly inhibited leukocytes and eosinophil recruitment to the lungs and decreased eosinophil peroxidase and histamine levels in bronchoalveolar lavage fluid. These observations may suggest that curcumin (intranasal drop or spray) may reduce the need for inhaled or oral steroids and β-agonists by reducing chronic allergic airway inflammation in pets and people. Later these investigators demonstrated that intranasal curcumin (2.5 and 5.0 mg/kg) reduces airway inflammation and bronchoconstriction by modulating cytokine levels (IL-4 and IL-5 and IFN-γ and TNF-α) and sPLA2 activity and by inhibiting PGD2 release and COX-2 expression. Curcumin's anti-allergic (antiasthma) activity is mediated by the suppression of p38 MAPK, ERK 42/44, and JNK54/56 activation during allergic response in the lung. They suggested that curcumin may offer an alternative for the development of nasal formulations and inhalers for asthma management (Subhashini et al. 2016).

Shin et al. (2015) immunized mice with intraperitoneal injection of ovalbumin (OVA) and alum. The OA-sensitized mice were challenged orally with 50 mg OVA and treated with turmeric extract (100 mg/kg) or curcumin (3 mg/kg or 30 mg/kg) for 16 days. Food allergy symptoms were decreased, as were rectal temperature, diarrhea, and anaphylaxis. Turmeric extract significantly decreased food allergy symptoms (decreased rectal temperature and anaphylactic response). However, curcumin treatment showed little improvement. Turmeric extract also inhibited IgE, IgG1, and mMCP-1 levels. Turmeric extract reduced type 2 helper cell (Th2)-related cytokines and enhanced Th1-related cytokines. Turmeric extract ameliorated OVA-induced food

allergy in mice by restoring Th1/Th2 balance. They concluded that turmeric extract significantly ameliorated food allergic symptoms in this mouse model of food allergy through promoting Th1 responses over Th2-dominant immune responses. The orally administered turmeric extract including various components may be useful to ameliorate Th2-mediated allergic disorders such as food allergy, atopic dermatitis, and asthma.

Interleukin-10 (IL-10) is a pleiotropic anti-inflammatory and immunosuppressive cytokine that is produced by innate and adaptive immunity cells including macrophages, dendritic cells, mast cell, natural killer cells, eosinophils, neutrophils, B cells, CD8+ T cells, and TH1, TH2, TH17, and regulatory T cells. CNS, astrocytes, microglia, and neurons are the major sources of IL-10 production. The major source of IL-10 in the periphery is macrophages. Curcumin has been reported to enhance IL_{10} release. This mechanism may play a role in curcumin's actions—managing or treating immune inflammatory conditions of the gut, joints, lungs, heart, blood vessels, and brain (Mollazadeh et al. 2017).

16 Psoriasis

Dendritic cells (antigen-presenting cells) play a critical role for initiating the activation and differentiation of T cells in inflammatory diseases including psoriasis. Diarylheptanoid from *C. kwangsiensis* (DCK) modulated multiple functions of dendritic cells in the immunopathogenesis of psoriasis. Many steps were modified by DCK including antigen uptake, maturation, migration, and pro-inflammatory cytokine production, and it also decreased proliferation and differentiation of Th1 and TH17 and their effector cytokine production. These mechanisms in part may contribute to turmeric efficacy in treating psoriasis (Liu et al. 2018a, b).

17 Kidney Stones

Bas et al. (2009) administered curcumin orally at 75 mg/kg/day dissolved in 10% ethyl alcohol for 6 days (1 day before and 5 days after shock-wave lithotripsy, SWL) in rats. This treatment produced significant differences in histological changes under light microscope ($P < 0.02$) between SWL and control groups on days 7 and 35. This treatment was found to reduce tissue fibrosis, expressions of iNOS and p65, and serum nitric oxide levels and also prevented interstitial, glomerular, tubular epithelial, and endothelial cellular injuries. They suggested that curcumin may be used as a protective anti-oxidative agent to prevent SWL-induced renal injury.

In another study, curcumin (60 mg/kg body weight) was orally administered once daily for 28 days in rats. The calcium and oxalate levels in urine and kidney tissue homogenate were measured, and kidney histopathological examination was performed. Curcumin treatment inhibited the development of kidney stones but failed to reverse the changes caused by the kidney stones (Ghodasara et al. 2010). Herbal extract of turmeric (curcumin), among many other plants' extracts, was found to inhibit struvite formation (Das et al. 2017). Dietary polyphenols, including curcumin, may be promising dietary supplements for the prevention of urolithiasis (Nirumand et al. 2018). This limited research suggests that curcumin may slow the progression of kidney stone formation and fibrosis and may also exert protective effects in the kidneys.

18 Cancer

Cancer is one of the most common causes of death worldwide. There appears to be a link between cancer and diet. The dietary modulation of gut microbiota and miRNAs may play an important role in cancer development and prevalence. Various dietary components such as turmeric (curcumin), fatty acids, resveratrol, and isothiocyanate are often utilized in cancer prevention and treatment; dietary components and fiber serve as probiotics and alter miRNA expression. This vital interaction of functional foods, herbs, fiber, and dietary supplement modulates the vital pathways involved in metastasis, invasion, apoptosis, tumor growth, and cell proliferation (Riaz Rajoka et al. 2018).

Curcumin could enhance the effect of radiation therapy, inhibit angiogenesis and cell proliferation by suppressing NF-κB and its target genes in colon cancer cells, and inhibit cell growth by modulating Akt/mTOR pathways via the downregulation of EGFR. In addition, pancreatic cells treated with curcumin resulted in the downregulation of 18 miRNAs and upregulation of 11 miRNAs; the upregulation of miR-22 led to the suppression of ESR-1 (estrogen receptor 1) and SP1 transcription factors.

Glioma is the most aggressive, lethal, and most prevalent of primary brain tumors. Glioblastoma (glioblastoma multiforme) is a malignant glioma that is almost impossible to cure because of poor drug transportation across the blood-brain barrier (BBB) and the existence of glioma stem cells. Recently, Zhao et al. (2018) discovered that curcumin-loaded RDP-modified liposome (RCL) inhibited glioma cell proliferation and tumor growth using an intracranial glioma mouse model. RCL prolonged the survival time of the glioma-bearing mice from 23 to 33 days; the inhibition mechanism of RCL on glioma cells may involve cell cycle arrest at the S phase and induction of cell apoptosis. This study provides

evidence that nanotechnology (delivery of curcumin to brain cancer cells) has potential for the treatment of human malignant gliomas.

19 Translation from Population Epidemiology and Clinic to Bench

The average estimated daily human consumption of turmeric (ST) in India is approximately 200–600 mg for life (~1–3 mg/kg, BID, TID). This may be translated into curcuminoids estimated daily human dose of ~3–30 mg for life. This consumption of a low dose of turmeric and its active ingredients may explain the low prevalence of Alzheimer's disease (AD) in India (1/4th of USA) and subsequent low cost of healthcare in India. Traditionally, turmeric powder is first seasoned in oil or ghee until it is brown for 1–2 min before it is used in beans, whole grains, rice, or vegetables (personal observation of Indian cooking during childhood). This method may be a reflection of the ancient art of nanoformulation and activation of >235 ingredients in turmeric and other species.

In non-turmeric-consuming nations, including the USA, many people are using higher doses of dietary supplements (curcumin or turmeric) as recommended by the nonscientist and nonbeliever in Ayurveda. High doses may negate its own beneficial health effects. Therefore, there is a need for developing low-dose formulations consisting of turmeric or curcumin/curcuminoids alone or in combination with other herbs or medications. Such novel combinations need to be clinically evaluated in aging dogs, cats, and people suffering with osteoarthritis, pain, and/or mild-to-moderate cognitive deficits (AD) and comorbidities, such as depression. However, such clinical studies require funding and the incentive to pursue this long course for finding an affordable herb for the prevention of chronic inflammatory diseases of the joints, gut, brain, and skin.

As an example, aspirin (81 mg daily for decades) lowers the risk of heart attack and stroke. Looking at aspirin's pharmacology, no one could have predicted such a great clinical outcome. A combination of salicylic acid (and other tricarboxylic acids) and turmeric at low doses used over decades in mid-age may reduce the progression of AD and OA in high-risk populations and pets.

20 Translation: Preclinical to Clinic

Preclinical data from earlier research from UCLA clearly demonstrate that curcumin at low doses has selective distribution in the brain. This finding may have great clinical implications in terms of its nonconventional PK and efficacy. The low (500 ppm) and high (2000 ppm) dose of curcumin in the feed for 6 months increases plasma level in dose-related fashion in mice, yielding 35 ± 14 and 171 ± 19 ng/ml, respectively. However, the brain level of curcumin following low and high dose was 469 ± 22 and 525 ± 125 ng/g, respectively. The brain-to-plasma ratio was 13.4 at low dose and 3 at high dose. Curcumin's half-life is >10 days in the brain and less than 7 h in the plasma (Dr. S. Frautschy, UCLA, personal communication). Long half-life suggests that active ingredients (curcumin and others) will accumulate in the brain and other fatty tissues over days or weeks.

These data may suggest that there is dose-related PK in the plasma, but not in the brain. The pGp transporters in the BBB may be activated by high plasma levels leading to promotion of curcumin's efflux from the brain. This phenomenon may explain the lack of efficacy in clinical trials using "industrial" doses of curcumin. The efficacy of 160 ppm and 500 ppm in feed for 6 months offered almost similar efficacy in a mouse model of Alzheimer's disease (references). Increasing the dose to 2000 or 5000 ppm did not yield better efficacy in mouse model. The translation effort from mice to human has failed in developing curcumin as a potential therapy for Alzheimer's disease. However, the failure in human clinical trials may help us in developing safer and more effective turmeric formulations for veterinary use, particularly for osteoarthritis and cognitive decline (dementia) in aging dogs and cats.

In depression, AD, pain, and ocular inflammation in animal model demonstrate that the low dose of turmeric or curcumin in feed or foods, for the duration of life, may offer an affordable way to reduce the burden of chronic inflammatory disease in aging pets and people. Along with lifestyle changes—exercise and healthy eating—a low dose of turmeric (ST) may reduce the need for opiates and other medications.

21 Gaps and Opportunities

- Recently, Cavaleri (2018) elegantly discussed and summarized the gaps, challenges, and opportunities in making turmeric or its major phytochemicals (e.g., curcumin, curcuminoids) and their active metabolites effective and safe dietary supplements (nutraceuticals) and medications.
- The purity, adulteration, heavy metal lead contamination, stability, and shelf-life of turmeric (standardized turmeric) and its major components in dietary supplements need to be kept forefront in R&D.
- The content of phytochemicals in turmeric may vary drastically from region to region within a country or around the world. This variation may contribute to the differences

in efficacy of many herbal dietary supplements (such as nutraceuticals, including standardized turmeric and its major components). Therefore, standardization of turmeric preparations (combinations) is not only desirable but is warranted.

- Tissue distribution studies (ADME, PK) following chronic administration of therapeutic (low) doses of turmeric (standardized turmeric) and its major components at steady state after 3, 7, or 30 days of dosing in chronic disease states need to be evaluated in dogs, cats, horses, and zoo animals.
- The in vitro and in vivo activities of curcumin in various experimental models so far have not been reproducible in clinical settings. The study of larger populations of cats, dogs, and horses living with natural progression of chronic dysregulated age-related diseases, and a longer duration of treatment with a wide range of doses of ST and varied formulations of curcumin, may offer more reliable data, which may have a better predictive value in the design of long-term clinical trials in pets and people.
- The use of standardized turmeric and its major components, alone or at various doses, in long-term clinical studies is warranted in aging pets and horses.
- The neuroprotective effects of IV, nasal, or oral formulations of ST or curcumin in stroke and concussion and in PTSD victims need to be evaluated.
- Standardized turmeric and its major components may be preferentially distributed to the therapeutic site (immune inflammation) and may serve a preventive (modulatory) role in aging pets and horses.
- Dogs, cats, and horses, as well as zoo animals, suffering with OA, age-related chronic dysregulated immune inflammation, and AD-type conditions are natural disease models. The efficacy of standardized turmeric and its major components needs to be established in aging pets and horses. Such studies are warranted for establishing more reliable and reproducible changes in the biomarkers of diseases over time.
- Long-term studies using turmeric (standardized turmeric) and its major components, alone or in combination, with other nutraceuticals (herbal supplements) and medications in pets, farm, and zoo animals suffering with chronic dysregulated immune inflammatory conditions are warranted. The long-term treatment with turmeric (standardized turmeric) and its major components would produce significant alterations (up- and downregulation of transcription and translation of genes and epigenetic mechanisms, proteins, mRNAs, and enzymes and their receptors and pathways). These kinds of precision clinical medicine studies are still lacking. This therapeutic approach in R&D may offer better and more reliable biomarkers of disease modification in the saliva, lymphocytes, blood, brain, joints, and eyes of dietary supplement-treated and placebo (vehicle)-treated animals and people.
- The aging people and pets at high risk of AD, OA, and other immune inflammatory conditions may be getting little or no amount (and sometimes too much) of standardized turmeric and its major components. Prevention studies are warranted in these high-risk populations to identify appropriate doses and duration of treatment.
- Population-based, broad dose-response, and PK/PD modeling in the presence and absence of medications for pain, depression, and other common illnesses in aging populations is needed. There is potential activation of drug transporters (Pgp) in target tissues such as the BBB and the gastrointestinal system and in joints while using a high dose of turmeric (standardized turmeric) and its major components. This field needs to be further explored.
- The use of turmeric (standardized turmeric) may be an affordable preventive approach for slowing the progression of aging-related chronic diseases. Its lifetime use as a food, rather than a pill or capsule, may be a viable approach to reduce the need for expensive medications with serious side effects in pets and people. Turmeric (standardized turmeric) and its major components may serve as an adjunct therapy in treating diseases of the digestive system, brain, joints, eyes, and body in aging pets and people.
- Nanotechnology and other medication delivery systems in aging pets may offer new approaches in veterinary practice.

22 Concluding Remarks and Future Directions

Turmeric is the dried rhizome (root) of *Curcuma longa* or *Curcuma aromatica* (wild turmeric), *Curcuma wenyujin* (China), and *Curcuma domestica* (Thailand). It is a rhizomatous herbaceous perennial plant belonging to the ginger family, *Zingiberaceae*. More than 133 different species of turmeric have been identified. It is native to the Indian subcontinent and Southeast Asia. Turmeric contains more than 235 active ingredients naturally packed into its root, optimistically in appropriate proportions. These complex phytochemicals (>235), including essential oils, curcuminoids (>89), and turmerosaccharides, as well as curcuminoid-free ingredients, fiber, and their metabolites and products of microbial degradation of standardized turmeric, may act in additive or synergistic fashion as a modulator of dysregulated chronic immune inflammation and pain in disease states in people, pets, and horses.

The average human consumption of turmeric in India is approximately 81 mg twice or three times a day for life (~1–3 mg/kg, BID, TID). This may be translated into curcuminoids' estimated human dose of ~3–15 mg BID, TID for life. The consumption of low dose of turmeric in foods may be responsible for low prevalence of AD in India (one-fourth of USA). A caution must be exercised when statistics and epidemiological data are being considered for possible translation from one culture or nation to others.

One of the Ayurvedic medicine's basic principles is that efficacy cannot be related to plasma level of a major active ingredient in a spice or an herb, and the whole herb is often more efficacious than its individual ingredients. Contrary to this basic principle, R&D teams over the past decades drastically improved the bioavailability of curcumin, turmeric's major active ingredient. Besides improving curcumin's pharmacokinetics, researchers also increased the dose of curcumin in most clinical trials conducted so far without improving its efficacy in cancer and AD.

Standardized turmeric containing curcumin (3, 9, 27, or 81 mg once a day or BID or TID) could achieve steady-state therapeutic level within 3–10 days in the brain of patients or pets suffering with AD or other persistent dysregulated chronic immune inflammatory conditions. This concept may be explored in aging dogs, cats, and horses living with OA and mild dementia. This concept is based on published preclinical studies—similar effects of aspirin and curcumin in vitro and in vivo. Twenty to thirty years ago, it was unforeseeable that 81 mg aspirin could reduce the risk of stroke and heart attack.

Low dose of standardized turmeric (3–4 mg/kg, BID, TID) blended in coconut oil or fish oil may tame age-related, persistent, dysregulated chronic immune inflammation in the brain, eyes, skin, muscles, gut, and other internal organs in pets. It may slow the progression of cognitive decline disorder (CCD) in dogs and cats. Low dose of standardized turmeric may serve as an adjunct therapy in managing many disease states in aging pets—in dogs and cats as well as in horses. The ingredients in turmeric may restore homeostasis in the brain, joints, gut, and other tissues only under disease states acting via many interrelated mechanisms of action.

In non-turmeric-consuming nations, including the USA, many people and pets may be using higher doses of dietary supplements containing curcumin or turmeric. This may negate its own beneficial health effects. Therefore, there is a need for developing low-dose formulations consisting of turmeric or curcumin/curcuminoids, alone or in combination with other herbs or medications. Such novel combinations need to be clinically evaluated in aging dogs, cats, horses, and people suffering with osteoarthritis and/or with mild-to-moderate dementia/Alzheimer's disease and comorbidities.

Standardized turmeric's novel formulations at low doses may exert mild-to-moderate beneficial effects on osteoarthritis, pain, depression, and neurodegenerative diseases. It may reduce the need for analgesics (opiates), antidepressants, anti-AD, steroids, and anticancer medications. The possibility that many active ingredients in turmeric formulations may be acting in additive or synergistic fashion needs to be explored and addressed. The preclinical data support such a concept. Using the latest drug-targeted delivery (nanotechnology) and reliable clinical trial strategies, standardized turmeric may be considered for R&D for the prevention, and possibly for the treatment, of OA and dementia and other aging-related diseases of the eyes, brain, gut, and joints in pets and humans.

References

Agarwal KA, Tripathi CD, Agarwal BB et al (2011) Efficacy of turmeric (curcumin) in pain and postoperative fatigue after laparoscopic cholecystectomy: a double-blind, randomized placebo-controlled study. Surg Endosc 25(12):3805–3810

Aggarwal BB, Yuan W, Li S et al (2013) Curcumin-free turmeric exhibits anti-inflammatory and anticancer activities: identification of novel components of turmeric. Mol Nutr Food Res 57 (9):1529–1542

Akuri MC, Barbalho SM, Val RM et al (2017) Reflections about osteoarthritis and Curcuma longa. Pharmacogn Rev 1(21):8–12

Alipour A, Elte JW, van Zaanen HC et al (2007) Postprandial inflammation and endothelial dysfunction. Biochem Soc Trans 35 (Pt 3):466–469

Arun N, Nalini N (2002) Efficacy of turmeric on blood sugar and polyol pathway in diabetic albino rats. Plant Foods Hum Nutr 57(1):41–52

Ashraf K, Mujeeb M, Ahmad A et al (2015) Determination of curcuminoids in Curcuma longa Linn. by UPLC/Q-TOF–MS: an application in turmeric cultivation. J Chromatogr Sci 53 (8):1346–1352

Bas M, Tugcu V, Kemahli E et al (2009) Curcumin prevents shock-wave lithotripsy-induced renal injury through inhibition of nuclear factor kappa-B and inducible nitric oxide synthase activity in rats. Urol Res 37(3):159–164

Bastaki SMA, Ahmed MMA, Zaabi AA et al (2016) Effect of turmeric on colon histology, body weight, ulcer, IL-23, MPO and glutathione in acetic-acid-induced inflammatory bowel disease in rats. BMC Complement Altern Med 16:72

Begum AN, Jones MR, Lim GP et al (2008) Curcumin structure-function, bioavailability, and efficacy in models of neuroinflammation and Alzheimer's disease. J Pharmacol Exp Ther 326(1):196–208

Bethapudi B, Murugan S, Illuri R et al (2017) Bioactive turmerosaccharides from *Curcuma longa* extract (NR-INF-02): potential ameliorating effect on osteoarthritis pain. Pharmacogn Mag 13 (Suppl 3):S623–S627

Betts JW, Sharili AS, La Ragione RM (2016) In vitro antibacterial activity of curcumin–polymyxin B combinations against multidrug-resistant bacteria associated with traumatic wound infections. J Nat Prod 79(6):1702–1706

Bland SD, Venable EB, McPherson JL et al (2017) Effects of liposomal-curcumin on five opportunistic bacterial strains found in the equine hindgut – preliminary study. J Anim Sci Technol 59:15

Bolger GT, Licollari A, Tan A et al (2017) Distribution and metabolism of Lipocurc™ (liposomal curcumin) in dog and human blood cells:

species selectivity and pharmacokinetic relevance. Anticancer Res 37(7):3483–3492
Bolger GT, Licollari A, Tan A et al (2018) Distribution of curcumin and THC in peripheral blood mononuclear cells isolated from healthy individuals and patients with chronic lymphocytic leukemia. Anticancer Res 38(1):121–130
Brumatti LV, Marcuzzi A, Tricarico PM et al (2014) Curcumin and inflammatory Bowel disease: potential and limits of innovative treatments. Molecules 19:21127–21153
Cavaleri F (2018) Presenting a new standard drug model for turmeric and its prized extract, curcumin. Int J Inflam 1(15):5023429
Chand N (2018) Composition of containing standardized turmeric for reducing inflammation. Provisional Patent Application # 626136994, Jan 4, 2018
Cheppudira B, Fowler M, McGhee L et al (2013) Curcumin: a novel therapeutic for burn pain and wound healing. Expert Opin Investig Drugs 22:295–1230
Colitti M, Gaspardo B, Della Pria A et al (2012) Transcriptome modification of white blood cells after dietary administration of curcumin and non-steroidal anti-inflammatory drug in osteoarthritic affected dogs. Vet Immunol Immunopathol 147:136–146
Das P, Gupta G, Velu V et al (2017) Formation of struvite urinary stones and approaches towards the inhibition – a review. Biomed Pharmacother 96:361–370
Del Grossi Moura M, Lopes LC, Biavatti MW et al (2017) Oral herbal medicines marketed in Brazil for the treatment of osteoarthritis: a systematic review and meta- analysis. Phytother Res 31:1676–1685
Dende C, Meena J, Nagarajan P et al (2017) Nanocurcumin is superior to native curcumin in preventing degenerative changes in Experimental Cerebral Malaria. Sci Rep 7(1):10062
Di Pierro F, Bressan A, Ranaldi D et al (2015) Potential role of bioavailable curcumin in weight loss and omental adipose tissue decrease: preliminary data of a randomized, controlled trial in overweight people with metabolic syndrome. Preliminary study. Eur Rev Med Pharmacol Sci 19:4195–4202
Dou Y, Luo J, Wu X (2018) Curcumin attenuates collagen-induced inflammatory response through the "gut-brain axis". J Neuroinflammation 15(1):6
Farinacci M, Colitti M, Stefanon B (2009) Modulation of ovine neutrophil function and apoptosis by standardized extracts of *Echinacea angustifolia*, *Butea frondosa* and *Curcuma longa*. Vet Immunol Immunopathol 128(4):366–373
Fujiwara H (2000) Curcumin inhibits glucose production in isolated mice hepatocytes. Diabetes Res Clin Pract 80:185–190
Gaffey A, Campbell J, Porritt K et al (2015) The effects of curcumin on musculoskeletal pain: a systematic review protocol. JBI Database System Rev Implement Rep 13:59–73
Gera M, Sharma N, Ghosh M et al (2017) Nanoformulations of curcumin: an emerging paradigm for improved remedial application. Oncotarget 8:66680–66698
Ghodasara J, Pawar A, Deshmukh C et al (2010) Inhibitory effect of rutin and curcumin on experimentally-induced calcium oxalate urolithiasis in rats. Pharmacogn Res 2:388–392
Ghosh SS, He H, Wang J et al (2018) Curcumin-mediated regulation of intestinal barrier function: the mechanism underlying its beneficial effects. Tissue Barriers 6(1):e1425085
Gopi S, Jacob J, Varma K et al (2017) Comparative oral absorption of curcumin in a natural turmeric matrix with two other curcumin formulations: an open-label parallel-arm study. Phytother Res 31:1883–1891
Gopinath H, Karthikeyan K (2018) Turmeric: a condiment, cosmetic and cure. Indian J Dermatol Venereol Leprol 84:16–21
Gupta SC, Kismali G, Aggarwal BB (2013) Curcumin, a component of turmeric: from farm to pharmacy. Biofactors 39:2–13
Gutierres VO, Campos ML, Arcaro CA et al (2015) Curcumin pharmacokinetic and pharmacodynamic evidences in streptozotocin-diabetic rats support the antidiabetic activity to be via metabolite (s). Evid Based Complement Alternat Med 2015:678218
Han S, Xu J, Guo X et al (2018) Curcumin ameliorates severe influenza pneumonia via attenuating lung injury and regulating macrophage cytokines production. Clin Exp Pharmacol Physiol 45(1):84–93
Hanai H, Sugimoto K (2009) Curcumin has bright prospects for the treatment of inflammatory Bowel disease. Curr Pharm Des 15 (18):2087–2094
Hanai H, Iida T, Takeuchi K et al (2006) Curcumin maintenance therapy for ulcerative colitis: randomized, multicenter, double-blind, placebo-controlled trial. Clin Gastroenterol Hepatol 4(12):1502–1506
Haroyan A, Mukuchyan V, Mkrtchya N et al (2018) Efficacy and safety of curcumin and its combination with boswellic acid in osteoarthritis: a comparative, randomized, double- blind, placebo-controlled study. BMC Complement Altern Med 18(1):7
Heaton LE, Davis JK, Rawson ES et al (2017) Selected in-season nutritional strategies to enhance recovery for team sport athletes: a practical overview. Sports Med 47(11):2201–2218
Hu X, Huang F, Szymusiak M et al (2016) PLGA-Curcumin attenuates opioid-induced hyperalgesia and inhibits spinal CaMKIIα. PLos One 11:e0146393
Innes JF, Fuller CJ, Grover ER et al (2003) Randomized, double- blind, placebo controlled parallel group study of P54FP for the treatment of dogs with osteoarthritis. Vet Rec 152:457–460
Javeri I, Chand N (2016) Curcumin. In: Gupta RC (ed) Nutraceuticals: efficacy, safety and toxicity. Academic Press, Amsterdam, pp 435–445
Jeengar MK, Shrivastava S, Mouli Veeravalli SC (2016) Amelioration of FCA induced arthritis on topical application of curcumin in combination with emu oil. Nutrition 32:955–964
Jia S, Du Z, Song C et al (2017) Identification and characterization of curcuminoids in turmeric using ultra-high-performance liquid chromatography-quadrupole time of flight tandem mass spectrometry. J Chromatogr A 1521:110–122
Jin TR (2018) Curcumin and dietary polyphenol research: beyond drug discovery. Acta Pharmacol Sin 39(5):779–786
Krausz AE, Adler BL, Cabral V (2015) Curcumin-encapsulated nanoparticles as innovative antimicrobial and wound healing agent. Nanomedicine 11:195–206
Kumar SSD, Houreld NN, Abrahamse H (2018) Therapeutic potential and recent advances of curcumin in the treatment of aging-associated diseases. Molecules 23(4):835–849
Kurup V, Barrios CS, Barrios R et al (2007) Immune response modulation by curcumin in a latex allergy model. Clin Mol Allergy 5(1):1
Lang A, Salomon N, Wu JC et al (2015) Curcumin in combination with mesalamine induces remission in patients with mild-to-moderate ulcerative colitis in a randomized controlled trial. Clin Gastroenterol Hepatol 13(8):1444–1449
Lim GP, Chu T, Yang F et al (2001) The curry spice curcumin reduces oxidative damage and amyloid pathology in an Alzheimer transgenic mouse. J Neurosci 21:8370–8377
Lin J-A, Chen J-H, Lee Y-W et al (2011) Biphasic effect of curcumin on morphine tolerance: a preliminary evidence from cytokine/chemokine protein array analysis. Evid Based Complement Alternat Med 2011:452153. https://doi.org/10.1093/ecam/neq018
Liu Q, Yin W, Han L et al (2018a) Diarylheptanoid from rhizomes of *Curcuma kwangsiensis* (DCK) inhibited imiquimod-induced dendritic cells activation and Th1/Th17 differentiation. Int Immunopharmacol 56:339–348
Liu X, Machado GC, Eyles JP et al (2018b) Dietary supplements for treating osteoarthritis: a systematic review and meta-analysis. Br J Sports Med 52:167–175

Lone PA, Ahmed SW, Prasad V et al (2018) Role of turmeric in management of alveolar osteitis (dry socket): a randomized clinical study. J Oral Biol Craniofac Res 8:44–47

Mantzorou M, Pavlidou E, Vasios G et al (2018) Effects of curcumin consumption on human chronic diseases: a narrative review of the most recent clinical data. Phytother Res 32:957–975. https://doi.org/10.1002/ptr.6037

Maradana MR, Thomas R, O'Sullivan BJ (2013) Targeted delivery of curcumin for treating type 2 diabetes. Mol Nutr Food Res 57:550–1556

Matabudul D, Pucaj K, Bolger G et al (2012) Tissue distribution of (Lipocurc™) liposomal curcumin and tetrahydrocurcumin following two- and eight-hour infusions in Beagle Dogs. Anticancer Res *32*:4359–4364

Mazzanti G, Di Giacomo S (2016) Curcumin and resveratrol in the management of cognitive disorders: what is the clinical evidence? Molecules 21:1243–1270

McCann MJ, Johnston S, Reilly K et al (2014) The effect of turmeric (Curcuma longa) extract on the functionality of the solute carrier protein 22 A4 (SLC22A4) and interleukin-10 (IL-10) variants associated with inflammatory bowel disease. Nutrients 6:4178–4190

McCubrey JA, Lertpiriyapong K, Steelman LS et al (2017) Regulation of GSK-3 activity by curcumin, berberine and resveratrol: potential effects on multiple diseases. Adv Biol Reg 65:77–88

McFarlin BK, Venable AS, Henning AL et al (2016) Reduced inflammatory and muscle damage biomarkers following oral supplementation with bioavailable curcumin. BBA Clin 5:72–78

Mollazadeh H, Cicero AFG, Blesso CN et al (2017) Immune modulation by curcumin: the role of interleukin-10. Crit Rev Food Sci Nutr:1–13. https://doi.org/10.1080/10408398.2017.1358139

Motaghinejad M, Bangash MY, Hosseini P (2015) Attenuation of morphine withdrawal syndrome by various dosages of curcumin in comparison with clonidine in mouse: possible mechanism. IJMS 40:125–132

Murugan S, Bethapudi B, Purusothaman D et al (2017) Anti-arthritic effect of polar extract of Curcuma longa on monosodium iodoacetate induced osteoarthritis in rats. Antiinflamm Antiallergy Agents Med Chem 16(3):193–202

Nalli M, Ortar G, Schiano Moriello A et al (2017) Effects of curcumin and curcumin analogues on TRP channels. Fitoterapia 122:126–131

Neto FC, Marton LT, de Marqui SV et al (2018) Curcuminoids from Curcuma Longa: new adjuvants for the treatment of Crohn's disease and ulcerative colitis? Crit Rev Food Sci Nutr 22:1–36

Nirumand MC, Hajialyani M, Rahimi R et al (2018) Dietary plants for the prevention and management of kidney stones: preclinical and clinical evidence and molecular mechanisms. Int J Mol Sci 19:765

Ohno M, Nishida A, Sugitani S et al (2017) Nanoparticle curcumin ameliorates experimental colitis via modulation of gut microbiota and induction of regulatory T cells. PLoS One 12(10):e0185999

Peddada KV, Brown A, Verma V et al (2018) Therapeutic potential of curcumin in major retinal pathologies. Int Ophthalmol. https://doi.org/10.1007/s10792-018-0845-y

Ponnusamy S, Zinjarde S, Bhargava S et al (2012) Discovering Bisdemethoxycurcumin from Curcuma longa rhizome as a potent small molecule inhibitor of human pancreatic α-amylase, a target for type-2 diabetes. Food Chem 135:2638–2642

Prasad S, Aggarwal BB (2011) Chapter 13: Turmeric, the golden spice. From traditional medicine to modern medicine. In: Benzie IFF, Wachtel-Galor S (eds) Herbal medicine: biomolecular and clinical aspects. CRC Press, Boca Raton, FL

Ramirez BA, Soler A, Carrion-Gutierrez MA et al (2000) An hydroalcoholic extract of Curcuma longa lowers the abnormally high values of human-plasma fibrinogen. Mech Ageing Dev 114 (3):207–210

Ramkumar M, Rajasankar S, Gobi VV et al (2018) Demethoxycurcumin, a natural derivative of curcumin abrogates rotenone-induced dopamine depletion and motor deficits by its antioxidative and anti-inflammatory properties in Parkinsonian rats. Pharmacogn Mag 14(53):9–16

Riaz Rajoka MS, Jin M, Haobin Z et al (2018) Impact of dietary compounds on cancer-related gut microbiota and microRNA. Appl Microbiol Biotechnol 102(10):4291–4303

Sarker MR, Franks SF (2018) Efficacy of curcumin for age-associated cognitive decline: a narrative review of preclinical and clinical studies. Geroscience 40(2):73–95

Schiborr C, Kocher A, Behnam D et al (2014) The oral bioavailability of curcumin from micronized powder and liquid micelles is significantly increased in healthy humans and differs between sexes. Mol Nutr Food Res 58:516–527

Seo HJ, Wang SM, Han C et al (2015) Curcumin as a putative antidepressant. Expert Rev Neurother 15(3):269–280

Shehzad A, Qureshi M, Anwar MN et al (2017) Multifunctional curcumin mediate multitherapeutic effects. J Food Sci 82:2006–2015

Shen L, Liu L, Ji HF (2017) Regulative effects of curcumin spice administration on gut microbiota and its pharmacological implications. Food Nutr Res 61:1361780

Shin HS, See HJ, Jung SY et al (2015) Turmeric (Curcuma longa) attenuates food allergy symptoms by regulating type 1/type 2 helper T cells (Th1/Th2) balance in a mouse model of food allergy. J Ethnopharmacol 175:21–29

Siard MH, McCurry KE, Adams AA (2016) Effects of polyphenols including curcuminoids, resveratrol, quercetin, pterostilbene, and hydroxypterostilbene on lymphocyte pro-inflammatory cytokine production of senior horses in vitro. Vet Immunol Immunopathol 173:50–59

Small GW, Siddarth P, Li Z et al (2017) Memory and brain amyloid and tau effects of a bioavailable form of curcumin in non-demented adults: a double-blind, placebo-controlled 18-month trial. Am J Geriatr Psychiatr 26:266–277

Sohrabi M, Alahgholi-Hajibehzad M, Mahmoodian ZM et al (2018) Effect of cinnamon and turmeric aqueous extracts on serum interleukin-17F level of high fructose-fed rats. Iran J Immunol 15:38–46

Su L-Q, Di Wang Y, Chi H-Y (2017) Effect of curcumin on glucose and lipid metabolism, FFAs and TNF-α in serum of type 2 diabetes mellitus rat models. Saudi J Biol Sci 24:1776–1780

Subhashini PS, Chauhan S, Kumari S et al (2013) Intranasal curcumin and its evaluation in murine model of asthma. Int Immunopharmacol 17(3):733–743

Subhashini PS, Chauhan S, Dash D et al (2016) Intranasal curcumin ameliorates airway inflammation and obstruction by regulating MAPKinase activation (p38, Erk and JNK) and prostaglandin D2 release in murine model of asthma. Int Immunopharmacol 31:200–200

Sundaram JR, Poore CP, Sulaimee NHB et al (2017) Curcumin ameliorates neuroinflammation, neurodegeneration, and memory deficits in p25 transgenic mouse model that bears hallmarks of Alzheimer's disease. J Alzheimers Dis 60:1429–1442

Suresh D, Srinivasan K (2010) Tissue distribution and elimination of capsaicin, piperine and curcumin following oral intake in rats. Indian J Med Res 131:682–691

Tanabe Y, Maeda S, Akazawa N (2015) Attenuation of indirect markers of eccentric exercise-induced muscle damage by curcumin. Eur J Appl Physiol 115(9):1949–1957

Tian L, Zeng K, Shao W et al (2015) Short-term curcumin gavage sensitizes insulin signaling in dexamethasone-treated C57BL/6 mice. J Nutr 145:2300–2307

Velusami CC, Richard EJ, Bethapudi B (2018) Polar extract of Curcuma longa protects cartilage homeostasis: possible mechanism of action. Inflammopharmacology 26(5):1233–1243

Verma S, Mundkinajeddu D, Agarwal A et al (2017) Effects of turmeric curcuminoids and metformin against central sensitivity to pain in mice. J Traditional and Compl Med 7:145–151

Vors C, Couillard C, Paradis ME et al (2018) Supplementation with resveratrol and curcumin does not affect the inflammatory response to a high-fat meal in older adults with abdominal obesity: a randomized, placebo-controlled crossover trial. J Nutr 148: 379–388

Wojcikowski K, Vigar VJ, Oliver CJ (2018) New concepts of chronic pain and the potential role of complementary therapies. Altern Ther Health Med. https://www.ncbi.nlm.nih.gov/pubmed/29428928

Xu X, Cai Y, Yu Y (2018) Effects of a novel curcumin derivative on the functions of kidney in streptozotocin-induced type 2 diabetic rats. Inflammopharmacology. https://europepmc.org/abstract/med/29582239

Yang JY, Zhong X, Kim SJ et al (2018) Comparative effects of curcumin and tetrahydrocurcumin on dextran sulfate sodium-induced colitis and inflammatory signaling in mice. J Cancer Prev 23(1):18–24

Yeon KY, Kim SA, Kim YH et al (2010) Curcumin produces an antihyperalgesic effect via antagonism of TRPV1. J Dent Res 89: 170–174

Zhang DW, Fu M, Gao SH et al (2013) Curcumin and diabetes: a systematic review. Evid Based Complement Alternat Med:636053. https://doi.org/10.1155/2013/636053. https://www.hindawi.com/journals/ecam/2013/636053/

Zhang Z, Leong DJ, Xu L et al (2016) Curcumin slows osteoarthritis progression and relieves osteoarthritis-associated pain symptoms in a post-traumatic osteoarthritis mouse model. Arthritis Res Ther 18:128–140

Zhao M, Zhao M, Fu C et al (2018) Targeted therapy of intracranial glioma model mice with curcumin nanoliposomes. Int J Nanomedicine 13:1601–1610

Zhi L, Dong L, Kong D et al (2013) Curcumin acts via transient receptor potential vanilloid-1 receptors to inhibit gut nociception and reverses visceral hyperalgesia. Neurogastroenterol Motil 25(6): e429–e440

Zhu Q, Sun Y, Yun Z et al (2014) Antinociceptive effects of curcumin in a rat model of postoperative pain. Sci Rep 4:4932–4936

Fenugreek in Health and Disease

Dinesh Kumar, Ramdas Singh Wangkheirakpam, Anu Rahal, and Jitendra K. Malik

Abstract

Fenugreek is an herb which has been used in traditional medicines for centuries in wound healing, as an aphrodisiac, for promotion of lactation, etc. The consumption of the seeds as a spice results in different medicinal effects such as hypocholesterolemic, antidiabetic, hepatoprotective, antibacterial, anthelmintic, anticancer, and galactogogue. Flavonoids, saponins, pyridine alkaloids, and steroidal sapogenins are some of the phytochemicals present in the plant. The plant is also embraced for its high content of important vitamins, minerals, protein and amino acids, and fibers making it a nutritious fodder for livestock. Extracts of the leaves and seeds of fenugreek are considered safe and are found to have potential therapeutic explicabilities in the treatment and/or management of diabetes, cancer, toxicities, cardiovascular diseases, physical injuries, and hormonal imbalances. The seeds and leaves of this plant are now being incorporated into animal, bird, and fish foods to increase feed intake, to promote weight gain, and to decrease the feed conversion ratio. The addition of fenugreek in the drinking water of poultry reduces stress, and this can be an important strategy to replace the use of antibiotics such as enrofloxacin as an anti-stress agent, and thus the issues of antibiotic residues in meat, as well as widely developing antibiotic resistance, would be less.

Keywords

Nutraceutical · Fenugreek (*Trigonella foenum-graecum*) · Phytochemicals · Sapogenins · Trigonelline · Diosgenin · Galactomannan

1 Introduction

Fenugreek (*Trigonella foenum-graecum* L.) is an annual forage aromatic leguminous herb. The plant was once native to the Mediterranean region, India, China, Northern Africa, and Ukraine but is now cultivated widely in many parts of the world. It is about 30–60 cm tall with smoothed erect untoothed stipulate and 2–2.5-cm-long leaflets. There are 1–2 flowers which are axillary and sessile. Calyx-teeth is linear and pods measure about 5–7.5 cm in length with a long persistent beak often falcate with 10–29 small size seeds without transverse reticulations (Kirtikar and Basu 2002). The seed is 4.01–4.19 mm long, 2.35–2.61 mm wide, and 1.49–1.74 mm thick (Altuntaş et al. 2005). The leaves and seeds of the plant are used as an herb and the seeds are used as a spice. India leads among the countries which produce fenugreek by sharing 70–80% of the global export (Edison 1995).

Fenugreek is also considered as one of the oldest known medicinal plant in recorded history (Lust 1986). This medicinal plant is used in various traditional medicines including Indian Ayurvedic, traditional Chinese medicines, and Egyptian medicine for wound healing, as an aphrodisiac, for promotion of lactation, and many more (Tiran, 2003). Phytochemicals like flavonoids, saponins, steroidal sapogenins, amino acids, and alkaloids are some of the important constituents found in the extracts of leaves, stem, and seeds of *Trigonella foenum-graecum* L. (fenugreek). The consumption of the seeds as a spice results in different medicinal effects such as hypocholesterolemic (Mathern et al. 2009), antidiabetic (Ajabnoor and Tilmisany 1988), hepatoprotective (Pribac et al. 2009), antibacterial (Sharma

D. Kumar (✉)
Division of Pharmacology & Toxicology, Indian Veterinary Research Institute, Bareilly, Uttar Pradesh, India

R. S. Wangkheirakpam
Department of Pharmacology & Toxicology, College of Veterinary Sciences & Animal Husbandry, R.K. Nagar, West Tripura, India

A. Rahal
Central Institute for Research on Goat (CIRG), Mathura, Uttar Pradesh, India

J. K. Malik
IVRI, Dehradun, Uttarakhand, India

R. C. Gupta et al. (eds.), *Nutraceuticals in Veterinary Medicine*, https://doi.org/10.1007/978-3-030-04624-8_2

et al. 2017), anthelmintic (Khadse and Kakde 2010), anticancer (Alsemari et al. 2014), and galactogogue (Betty 2008). These phytochemicals also now serve as raw materials for the manufacture of various therapeutic and hormonal drugs (Priya et al. 2011). Fenugreek is also a good source of dietary fiber where the proportions of soluble and insoluble fibers present in its seeds are 13% and 32%, respectively (Roberts 2011).

The antidiabetic and hypocholesterolemic effects of fenugreek are attributed to multiple components. However, these effects, especially the hypoglycemic effect, may partly be secondary to the fiber content which is known to affect gastric emptying and subsequently decreasing the postprandial glucose level in blood (Srinivasan 2006; Benzie and Wachtel-Galor 2011).

Scientific Classifications

Kingdom:	Plantae
Subkingdom:	Tracheobionta
Super-kingdom:	Spermatophyta
Division:	Magnoliophyta
Class:	Magnoliopsida
Subclass:	Rosidae
Order:	Fabales
Family:	Leguminosae/Fabaceae
Genus:	*Trigonella*
Species:	*T. foenum-graecum* (Kirtikar and Basu 2003; Dymock et al. 2005)

2 Phytoconstituents

2.1 Leaves

The green leaves of fenugreek contain numerous phytochemicals, including various nutrients and vitamins. The fresh leaves are used as vegetables in diets mainly for their rich content of vitamins and minerals, and they have also been used as green fodder for livestock. The moisture, nutrient, and mineral contents in fenugreek leaves are shown in Table 1. Ascorbic acid and β-carotene contents in the fresh leaves of fenugreek are about 220.97 mg and 19 mg/100 g of leaves, respectively (Yadav and Sehgal 1997). Minerals like zinc, iron, phosphorous, calcium, etc. and vitamins like riboflavin, carotene, thiamine, niacin, vitamin C, etc. are also present in the leaves (Rao 2003) (Table 2).

2.2 Seeds

Phytochemical constituents in the seeds, husk, and cotyledons of fenugreek differ. The endosperm shows the highest saponin and protein content, while the husk shows a higher polyphenols content. The mature seeds contain about 0.1–1.5% of diosgenin (a steroidal sapogenin) and are extracted commercially (Saxena et al. 2013). Volatile and fixed oils are also present in fenugreek seeds in small amounts (Sowmya and Rajyalakshmi 1999). Among multiple flavonoid glycosides isolated from the seeds of fenugreek, isoorientin has been found in significant amount (Luan et al. 2018). Tables 3 and 4 show the list of chemicals present in fenugreek seeds.

Other constituents of the seed extracts include fibers, gum and neutral detergent fiber (Yadav et al. 2011), and lipids, triacylglycerols, diacylglycerols, monoacylglycerols, phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, free fatty acids (Chatterjee et al. 2010), and many others. The chemical structures of some of the bioactive phytochemicals present in fenugreek are shown in Figs. 1 and 2.

Table 1 Nutrient content of fenugreek leaves (Rao 2003)

Moisture	86.1%
Protein	4.4%
Fat	0.9%
Minerals	1.5%
Fiber	1.1%
Carbohydrates	6%

Table 2 Saponins, pyridine alkaloids, and steroidal sapogenins in fenugreek seeds

Flavonoids	Vitexin
	Tricin
	Naringenin
	Quercetin
	Luteolin
Saponins	Graecunins
	Fenugrin B
	Fenugreekine
	Trigofoenosides A–G
Pyridine alkaloids	Trimethylamine
	Neurin
	Choline
	Gentianine
	Carpaine
	Betain
	Trigonelline
Steroidal sapogenins	Yamogenins
	Diosgenin
	Smilagenin
	Sarasapogenin
	Trigogenin
	Neotigogenin
	Gitogenin
	Yuccagenin
	Saponaretin

Source: Review article of Khorshidian et al. (2016)

Table 3 Proteins and amino acids, vitamins, and minerals in fenugreek seeds

Chemical composition		Nutrient value (per 100 g)
Protein and amino acids	Globulin	–
	Albumin	–
	Lecithin	Totally 25.4 g
	Histidine	–
	Lysine	–
	4-Hydroxyisoleucine	–
Vitamins	Vitamin A	1040 IU
	Vitamin C	12 mg
	Niacin	6 mg
	Pyridoxine	0.6 mg
	Thiamine	0.41 mg
	Riboflavin	0.36 mg
	Nicotinic acid	1.1 mg
	Folate	57 μg
Minerals	Calcium	176 mg
	Iron	33.5 mg
	Zinc	2.5 mg
	Phosphorus	296 mg
	Magnesium	191 mg
	Manganese	1.22 mg
	Selenium	6.3 μg

Source: Review article of Khorshidian et al. (2016)

Table 4 Chemical Composition of fenugreek (FK) seed (AOAC 1990)

Items	Percentage
Moisture	7.15
Dry matter	92.85
Organic matter	33.03
Crude protein	16.51
Ether extract	9.49
Total ash	7.15
NFE	33.82
ME(kcal/kg)	38.52

3 Uses of Fenugreek

Food is a major determinant for the health of animals including birds and fish. It not only helps in maintaining normal body functioning and metabolic status, but also the various constituents in feeds such as antioxidants, minerals, vitamins, fibers, etc. aid in disease prevention.

3.1 Ethnohistorical Uses of Fenugreek

Fenugreek is one of the oldest medicinal plants used for many ailments. The plant was traditionally used as galactogogue in Indian subcontinent (Betty 2008), as an analgesic in labor/delivery in ancient Rome, as a health tonic, and in treatment of edema and leg weakness in traditional Chinese medicine (Yoshikawa et al. 2000). The leaves and seeds are used as vegetable or green fodder for livestock (Ahmad et al. 2016) and as a spice (Wani and Kumar 2016), respectively, in many parts of the world.

3.2 Fenugreek Uses in Animal Health

Extracts of the seeds, shoots, roots, and leaves of fenugreek have shown multiple pharmacological properties, such as antimicrobial (Wagh et al. 2007; Norziah et al. 2015; Adil et al. 2015), antifungal (Haouala et al. 2008), anticancer (Raju et al. 2004; Shabbeer et al. 2009; Alsemari et al. 2014), hepatoprotective (Pribac et al. 2009), antidepressant (Kalshetti et al. 2015), antidiabetic (Sauvaire et al. 1998; Naicker et al. 2016), antiulcerogenic (Pandian et al. 2002), hypotensive (Moradi and Moradi 2013), anti-inflammatory, antipyretic, and analgesic (Malviya et al. 2010).

3.2.1 Diabetes

Diabetes, a group of metabolic disorders, is not limited to humans. Many animals, including pets, birds, and wild animals, also suffer diabetes naturally or by other influences. The hypoglycemic effect of fenugreek seeds in the human patient, as well as in chemically induced diabetic animals (rats, dog), has been described by many researchers. Decoction and ethanol extract of fenugreek seeds produced anti-hypoglycemic effects in alloxanized mice in a dose-dependent manner (Ajabnoor and Tilmisany 1988). This effect on blood glucose level in part has been attributed to the presence of steroids, saponins, alkaloids, and fiber content in the seeds. Soluble dietary fiber (SDF)—galactomannan of the plant—can improve glucose homeostasis in type I and type II diabetes by delaying carbohydrate digestion and absorption and enhancing insulin action. The viscous and gel-forming properties of SDF prevent macronutrients absorption, reduce postprandial glucose response, and beneficially affect certain blood lipids (Ou et al. 2001). Trigonelline, a pyridine alkaloid, apart from its antioxidative effects, can alter the activities of enzymes involved in glucose metabolism, β-cell regeneration, and insulin secretion (Zhou et al. 2012). The treatment of alloxan-induced diabetic rats with fenugreek seed powder modulated key enzymes like glycolytic, gluconeogenic, and NADH-linked lipogenic enzymes in the liver and kidney necessary for normalizing glucose level (Raju et al. 2001). Furostanol, a saponin constituent of fenugreek extract, increases feed intake and weight gain in diabetic rats (Petit et al. 1995). Saponins also modulate the disaccharidase and glycogen enzyme activities in the intestine, which results in increased hepatic glycogen content and suppression of blood glucose level. Diosgenin, a

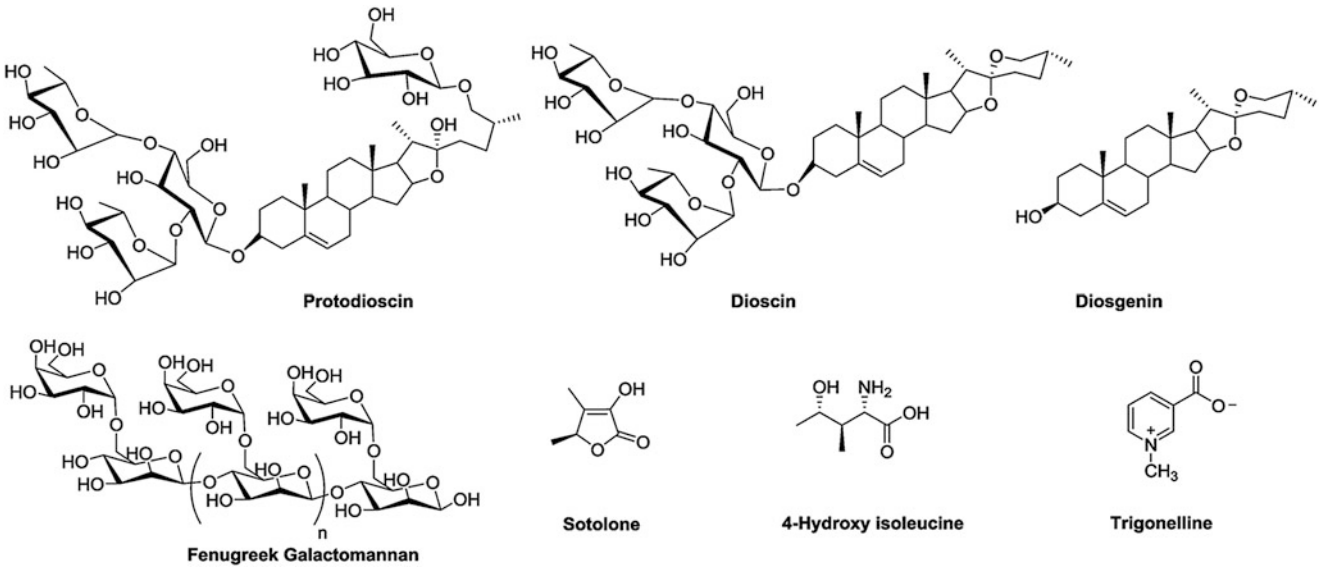


Fig. 1 Structures of some of the important phytochemicals present in fenugreek. Source: Review article of Venkata et al. (2017)

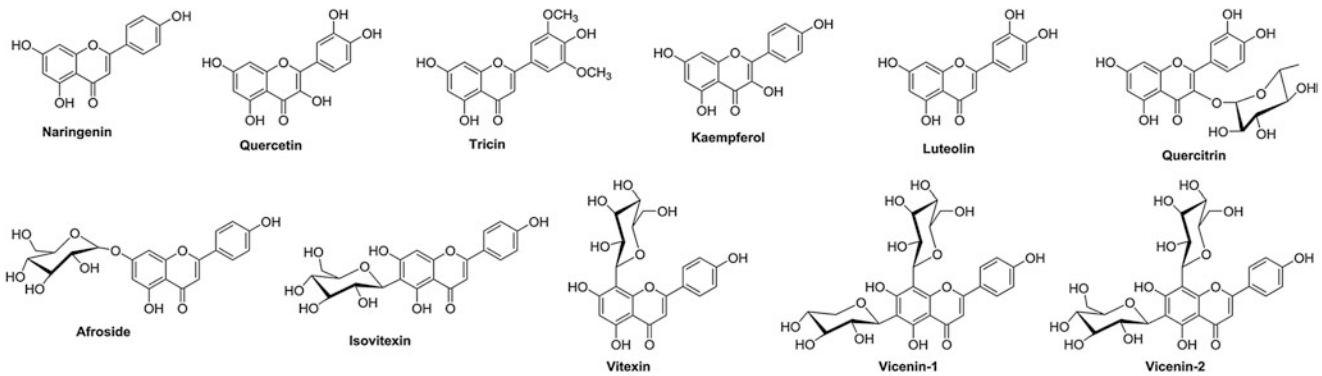


Fig. 2 Flavonoids and their derivatives present in fenugreek. Source: Review article of Venkata et al. (2017)

bioactive steroidal sapogenin belonging to the triterpene group, is a product of the hydrolysis of saponins. Diosgenin content in fenugreek seeds is higher than in its aerial parts (Dangi et al. 2014). This compound is a precursor for several hormones and is extracted commercially for producing sex hormones and other important steroidal drugs. The antidiabetic potential of diosgenin may be attributed to its multiple effects including renewal of pancreatic β-cells, stimulation of insulin secretion, antioxidative effects, stimulation of differentiation of adipocytes, and enhancement of insulin-dependent glucose uptake (Son et al. 2007; Uemura et al. 2010; Kalailingam et al. 2014). Diosgenin also exhibits renal protection in diabetic rats through its anti-inflammatory and antioxidative actions (Kanchan et al. 2016). Subsequent reduction of lipid peroxidation in the liver of diabetic rats after treating with fenugreek has also been attributed to its antioxidative actions (Jin et al. 2014). A nonprotein amino acid, 4-hydroxyisoleucine (4-HIL), is one of the extensively studied phytochemicals present in fenugreek which has insulin-like action and can stimulate insulin production, thereby controlling blood sugar levels in diabetic patient as well as in vitro studies (Gupta et al. 2001). This unusual amino acid is even safer and more effective than many of the current medications available for the treatment of type 2 diabetes mellitus (Zafar and Gao 2016).

The neurological consequences associated with this metabolic disease in the CNS are now receiving considerable attention (Kamboj et al. 2009). Oxidative stress has been implicated in the pathogenesis of many neurodegenerative diseases (Chen et al. 2012). Hyperglycemia generates many free radicals in the diabetic patient, ultimately leading to increased damage of plasma membranes and simultaneous reduction in antioxidant levels (Preet et al. 2005). There is also an increase in Ca^{2+} levels concomitantly with free radicals, which actually correlates to the increase in cellular lipid peroxidation of the synaptosomal membrane and

inhibition of Ca^{2+}ATPase activity (Pekiner et al. 2005; Kamboj et al. 2009). Administration of fenugreek also reduces some of the aberrations that occur in the brain during diabetes, mainly due to its antioxidative activities and neuroprotective effects.

3.2.2 Cholesterol Lowering and Cardiovascular Protection

Cardiovascular diseases (CVD) are the leading cause of human death and morbidity globally (Mendis et al. 2011). Proper management of the cholesterol level in the hypercholesterolemic patient is essential to prevent cardiovascular diseases. Statins alone or in combination with some other drugs are commonly used for controlling increased cholesterol levels. Hundreds of plant-based medicines are also used either singly or in combinations in traditional systems of medicine for the treatment of coronary heart diseases (Mahady 2009). Fenugreek seeds lower serum cholesterol, triglyceride, and low-density lipoprotein in hypercholesterolemic and diabetic patients (Sharma et al. 1996; Mathern et al. 2009) and animals (Sauvaire et al. 1991; Boban et al. 2009). Administration of fenugreek in obsessed rats also reduces triglyceride accumulation in liver while increasing the fecal bile and cholesterol excretion (Rashmi and Rahul 2011). This increased excretion of bile and cholesterol is considered to be a consequence of the reaction between bile acid and fenugreek-derived saponins in the gut causing formation of large micelles which cannot be absorbed easily from the gut (Olaiya and Soetan 2014). The cholesterol-lowering potential of fenugreek is also attributed to its high fiber content. Soluble fiber from fenugreek seemed to reduce reabsorption of bile constituents in the small intestine through binding cholesterol and bile acids and disruption in the enterohepatic cycle in vivo. This enhances utilization of cholesterol in bile acid biosynthesis, subsequently reducing its level (Muraki et al. 2011).

3.2.3 Cancer

Many constituents in fenugreek have shown to exhibit antitumor or anticancer activities in vivo and in vitro. Some of these important constituents include diosgenin (Raju et al. 2004), trigonelline (Bhalke et al. 2009), and flavonoids (Ahmed et al. 2017). Phytoestrogens and saponins present in fenugreek extracts are found to possess anticancer activity in vitro (Raju et al. 2004). Saponins in the extracts not only selectively inhibit cell division in tumor cells but also can initiate apoptosis of the cells (Francis et al. 2002). Diosgenin, a steroidal saponin, has shown antitumorigenic activities in colorectal cancer, osteosarcoma, hepatocellular carcinoma, breast cancer, and leukemia. The effects of diosgenin are mediated through various pathways such as the STAT pathway (Li et al. 2010), activation of p53 and caspase-3 (Liu et al. 2005), and the induction of the tumor necrosis factor-related *apoptosis*-inducing ligand (TRAIL) death receptor DR5 (Lepage et al. 2011). A study in rats revealed the anticancer activity of diosgenin from its ability to inhibit the formation of aberrant crypt foci (ACF), which are clusters of abnormal tube-like glands in the lining of the colon and rectum and can be observed as preneoplastic lesion (Raju et al. 2004). Diosgenin suppressed the expression of bcl-2, a proapoptotic protein, and increased the expression of caspase-3, an anti-apoptotic protein (Raju et al. 2004). Cytokine TNF-α is known to promote cell proliferation, an event common in the initiation and promotion of malignant disease. Diosgenin may also act against bone cancer through the inhibition of TNF-α, thus suppressing proliferation and development of bone cells (Shishodia and Aggarwal 2006). The effectiveness of the fenugreek plant was also seen in colon cancer through the modulation of β-glucuronidase and mucinase activities (Devasena and Menon 2003). Limiting the activities of β-glucuronidase and mucinase in the colonic mucosa may enhance the effectiveness of chemotherapy in colon cancer. The increased activities of β-glucuronidase promote the release of free carcinogens from carcinogen-glucuronide conjugates by enhancing the process of hydrolysis within the colonic lumen, and mucinase assists by hydrolyzing the protective mucin in the gut (Beaud et al. 2005; Booupathy et al. 2016). Fenugreek can decrease the activities of both β-glucuronidase and mucinase in colonic mucosa and may subsequently prevent free carcinogens from acting on the colonocytes (Devasena and Menon 2003).

3.2.4 Antibacterial and Antifungal Effects

The antibacterial and antifungal activities of fenugreek have been reported by many investigators in recent years. The examination of methanol, acetone, and aqueous extracts of fenugreek leaves, seeds, and stems against *E. coli* and *Staphylococcus* isolated from spoiled cabbage revealed the antibacterial property of the herb. The methanol extract of the leaves demonstrated the highest effect, while the aqueous extracts showed the least (Sharma et al. 2017). Mercan et al. (2007) reported an interesting finding that honey samples with the highest antibacterial activity against several bacteria such as *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* displayed maximum pollens from fenugreek as compared to other plants. The extracts were also effective against *Helicobacter pylori* (Randhir et al. 2004).

Fenugreek extracts are also effective against several fungal strains including *Fusarium graminearum*, *Rhizoctonia solani*, *Botrytis cinerea*, *Alternaria* sp., and *Pythium aphanidermatum* (Haouala et al. 2008). However, the potency of the extracts varies with different parts of the fenugreek plant and also the species of fungus. Defensins are small cysteine-rich cationic proteins and function as host defense peptides. A defensin-like peptide, Tf-AFP, with a molecular mass of 10.3 kDa is present in fenugreek and was

isolated from fenugreek seeds by Oddepally and Guruprasad (2015). These defensins are active against bacteria, fungi, and many viruses (Kagan et al. 1990).

3.2.5 Cutaneous Wound Healing

Many herb and spice extracts contain numerous constituents which enhance cutaneous wound healing. Antioxidant activity is one of the major effects of such bioactive constituents which can eventually reduce excessive or chronic inflammation during injury and subsequently promote wound healing. Topical application of 10 % fenugreek seed ointment promoted formation of cellular fibrous connective tissue, granulation tissue, and early maturation of fibrous connective tissues and thus enhanced wound healing in rabbits (Muhammed and Salih 2012). In another study in rats, topical or oral administration of the fenugreek seed suspension quickened contraction and epithelization of the cutaneous wound (Sumitra et al. 2000). Apart from its antioxidative actions, several other constituents present in the extracts are capable of modulating the different phases of healing, which include inflammation, cell proliferation and migration, angiogenesis, and maturation. Antioxidative and antibacterial activities exerted by several constituents of fenugreek extracts are considered important factors augmenting the healing processes (Muhammed and Salih 2012; Ktari et al. 2017). Moreover, fatty acids present in fenugreek seed help in building collagen and consequently promote wound healing and maintenance of skin elasticity (Dixit et al. 2005).

3.2.6 Toxicity Amelioration

Fenugreek is hepatoprotective (Kaviarasan and Anuradha 2007). The antioxidative (Reddy and Srinivasan 2011), anti-radical, and iron metabolism normalizing effect of fenugreek are thought to impart hepatoprotection (Kaviarasan et al. 2007). Incorporation of fenugreek seeds powder (FSP) (5%) in pelleted diet ameliorated chronic liver injury induced by $AlCl_3$ in Wistar rats (Belaïd-Nouira et al. 2013a). The altered liver enzymes and protein levels returned to normal after feeding FSP. Moreover, fenugreek could reduce nephrotoxicity (Belaïd-Nouira et al. 2013b). Fenugreek has shown effectiveness in preventing liver cell necrosis in primary rat hepatocytes culture against *N*-methyl- *N*-nitro-*N*-nitrosoguanidine (MNNG) toxicity in vitro (Khader et al. 2007). Furthermore, the plant has potential for initiating regeneration of hepatocytes during injury. Kaviarasan et al. (2006) reported that in ethanol-induced liver damage, protection of hepatocyte structure and function by fenugreek seed aqueous extract occurred in a dose-dependent manner. Ethanolic extract of fenugreek seed reduced dimethoate (an OP compound)-induced pancreatic damage (Mesallam et al. 2018). Nevertheless, fenugreek extracts were found to potentiate apoptosis of cells induced by radiation, and this cytotoxicity was pronounced in T cells of humans. The cytotoxic potentiative effect of this extract can be of great use in cancer research and treatment by reducing unwanted side effects in those patients who are more sensitive to radiation. However, more in vivo and in vitro studies are needed to support these findings for final validation of effects (Tavakoli et al. 2015).

3.2.7 Gastroprotection

The aqueous extract of fenugreek seeds and a gel fraction isolated from the seeds have ulcer protective potential when compared with omeprazole on ethanol-induced gastric ulcer in experimental rats (Pandian et al. 2002). A similar result was observed on aspirin-induced gastric ulcer in rats using ranitidine as the standard drug (Thirunavukkarasu and Anuradha 2007). This cytoprotective effect was not only due to anti-secretory action of the seed but also attributed to the effects on mucosal glycoproteins. Development of a mucin-like gel layer of galactomannan on the surface of the gastric mucosa forms a barrier, protecting the mucosa from ulcerogenic agents as well as from the gastric juice pepsin in the stomach (Madar and Shomer 1990). Moreover, the antioxidative actions of the seed extract may also contribute to diminishing mucosal injury (Narender et al. 2006). Figer et al. (2017) demonstrated that fenugreek aqueous extract at different concentrations significantly inhibited cell death better than misoprostol sodium against ethanol-induced damage in human gastric carcinoma epithelial cell line in vitro. Higher concentrations beyond 5.0 μg/ml resulted in a decrease in activity. In silico analysis revealed a remarkable degree of interaction of flavonoid constituents with H^+/K^+ ATPase receptor binding sites demonstrating the promising therapeutic potential of fenugreek seed extract as gastroprotective (Figer et al. 2017).

3.2.8 Other Benefits of Fenugreek

Apart from the uses discussed above, fenugreek is well known for its multiple pharmacological actions. Changes in hepatic lipid metabolism can result in development of chronic liver disease (Corey and Cohen 2015). Fenugreek can lower hepatic lipids in the body because of its potential to modify the activities of several enzymes including enzymes related to glucose and lipid metabolism (Madar and Shomer 1990).

Fenugreek is anthelmintic as it causes the evacuation of parasitic intestinal worms. Alcoholic extract of fenugreek seeds has shown anthelmintic activity against earth worm comparable to albendazole in vitro (Khadse and Kakde 2010). However, the aqueous extract was less potent than albendazole (Buchineni and Kondaveti 2016). The effectiveness of the extracts is also reported against *Hymenolepis nana*, *Syphacia obvelata*, and *Moniezia expansa* (Ghafagaai et al. 1980).

Fenugreek is a potent immunostimulant which can stimulate both humoral (Tripathi et al. 2012) and cell-mediated

immune mechanisms (Anarthe et al. 2014). The immunomodulatory effect has been reported by many investigators (Bin-Hafeez et al. 2003; Tripathi et al. 2012; Meghwal and Goswamy 2012; Wani and Kumar 2016). The extract increased phagocytic index, phagocytic capacity of macrophages, as well as lymphoproliferation which strongly suggest its stimulatory effect on immune functions in mice (Bin-Hafeez et al. 2003).

The neuroprotective action of fenugreek has also been reported (Moghadam et al. 2013; Hamden et al. 2010; Ahmed et al. 2017). The powder made from this herb has shown neuroprotective effect in aluminum chloride-induced Alzheimer's disease in rats which might be the result of synergistic activities of several constituents present in the seed powder. This effect resulted in attenuation of AlCl3-induced memory deficits, amyloid and tau pathology, oxidative stress, and inflammation in Alzheimer's disease in rats (Prema et al. 2017).

4 Fenugreek in Animal, Poultry, and Fish Feeds

The use of antibiotics, hormones, and many more chemicals as feed additives in livestock, poultry, and fish productions is usually associated with many untoward effects, as well as residue issues in meat, milk, eggs, and fish for human consumption. Many natural materials like medicinal plants/herbs could be used as feed additives in animal, poultry, and fish diets to increase feed utilization efficacy and production performance. Herbs or spices have been reported to have the potential of enhancing various physiological functions like appetite stimulation, growth, anti-stress, immune functions, and so on. Incorporation of 10% of an extract of a mixture of herbal plants including fenugreek seeds in animal feed reduced the production of aflatoxin by *Aspergillus flavus* by about 85–90% (El-Shayeb and Mabrouk 1984). Fenugreek stimulates bile secretion by increasing the conversion of cholesterol to bile salts (Bhat et al. 1985). Improvement in feed intake after incorporation of fenugreek seed in the diet has also been reported in rats (Petit et al. 1993).

4.1 Poultry Production

Incorporation of many herbs or spices in feeds improves digestibility, nutrient absorption, and even elimination of pathogens from the GI tract, and this in return enhances growth and productivity in poultry. Supplementation of broiler chick feed with fenugreek increased feed intake and body weight gain and decreased the feed conversion ratio (Elbushra 2012). Beside these, there was also a reduction in the mortality rate of poultry (Alloui et al. 2012). Fenugreek can also be added to drinking water as an anti-stress agent. This would be an important step in replacing the use of an antibiotic like enrofloxacin (Saber et al. 2017).

The inclusion of fenugreek seeds in broiler diet is not only economical by increasing the feed conversion ratio, but it also contributes to reduced abdominal fat deposition in the birds (Yesuf et al. 2017). This is an important finding which would be of great benefit for lean broiler meat production.

4.2 Livestock Production

Fenugreek produces high quality forages in all growth stages. It has a high content of many nutrients as well as phytochemicals (diosgenin) which promote growth and milk production in livestock. (Acharya et al. 2007; Żuk-Gołaszewska and Wierzbowska 2017). The nutritive value of the forage in all growth stages is comparable to early-bloom alfalfa (*Medicago sativa* L.) (Mir et al. 1998). The incorporation of fenugreek in cattle diets resulted in improvement of milk quality parameters and animal metabolism (Rjat and Taparia 1990). Fenugreek seed in buffalo diet improves total dry matter and daily consumption of concentrates thereby increasing milk production (Degirmencioglu et al. 2016). Fenugreek insignificantly affects milk constituents such as SNF, proteins, somatic cell count (SCC), fats, etc. (El-Nor et al. 2007; Degirmencioglu et al. 2016). However, a slight reduction in milk fat content as reported by some researchers might be related to increasing milk yield (Degirmencioglu et al. 2016). The enhanced performance of sheep after addition of fenugreek seeds in their feed has also been reported (Ismail 2000).

4.3 Fish Production

Fish are considered one of the best and least expensive sources of lean meat. In a study from 2010, 16.7% of animal protein and 6.5% of all protein consumed globally comes from fish (Barik 2017). In commercial fish production, antibiotics and hormones are increasingly added to the fish diet for a faster growth rate and other purposes. However, strict regulations on the application of antibiotics and chemotherapeutics in aquatic feeds exist due to concern for bioaccumulation (Lim et al. 2013). Hence, replacement of such chemicals with many natural materials such as herbs/spices which have medicinal value should be encouraged and practiced. The addition of different percentages of fenugreek seed meal (FSM) in the diet of common carp fingerlings has resulted in a decrease in the feed conversion ratio and a faster growth rate (Roohi et al. 2017).

5 Toxicology and Safety Profile

The various extracts of fenugreeks have been used since ancient times in different medical conditions of humans and animals and are generally considered safe. The acute and subacute toxicity studies of fenugreek in rats (up to 5g/kg body weight) and mice (up to 2g/kg body weight) did not produce significant toxicity in either sex (Narasimhamurthy et al. 1999). The LD_{50} of the aqueous extract of fenugreek in mice is 10 g/kg body weight p.o. (Abdel-Barry et al. 1997). Administration of a glycosidic extract orally to mice for 28 days has a lower LD_{50} (4.25 g/kg body weight) (Kandhare et al. 2015). Recent evidence suggests that fenugreek may have neurodevelopmental, neurobehavioral, and neuropathological side effects, and therefore its consumption should be avoided during pregnancy and lactation (human, rodent, rabbit, and chick data reviewed in Ouzir et al. 2016).

6 Concluding Remarks and Future Directions

Studies conducted over the last few years on fenugreek revealed its varied nutritive and medicinal values. Fenugreek, a rich source of protein, fiber, vitamins, and minerals, can be offered to animals, birds, and fish as a feed supplement for improving their health and performance. Antidiabetic, hypoglycemic, hypocholesterolemic, antioxidative, antiulcerogenic, antimicrobial, anticarcinogenic, and neuroprotective activities are some of the major medicinal effects exhibited by fenugreek. Replacement of harmful antibiotics and hormones with fenugreek and/or other medicinal plants/herbs in animal, bird, and fish feeds has shown definite benefit in the attempt to improve livestock, poultry, and fish production quantity and quality. Additionally, the issues of drug residues in meat, milk, fish, and eggs and their products for human consumption as well as development of antibiotic resistance which occur mainly due to indiscriminate use of antimicrobial agents may be able to be resolved to a certain extent. However, current knowledge on the molecular mechanisms involved in exhibiting the various pharmacological effects of most of the bioactive phytochemicals in fenugreek extracts is limited, and further research is needed for scientific validation of the multiple effects as well as to explore any other distinct therapeutic potentials of the herb.

Acknowledgments The authors are thankful to all the scientists, scholars, and staff of the Division of Pharmacology and Toxicology, Indian Veterinary Research Institute (IVRI), Izzatnagar, Bareilly, India, for their support and cooperation in completion of this book chapter.

References

Abdel-Barry JA, Abdel-Hassan IA, Al-Hakiem MH (1997) Hypoglycemic and antihyperglycemic effects of *Trigonella foenum-graecum* leaf in normal and alloxan induced diabetic rats. J Ethnopharmacol 58(3):149–155

Acharya SN, Basu SK, Thomas JE (2007) Medicinal properties of fenugreek (*Trigonella foenum-graecum* L.): a review of the evidence based information. Adv Med Plant Res:81–122

Adil S, Qureshi S, Pattoo RA (2015) A review on positive effects of fenugreek as feed additive in poultry production. Int J Poult Sci 14 (12):664–669

Ahmad A, Alghamdi SS, Mahmood K et al (2016) Fenugreek a multipurpose crop: potentialities and improvements. Saudi J Biol Sci 23 (2):300–310

Ahmed SI, Hayat MQ, Zahid S et al (2017) Isolation and identification of flavonoids from anticancer and neuroprotective extracts of *Trigonella foenum graecum*. Trop J Pharm Res 16(6):1391–1398

Ajabnoor MA, Tilmisany AK (1988) Effect of *Trigonella foenum graceum* on blood glucose levels in normal and alloxan-diabetic mice. J Ethnopharmacol 22(1):45–49

Alloui N, Aksa SB, Alloui MN et al (2012) Utilization of fenugreek (*Trigonella foenum-graecum*) as growth promoter for broiler chickens. J World Poult Res 2(2):25–27

Alsemari A, Alkhodairy F, Aldakan A et al (2014) The selective cytotoxic anti-cancer properties and proteomic analysis of *Trigonella foenum-graecum*. BMC Complement Altern Med 14(1):114

Altuntaş E, Özgöz E, Taşer ÖF (2005) Some physical properties of fenugreek (*Trigonella foenum-graceum* L.) seeds. J Food Eng 71 (1):37–43

Anarthe SJ, Sunitha D, Raju MG (2014) Immunomodulatory activity for methanolic extract of *Trigonella foenum graecum* whole plant in wistar albino rats. Am J Phytomed Clin Ther 2(9):1081–1092

Association of Official Analytical Chemists (A.O.A.C) (1990) Official methods of analysis, 15th edn. Association of Official Analytical Chemists, Washington, DC

Barik NK (2017) Freshwater fish for nutrition security in India: evidence from FAO data. Aquacult Rep 7:1–6

Beaud D, Tailliez P, Anba-Mondoloni J (2005) Genetic characterization of the β-glucuronidase enzyme from a human intestinal bacterium, *Ruminococcus gnavus*. Microbiology 151(7):2323–2330

Belaïd-Nouira Y, Bakhta H, Haouas Z et al (2013a) Fenugreek seeds, a hepatoprotector forage crop against chronic $AlCl_3$ toxicity. BMC Vet Res 9(1):22

Belaïd-Nouira Y, Bakhta H, Haouas Z et al (2013b) Fenugreek seeds reduce aluminum toxicity associated with renal failure in rats. Nutr Res Pract 7(6):466–474

Benzie IF, Wachtel-Galor S (2011) Herbal medicine: biomolecular and clinical aspects, 2nd edn. CRC Press, Boca Raton, FL, p 410

Betty R (2008) The many healing virtues of fenugreek. Spice India 1:17–19

Bhalke RD, Anarthe SJ, Sasane KD et al (2009) Antinociceptive activity of *Trigonella foenum graecum* leaves and seeds (Fabaceae). Int J Pharm Technol 8(2):57–59

Bhat BG, Sambaia K, Chandrasekhara N (1985) The effect of feeding fenugreek and ginger on bile composition in the albino rats. Nutr Rep Int 32:1145–1151

Bin-Hafeez B, Haque R, Parvez S et al (2003) Immunomodulatory effects of fenugreek (*Trigonella foenum graecum* L.) extract in mice. Int Immunopharmacol 3(2):257–265

Boban PT, Nambisan B, Sudhakaran PR (2009) Dietary mucilage promotes regression of atheromatous lesions in hypercholesterolemic rabbits. Phytother Res 23(5):725–730

Booupathy LK, Venkatachalam S, Natarajan N (2016) Chemopreventive effect of myrtenal on bacterial enzyme activity and the development of 1, 2-dimethyl hydrazine-induced aberrant crypt foci in Wistar rats. J Food Drug Anal 24(1):206–213

Buchineni M, Kondaveti S (2016) *In-vitro* anthelmintic activity of fenugreek leaves (aqueous extract) in Indian earthworms. Pharm Innov J 5(4, Part B):70–72

Chatterjee S, Variyar SP, Sharma A (2010) Bioactive lipid constituents of fenugreek. Food Chem 119(1):349–353

Chen X, Guo C, Kong J (2012) Oxidative stress in neurodegenerative diseases. Neural Regen Res 7(5):376–385

Corey KE, Cohen DE (2015) Lipid and lipoprotein metabolism in liver disease. In: De Groot LJ, Chrousos G, Dungan K, et al. (ed) Endotext (Internet), MDText.com, Inc., South Dartmouth, MA, 2000-. (Updated 2015 Jun 27). Available from https://www.ncbi.nlm.nih.gov/books/NBK326742

Dangi R, Misar A, Tamhankar S, Rao S (2014) Diosgenin content in some *Trigonella* species. Indian J Adv Plant Res 1:47–51

Degirmencioglu T, Unal H, Ozbilgin S et al (2016) Effect of ground fenugreek seeds (*Trigonella foenum-graecum*) on feed consumption and milk performance in Anatolian water buffaloes. Arch Anim Breed 59(3):345–349

Devasena T, Menon VP (2003) Fenugreek affects the activity of *β*-glucuronidase and mucinase in the colon. Phytother Res 17 (9):1088–1091

Dixit P, Ghaskadbi S, Mohan H et al (2005) Antioxidant properties of germinated fenugreek seeds. Phytother Res 19(11):977–983

Dymock W, Warden CJH, Hooper D (2005) Pharmacographic indica. Srishti Book Distributors, New Delhi, pp 401–404

Edison S (1995) Spices-research support to productivity. In: Ravi N (ed) The Hindu. survey of Indian agriculture. Kasturi and Sons Ltd., Madras, pp 101–105

Elbushra ME (2012) Effect of dietary Fenugreek seeds (*Trigonella foenum*) as natural feed addition on broiler chicks performance. J Sci Technol 13:27–31

El-Nor SAH, Khattab HM, Al-Alamy HA et al (2007) Effects of some medicinal plants seeds in the rations on the productive performance of lactating buffaloes. Int J Dairy Sci 2:348–355

El-Shayeb NMA, Mabrouk AM (1984) Utilization of some edible and medicinal plants to inhibit aflatoxin formation. Nutr Rep Int 29:273–282

Figer B, Pissurlenkar R, Ambre P et al (2017) Treatment of gastric ulcers with fenugreek seed extract; *In-Vitro*, *In-Vivo* and *In-Silico* approaches. Indian J Pharm Sci 79(5):724–730

Francis G, Kerem Z, Makkar PS et al (2002) The biological action of saponins in animals systems: a review. Br J Nutr 88(6):587–605

Ghafagaai T, Farid H, Pourafkari A (1980) *In-vitro* study of the anthelmintic action of *Trigonella foenum graecum* L. grown in Iran. Iran J Publ Health 9(1–4):21–26

Gupta A, Gupta R, Lal B (2001) Effect of *Trigonella foenum-graecum* (fenugreek) seeds on glycaemic control and insulin resistance in type 2 diabetes. J Assoc Physicians India 49:1057–1061

Hamden K, Masmoudi H, Carreau S et al (2010) Immunomodulatory, β-cell, and neuroprotective actions of fenugreek oil from alloxan-induced diabetes. Immunopharmacol Immunotoxicol 32(3):437–445

Haouala R, Hawala S, El-Ayeb A et al (2008) Aqueous and organic extracts of *Trigonella foenum-graecum* L. inhibit the mycelia growth of fungi. J Environ Sci (China) 20(12):1453–1457

Ismail A (2000) Effect of fenugreek seeds (*Trigonella Foenum-graecum* L.) as feed additive on sheep performance in the North Western coast of Egypt. In: Proc 3rd all Africa Conf. Anim. Agric. 811th Conf, Egyptian Soc. Anim. Prod. Alexandria, Egypt, pp 6–9

Jin Y, Shi Y, Zou Y et al (2014) Fenugreek prevents the development of STZ-induced diabetic nephropathy in a rat model of diabetes. Evid Based Complement Alternat Med 2014. https://doi.org/10.1155/2014/259368

Kagan BL, Selsted ME, Ganz T, Lehrer RI (1990) Antimicrobial defensin peptides form voltage-dependent ion-permeable channels in planar lipid bilayer membranes. Proc Natl Acad Sci 87 (1):210–214

Kalailingam P, Kannaian B, Tamilmani E et al (2014) Efficacy of natural diosgenin on cardiovascular risk, insulin secretion, and beta cells in streptozotocin (STZ)-induced diabetic rats. Phytomedicine 21(10):1154–1161

Kalshetti PB, Alluri R, Mohan V et al (2015) Effects of 4-hydroxyisoleucine from fenugreek seeds on depression-like behavior in socially isolated olfactory bulbectomized rats. Pharmacogn Mag 11(Suppl 3):S388

Kamboj SS, Chopra K, Sandhir R (2009) Hyperglycemia-induced alterations in synaptosomal membrane fluidity and activity of membrane bound enzymes: beneficial effect of N-acetylcysteine supplementation. Neuroscience 162(2):349–358

Kanchan DM, Somani GS, Peshattiwar VV et al (2016) Renoprotective effect of diosgenin in streptozotocin induced diabetic rats. Pharmacol Rep 68(2):370–377

Kandhare AD, Bodhankar SL, Mohan V et al (2015) Acute and repeated doses (28 days) oral toxicity study of glycosides based standardized fenugreek seed extract in laboratory mice. Regul Toxicol Pharmacol 72(2):323–334

Kaviarasan S, Anuradha CV (2007) Fenugreek (*Trigonella foenum graecum*) seed polyphenols protect liver from alcohol toxicity: a role on hepatic detoxification system and apoptosis. Pharmazie 62 (4):299–304

Kaviarasan S, Ramamurty N, Gunasekaran P et al (2006) Fenugreek (*Trigonella foenum graecum*) seed extract prevents ethanol-induced toxicity and apoptosis in Chang liver cells. Alcohol Alcohol 41 (3):267–273

Kaviarasan S, Naik GH, Gangabhagirathi R et al (2007) *In-vitro* studies on antiradical and antioxidant activities of fenugreek (*Trigonella foenum graecum*) seeds. Food Chem 103(1):31–37

Khader M, Eckl PM, Bresgen N (2007) Effects of aqueous extracts of medicinal plants on MNNG-treated rat hepatocytes in primary cultures. J Ethnopharmacol 112(1):199–202

Khadse CD, Kakde RB (2010) *In-vitro* anthelmintic activity of Fenugreek seeds extract against Pheritima posthuma. Int J Res Pharm Sci 1(3):267–269

Khorshidian N, Yousefi Asli M, Arab M et al (2016) Fenugreek: potential applications as a functional food and nutraceutical. Nutr Food Sci Res 3(1):5–16

Kirtikar KR, Basu BD (2002) Indian medicinal plants, vol I. International Book Distributors, Dehradun, India, pp 700–701

Kirtikar KR, Basu BD (2003) Indian medicinal plants with illustrations, vol 3, 2nd edn. Oriental Enterprises, Dehradun, India, pp 982–983

Ktari N, Trabelsi I, Bardaa S et al (2017) Antioxidant and hemolytic activities, and effects in rat cutaneous wound healing of a novel polysaccharide from fenugreek (*Trigonella foenum-graecum*) seeds. Int J Biol Macromol 95:625–634

Lepage C, Léger DY, Bertrand J et al (2011) Diosgenin induces death receptor-5 through activation of p38 pathway and promotes TRAIL-induced apoptosis in colon cancer cells. Cancer Lett 30(2):193–202

Li F, Fernandez PP, Rajendran P et al (2010) Diosgenin, a steroidal saponin, inhibits STAT3 signaling pathway leading to suppression of proliferation and chemosensitization of human hepatocellular carcinoma cells. Cancer Lett 292(2):197–207

Lim SJ, Jang E, Lee SH et al (2013) Antibiotic resistance in bacteria isolated from freshwater aquacultures and prediction of the persistence and toxicity of antimicrobials in the aquatic environment. J Environ Sci Health 48(6):495–504

Liu MJ, Wang Z, Ju Y et al (2005) Diosgenin induces cell cycle arrest and apoptosis in human leukemia K562 cells with the disruption of Ca^{2+} homeostasis. Cancer Chem Pharmacol 55(1):79–90

Luan G, Wang Y, Wang Z et al (2018) Flavonoid glycosides from fenugreek seeds regulate glycolipid metabolism by improving mitochondrial function in 3T3-L1 adipocytes *in vitro*. J Agric Food Chem 66:3169–3178

Lust JB (1986) The herb book. Bantam Books Inc, New York, pp 1–55

Madar Z, Shomer IJ (1990) Polysaccharide composition of a gel fraction derived from fenugreek and its effect on starch digestion and bile acid absorption in rats. J Agric Food Chem 38(7):1535–1539

Mahady GB (2009) Medicinal plants for the prevention and treatment of coronary heart disease. Ethnopharmacology II:75–99

Malviya KG, Babhulkar MW, Mali P et al (2010) Evaluation of anti-inflammatory potential of *Trigonella foenum-graecum* (fenugreek) seed extracts by using carrageenan induced rat paw edema. Drug Invent Today 2(2):109–111

Mathern JR, Raatz SK, Thomas W et al (2009) Effect of fenugreek fiber on satiety, blood glucose and insulin response and energy intake in obese subjects. Phytother Res 23(11):1543–1548

Meghwal M, Goswamy TK (2012) A review on the functional properties, nutritional content, medicinal utilization and potential application of fenugreek. J Food Process Technol 3:9

Mendis S, Puska P, Norrving B et al (2011) Global atlas on cardiovascular disease prevention and control. World Health Organization, Geneva, pp 3–18

Mercan N, Guvensen A, Celik A et al (2007) Antimicrobial activity and pollen composition of honey samples collected from different provinces in Turkey. Nat Prod Res 21(3):187–195

Mesallam DI, Hamid OIA, Ibrahem NE (2018) Ethanolic extract of fenugreek seeds moderates dimethoate-induced pancreatic damage in male rats. Environ Sci Pollut Res 25(4):3894–3904

Mir Z, Mir PS, Acharya SN et al (1998) Comparison of alfalfa and fenugreek (*Trigonella foenum-graecum*) silages supplemented with barley grain on performance of growing steers. Can J Anim Sci 78 (3):343–349

Moghadam FH, Vakili-Zarch B, Shafiee M et al (2013) Fenugreek seed extract treats peripheral neuropathy in pyridoxine induced neuropathic mice. Excli J 12:282–290

Moradi N, Moradi K (2013) Physiological and pharmaceutical effects of fenugreek (*Trigonella foenum-graecum* L.) as a multipurpose and valuable medicinal plant. Global J Med Plant Res 1(2):199–206

Muhammed DO, Salih NA (2012) Effect of application of Fenugreek (*Trigonella foenum-graecum*) on skin wound healing in rabbits. AL-Qadisiya J Vet Med Sci 11(2):86–93

Muraki E, Hayashi Y, Chiba H et al (2011) Dose-dependent effects, safety and tolerability of fenugreek in diet-induced metabolic disorders in rats. Lipids Health Dis 10(1):240

Naicker N, Nagiah S, Phulukdaree A et al (2016) *Trigonella foenum-graecum* seed extract, 4- hydroxyisoleucine, and metformin stimulate proximal insulin signaling and increase expression of glycogenic enzymes and GLUT2 in HepG2 cells. Metab Syndr Relat Disord 14 (2):114–120

Narasimhamurthy K, Viswanatha S, Ramesh BS (1999) Acute and subchronic toxicity assessment of debitterized fenugreek powder in the mouse and rat. Food Chem Toxicol 37(8):831–838

Narender T, Puri A, Khaliq T et al (2006) 4-Hydroxyisoleucine an unusual amino acid as antidyslipidemic and antihyperglycemic agent. Bioorg Med Chem Lett 16(2):293–296

Norziah MH, Fezea FA, Bhar R et al (2015) Effect of extraction solvents on antioxidant and antimicrobial properties of fenugreek seeds (*Trigonella foenum-graecum* L.). Int Food Res J 22(3): 1261–1271

Oddepally R, Guruprasad L (2015) Isolation, purification, and characterization of a stable defensin-like antifungal peptide from *Trigonella foenum-graecum* (fenugreek) seeds. Biochem (Moscow) 80(3):332–342

Olaiya CO, Soetan KO (2014) A review of the health benefits of fenugreek (*Trigonella foenum-graecum* L.): nutritional, Biochemical and pharmaceutical perspectives. Am J Soc Issues Human:3–12

Ou S, Kwok KC, Li Y et al (2001) *In-vitro* study of possible role of dietary fiber in lowering postprandial serum glucose. J Agr Food Chem 49(2):1026–1029

Ouzir M, El Bairi K, Amzari S (2016) Toxicological properties of fenugreek (*Trigonella foenum graecum*). Food Chem Toxicol 96:145–154

Pandian RS, Anuradha CV, Viswanathan P (2002) Gastroprotective effect of fenugreek seeds (*Trigonella foenum graecum*) on experimental gastric ulcer in rats. J Ethnopharmacol 81(3):393–397

Pekiner DB, Evcimen DN, Nebioğlu S (2005) Diabetes-induced decrease in rat brain microsomal Ca^{2+}-ATPase activity. Cell Biochem Funct 23(4):239–243

Petit P, Sauvaire Y, Ponsin G et al (1993) Effects of a fenugreek seed extract on feeding behaviour in the rat: metabolic endocrine correlates. Pharmacol Biochem Behav 45(2):369–374

Petit PR, Sauvaire YD, Hillaire-Buys DM et al (1995) Steroid saponins from fenugreek seeds: extraction, purification, and pharmacological investigation on feeding behavior and plasma cholesterol. Steroids 60(10):674–680

Preet A, Gupta BL, Siddiqui MR et al (2005) Restoration of ultrastructural and biochemical changes in alloxan-induced diabetic rat sciatic nerve on treatment with Na_3VO_4 and *Trigonella*—a promising antidiabetic agent. Mol Cell Biochem 278(1–2):21–31

Prema A, Justin Thenmozhi A, Manivasagam T et al (2017) Fenugreek seed powder attenuated aluminum chloride-induced tau pathology, oxidative stress, and inflammation in a rat model of Alzheimer's disease. J Alzheimer's Dis 60(s1):S209–S220

Pribac G, Ardelean A, Czapar M et al (2009) *Trigonella foenum-graecum* and *Trigonella policreata* seeds extract exert a protective action of alcohol toxicity in BRL3A rat liver cells. Stud Univ Vasile Goldis Arad Stiintele Vietii 19(1):87–93

Priya V, Jananie RK, Vijayalakshmi K (2011) GC/MS determination of bioactive components of *Trigonella foenum-grecum*. J Chem Pharm Res 3(5):35–40

Raju J, Gupta D, Rao AR et al (2001) *Trigonella foenum graecum* (fenugreek) seed powder improves glucose homeostasis in alloxan diabetic rat tissues by reversing the altered glycolytic, gluconeogenic and lipogenic enzymes. Mol Cell Biochem 224(1–2):45–51

Raju J, Patlolla JMR, Swamy MV et al (2004) Diosgenin, a steroid saponin of *Trigonella foenum graecum* (Fenugreek), inhibits azoxymethane-induced aberrant crypt foci formation in F344 rats and induces apoptosis in HT-29 human colon cancer cells. Cancer Epidemiol Biomarkers Prev 13(8):1392–1398

Randhir R, Lin YT, Shetty K (2004) Phenolics, their antioxidant and antimicrobial activity in dark germinated fenugreek sprouts in response to peptide and phytochemical elicitors. Asia Pac J Clin Nutr 13(3):295–307

Rao AV (2003) Herbal cure for common diseases. Fusion Books, New Delhi

Rashmi Y, Rahul K (2011) Study of phytochemical constituents and pharmacological actions of *Trigonella foenum-graecum*: a review. Int J Pharm Technol 3:1022–1028

Reddy RR, Srinivasan K (2011) Hepatoprotective and antioxidant effect of fenugreek (*Trigonella foenum-graecum*) seeds in mice under lithogenic condition. J Food Biochem 35(6):1619–1626

Rjat H, Taparia A (1990) Utilization of methi straw by cattle. Indian J Anim Sci 60(11):1380–1381

Roberts KT (2011) The potential of fenugreek (*Trigonella foenum-graecum*) as a functional food and nutraceutical and its effects on glycemia and lipidemia. J Med Food 14(12):1485–1489

Roohi Z, Imanpoor MR, Jafari V et al (2017) The use of fenugreek seed meal in fish diets: growth performance, haematological and

biochemical parameters, survival and stress resistance of common carp (*Cyprinus carpio* L.). Aquac Res 48(3):1209–1215
Saber B, Abdeldjelil MC, Benazzouz H et al (2017) Fenugreek (*Trigonella foenum-graecum*): an alternative antistress in broiler chickens in Algeria. Der Pharm Lett 9(1):64–69
Sauvaire Y, Ribes G, Baccou JC et al (1991) Implication of steroid saponins and sapogenins in the hypocholesterolemic effect of fenugreek. Lipids 26(3):191–197
Sauvaire Y, Petit P, Broca C et al (1998) 4-Hydroxyisoleucine: a novel amino acid potentiator of insulin secretion. Diabetes 47(2):206–210
Saxena R, Rathore SS, Barnwal P et al (2013) Effect of cryogenic grinding on recovery of diosgenin content in fenugreek (*Trigonella foenum-graecum* L.) genotypes. Int J Seed Spices 3(1):26–30
Shabbeer S, Sobolewski M, Anchoori RK et al (2009) Fenugreek: a naturally occurring edible spice as an anticancer agent. Cancer Biol Ther 8(3):272–278
Sharma RD, Sarkar A, Hazra DK et al (1996) Hypolipidemic effect of fenugreek seeds: a chronic study in non-insulin dependent diabetic patients. Phytother Res 10(4):332–334
Sharma V, Singh P, Rani A (2017) Antimicrobial activity of *Trigonella foenum-graecum* L. (Fenugreek). Eur J Exp Biol 7(1):4
Shishodia S, Aggarwal BB (2006) Diosgenin inhibits osteoclastogenesis, invasion, and proliferation through the downregulation of Akt, IκB kinase activation and NF-κB-regulated gene expression. Oncogene 25(10):1463–1473
Son IS, Kim JH, Sohn HY et al (2007) Antioxidative and hypolipidemic effects of diosgenin, a steroidal saponin of yam (*Dioscorea* spp.), on high-cholesterol fed rats. Biosci Biotechnol Biochem 71 (12):3063–3071
Sowmya P, Rajyalakshmi P (1999) Hypocholesterolemic effect of germinated fenugreek seeds in human subjects. Plant Food Hum Nutr 53(4):359–365
Srinivasan K (2006) Fenugreek (*Trigonella foenum-graecum*): a review of health beneficial physiological effects. Food Rev Int 22 (2):203–224
Sumitra M, Manikandan P, Suguna L et al (2000) Study of dermal wound healing activity of *Trigonella foenum graceum* seeds in rats. J Clin Biochem Nutr 28(2):59–67
Tavakoli MB, Kiani A, Roayaei M (2015) The effects of fenugreek on radiation induced toxicity for human blood T-cells in radiotherapy. J Med Signals Sens 5(3):176
Thirunavukkarasu V, Anuradha CV (2007) Gastroprotective effect of fenugreek seeds (*Trigonella foenum graecum*) on experimental gastric ulcer in rats. J Herbs Spices Med Plants 12(3):13–25
Tiran D (2003) The use of fenugreek for breast feeding woman. Comp Ther Nurs Midwifery 9(3):155–156
Tripathi S, Maurya AK, Kahrana M et al (2012) Immunomodulatory property of ethanolic extract of *Trigonella foenum-graecum* leaves on mice. Der Pharm Lett 4(2):708–713
Uemura T, Hirai S, Mizoguchi N et al (2010) Diosgenin present in fenugreek improves glucose metabolism by promoting adipocyte differentiation and inhibiting inflammation in adipose tissues. Mol Nutr Food Res 54(11):1596–1608
Venkata KCN, Bagchi D, Bishayee A (2017) A small plant with big benefits: fenugreek (*Trigonella foenum-graecum* Linn.) for disease prevention and health promotion. Mol Nutr Food Res 61(6). https://doi.org/10.1002/mnfr.201600950
Wagh P, Rai M, Deshmukh SK et al (2007) Bio-activity of oils of *Trigonella foenum-graecum* and *Pongamia pinnata*. Afr J Biotechnol 6(13):1592–1596
Wani SA, Kumar P (2016) Fenugreek: a review on its nutraceutical properties and utilization in various food products. J Saudi Soc Agric Sci 17(2):97–106
Yadav S, Sehgal S (1997) Effect of home processing and storage on ascorbic acid and β-carotene content of bathua (*Chenopodium album*) and fenugreek (*Trigonella foenum graecum*) leaves. Plant Food Hum Nutr 50(3):239–247
Yadav R, Kaushik R, Gupta D (2011) The health benefits of *Trigonella foenum-graecum:* a review. Int J Eng Res Appl 1(1):32–35
Yesuf K, Mersso B, Bekele T (2017) Effects of different levels of turmeric, fenugreek and black cumin on carcass characteristics of broiler chicken. J Livestock Sci 8:11–17. ISSN: 2277-6214
Yoshikawa T, Toyokuni S, Yamamoto Y et al (2000) Free radicals in chemistry biology and medicine. OICA International, London, p 580
Zafar MI, Gao F (2016) 4-Hydroxyisoleucine: a potential new treatment for type 2 diabetes mellitus. BioDrugs 30(4):255–262
Zhou J, Chan L, Zhou S (2012) Trigonelline: a plant alkaloid with therapeutic potential for diabetes and central nervous system disease. Curr Med Chem 19(21):3523–3531
Żuk-Gołaszewska K, Wierzbowska J (2017) Fenugreek: productivity, nutritional values and uses. J Elementol 22(3):1067–1080

Neem Extract

Anu Rahal, Dinesh Kumar, and Jitendra K. Malik

Abstract

Neem has become a cynosure of modern lifestyle and finds extensive use in Ayurvedic, Unani and homoeopathic medicine. It elaborates a vast collection of bioactive compounds that are chemically diverse and structurally complex which exhibit immunomodulatory, anti-inflammatory, antihyperglycaemic, antiulcer, antimalarial, antifungal, antibacterial, antiviral, antioxidant, antimutagenic and anticarcinogenic properties. The leaves are a habitual feed for ruminants in arid lands; the seeds are a rich source of fatty acids and proteins but remain largely unexploited owing to the bitter toxic principles present in it. If the bitters can be substantially reduced, the toxicity of neem parts can be overcome, and its different parts may be fully utilized as an excellent nutraceutical. Its bioactivity, livestock safety and ecofriendly temperament is quite encouraging to prospect its bright future in the field of nutraceuticals.

Keywords

Neem · Leaves · Seed kernel · Neem oil · Animal feed · Safety

1 Introduction

Neem (*Azadirachta indica*; family, Meliaceae; subfamily, Meliodeae; order, Meliales) is a nonleguminous evergreen tree native to the Indian subcontinent. It grows widely on almost all kinds of soils including saline and alkaline and other wastelands in several countries of Asia, Australia, Africa and Central and South America. It is, therefore, often used in afforestation programmes, especially in semiarid regions, and grows as boulevard, ornamental and agroforestry plantation or as roadside shade. It is a quick-growing tree, sometimes up to 100 feet high, with luxuriant spreading branches, and remains green throughout the year. At an age of 2–3 years, it bears white flowers, which smell like honey, and then fruits 1–2 years later. Fruits are green when raw, turning yellow after it ripened and aromatic with garlic-like odour. The ripe fruit is yellow drupes that are ellipsoid and glabrous, 12–20 mm long. Fresh leaves and flowers come in March–April. Fruits mature between April and August depending upon the environmental factors.

Neem is commonly known as village dispensary of India. It holds high value owing to its medicinal and insecticidal properties in Ayurvedic and Unani systems of medicines. Every part of neem (leaf, flower, fruit, seed, kernel bark, root, wood, twig and oil) and its fractioned products possess curative properties and find place in traditional remedies. United Nations has declared neem tree as the "Tree of the 21st century", while in a report published in 1992, the US National Academy of Science has designated it as "Neem: A tree of solving global problems". The European literature "Materia medica" has also regarded neem as "Panacea of all Disease". Neem finds varied use in ecological, medicinal and agricultural sectors, including animal feed, medicines, soap industry, nitrification inhibition, slow nutrient release manure, fuel, energy, pest control, etc. A large number of neem-based pest control and healthcare products have developed in the international market. Neem plant contents are effective bioinsecticide and are useful in the control of many insect species of medical and veterinary importance like head lice and *Anopheles* mosquito. Owing to their features of diverse activity and relative safety to non-target organisms, today, neem is being recognized as the single most important source of biopesticides and allied products. Medical research

A. Rahal (✉)
Division of Animal Health, ICAR-Central Institute for Research on Goats, Mathura, Uttar Pradesh, India

D. Kumar
Division of Pharmacology and Toxicology, Indian Veterinary Research Institute, Bareilly, Uttar Pradesh, India

J. K. Malik
Division of Pharmacology and Toxicology, Indian Veterinary Research Institute, Dehradun, Uttarakhand, India

R. C. Gupta et al. (eds.), *Nutraceuticals in Veterinary Medicine*, https://doi.org/10.1007/978-3-030-04624-8_3

has focused, of late, on its applications as a spermicide and a treatment for scabies, although anticarcinogenic properties of the tree have also received attention.

2 Extracts of Neem

Diverse chromatographic techniques such as simple column chromatography, preparative thin-layer chromatography, vacuum liquid chromatography and reverse phase medium- or high-pressure preparative liquid chromatography have been described in literature for isolating bioactive principles from neem on an industrial scale. Depending upon the final use, different types of extraction procedures are taken up for different parts of the plant. The choice of solvent and the method depends on the compound to be concentrated. Technologies for the preparation of azadirachtin concentrates of varying strengths have been developed for both household and industrial use. The indigenous methods include aqueous extraction, cold maceration, steam distillation, and organic solvent extraction. Maceration is a cold extraction process for defatted kernels by soaking in methanol with intermittent stirring for 3 days, after which it is filtered and the solvent evaporated under vacuum to obtain the dried crude extract. In batch stirring extraction, a magnetic pellet is dropped in the flask, and the mixture is stirred for 8 h on a magnetic stirrer plate followed by vacuum drying to obtain crude extract.

A single seeded mature neem fruit contains 23.8% skin, 47.5% pulp, 18.6% shell and 10.1% kernel. The depulped and decoated seed yields about 26% kernel, which gives 45–50% oil leaving the rest as neem kernel cake (NKC). NKC is prepared by soaking the dried neem fruits in water for 3–4 days and depulping using depulper machine. The seeds are then dried for 7 days before being decorticated using a winnowing machine and then crushed after further drying for 3 days (Bawa et al. 2007). NKC can also be prepared by spreading the neem fruit in the sun for 15 days and then soaking in water for 3 days followed by depulping. The depulped seeds are washed and sun-dried for a period of 10 days. The dry seeds are decorticated, further dried for 5 days and crushed followed by manual removal of oil to produce the neem kernel cake (Aruwayo 2011).

Another method of preparing NKC is by spreading the seeds in the sun to obtain a constant weight. The dried seeds are soaked in water in an open basin for 72 h. The seeds are placed into a jute bag to drain the water and later sun-dried to constant weight. Then, the water soaked and untreated seeds are milled separately for oil extraction. Cake obtained from the oil extraction is then ground in a hammer mill.

NKC is unpalatable in calves (Bedi et al. 1975), crossbred bulls (Ananthasubramainiam et al. 1979) and sheep (Gupta and Bhaid 1980) owing to its bitter taste and pungent odour but possess antiparasitic (Ogbuewu et al. 2011a), insect repellent, antifeedant, growth inhibitors and other insecticidal properties. The bioactive principles are slightly hydrophilic but freely lipophilic and highly soluble in organic solvents like hydrocarbon, alcohols, ketones and esters (Schmutterer 1995; NRC 1992). There are various methods of removing the oil from the neem seed cake. For the expeller NKC, the crushed kernel is steamed, and the oil is pressed out using expeller machine. The NKC processing by hydraulic press is devoid of heat. The milled kernel is cold-pressed using the hydraulic press machine until the oil content of the residue (cake) is minimal. The NKC can be further defatted using hexane.

To facilitate acceptance of NKC as animal feed, the removal of bioactive bitters from neem kernels is essential and can be made using simple techniques like

- *Water extraction*: This is the simplest technique to crush or grind the kernels and extract them with water. They may be steeped overnight in a cloth bag suspended in a barrel of water, or water can be poured into the bag, and extract can be collected as it emerges. By using water extraction, it has been estimated that 20–30 kg neem seeds can normally treat one hectare of land.
- *Hexane extraction*: Kernels are grated and steeped in the solvent hexane to extract only oil. The residue left after extraction with hexane still contains limonoid active ingredients, and subsequent water or alcohol extraction yields clean limonoids uncontaminated by oil.
- *Alcohol extraction*: It is the shortest process for generating neem-based pesticides in concentrated form. Limonoids are highly soluble in alcohol. The grated kernels are soaked in ethanol usually, but sometimes methanol is also used. It extracts the active ingredients to the range of 0.2–6.2%.

In addition to these techniques, further attempts can be made to remove the bitter principles of the cake for improved palatability. The oil from the crushed NKC can be extracted using organic solvent of high polarity mixed with water. Other methods include alcohol treatment and alkali treatment (0.8% sodium hydroxide, w/w) along with aqueous boiling (in the ratio 1:2.5w/v) of cake followed by water washing and draining off. Out of these several methods used to detoxify NKC, water washing is most effective despite the loss of 22% dry matter. To avoid this dry matter loss from water washing, alkali-treated (20 g sodium hydroxide/kg cake, w/w) NKC without washing is also quite palatable to adult cattle and buffaloes (Katiyar et al. 1993), promising future in the feeding of buffalo calves, Uda lambs and rams (Aruwayo 2011).

3 Phytoconstituents

Neem is a source of several bioactive triterpenoids including azadirachtin, nimbin, nimbinin, nimbidin, 6-desacetylnimbin, salannin and beta-sitosterol (Dasgupta et al. 2004). The most biologically active compound is azadirachtin, which is actually a mixture of seven isomeric compounds labelled as azadirachtin A–G, of which azadirachtin E is most effective (Verkerk and Wright 1993). Other compounds that have a biological activity are salannin, volatile oils, meliantriol and nimbin (NRC 1992).

Neem kernels contain 30–50% of oil rich in triterpene or limonoids mainly used by the soap, pesticide and pharmaceutical industries (Djenontin et al. 2012). The four best limonoids compounds are azadirachtin, salannin, meliantriol, and nimbin. Limonoids contain insecticidal and pesticidal activity. The seed also contains tignic acid responsible for the distinctive odour of the oil (Sharma et al. 2011).

The bark powder contains proteins, sugar, amino acids and oil (Subramanian and Lakshmanan 1996). Bark extract is also rich in phenols, unsaturated sterol, triterpene (including diterpenoids, limonoids, cseco-meliacins, cseco-limonoids, etc.) and saponin. Polysaccharides such as arabinofucoglucanes and fucogalactoglucoarabinanes have also been isolated (Fujiwara et al. 1984). Flavonoids, flavonolglycosides, dihydrochalcones, tannins and others are also important constituents of bark, leaves, fruits and flowers of neem.

4 Medicinal Uses

Indigenous literature indicates applications of various parts of neem as feed and in a large number of prescriptions and formulations to provide health cover to livestock in various forms. Pandava brothers Nakul and Sahadev used neem to treat sick and injured horses and elephants using poultices prepared from neem leaves and oil, during the battle of Mahabharata. Various neem preparations have been standardized in the form of powders, oils, liquids and liniments. Various preparations obtained from different parts of neem have been found to exert antibacterial, antiviral, antimalarial, antioxidant, antifungal, antimutagenic, anticarcinogenic, anti-inflammatory, contraceptive and antiulcer activities (Subapriya and Nagini 2005). Azadirachtin disrupts the metamorphosis of insect larvae and is thus used as a feeding deterrent (NRC 1992). Nimbidin is quite effective in the treatment of skin conditions such as eczema, furunculosis, arsenical dermatitis, scabies and seborrheic dermatitis (Dasgupta et al. 2004). Extracts from neem leaves, seeds, and bark also act as nitrification inhibitors (Abbasi et al. 2011).

4.1 Bacterial Diseases

Neem oil is effective against a wide spectrum of bacteria such as *Staphylococcus aureus*, *Bacillus cereus*, *Bacillus pumilus*, *Escherichia coli*, *Pasteurella vulgaris*, *Mycobacterium tuberculosis*, *Klebsiella pneumoniae*, *Salmonella typhi*, *Salmonella dysenteriae*, *Enterococcus faecalis*, *Streptococcus mutans*, *Streptococcus salivarius*, *Streptococcus mitis*, *Streptococcus sanguis* and even streptomycin-resistant strains (SaiRam et al. 2000; Prashant et al. 2007; Mehrotra et al. 2010; Sarmiento et al. 2011; Maragathavalli et al. 2012; Vinoth et al. 2012; Chava et al. 2012; Rosaline et al. 2013). It is a conventional precautionary measure against leptospiral epidemics in tropics, especially in waterlogged areas. It acts as an impermeable antibacterial film on skin coat that prevents the portal entry of bacteria. Moreover, when it is mixed with water, the neem oil, even in lower concentrations, becomes acidic and may turn leptospiricidal.

The bark extract is immunomodulatory. Extracts from bark, leaves, fruits, oil and root are used to control leprosy, intestinal ulcers and respiratory disorders (Ketkar and Ketkar 1995; Kartikar and Basu 1935). Anthraquinone fraction of leaf, dried flower and fruit is taken orally for leprosy. Tricyclic triterpenoids, margolone, margolonone and isomargolonone inhibit the growth of *Klebsiella*, *Staphylococcus* and *Serratia* species. Chloroform extracts of neem inhibit the growth of *Listeria monocytogenes*, while ethanolic extracts inhibit *Staphylococcus aureus* (Mahfuzul et al. 2007). Even extracts of neem cake, a waste by-product of oil extraction, can inhibit *Campylobacter jejuni* (Del Serrone and Nicoletti 2013).

Aquaneem, an emulsified product from neem kernels, inhibits pathogens of fish (*Aeromonas hydrophila*, *Pseudomonas fluorescens* and *Escherichia coli*) (Das et al. 1999). Neem leaf extract reduces bacterial load of *Streptococcus* sp., *Aeromonas hydrophila*, *Enterobacter* sp., *Escherichia coli*, *Pseudomonas* sp., *Proteus* sp., *Vibrio* sp. and *Yersinia enterocolitica* in marine decorative fishes (Dhayanithi et al. 2010). It inhibits the formation of biofilm in *Pseudomonas aeruginosa* (Harjai et al. 2013). Sulfonoquinovosyldiacylglyceride, a water-soluble glycolipid isolated from the leaves of neem, inhibits *Salmonella typhi*, *Shigella dysenteriae*, *Escherichia coli*, and *Vibrio cholerae* (Bharitkar et al. 2014).

4.2 Viral Diseases

An aqueous extract of tender leaves protects against vaccinia (viral disease in cattle), variola (smallpox), fowlpox and Newcastle diseases, while its paste is used for ulcerative lesions of cowpox. It inhibits poliovirus, herpes infectious virus, coxsackie B group virus and dengue virus at an early

step of viral genome replication (Badam et al. 1999; Parida et al. 2002; SaiRam et al. 2000). This is possibly by virus inactivation, in addition to interfering at an early event of its replication cycle. The bark extract also significantly blocks herpes simplex virus type 1 (HSV-1) access into cells at concentrations 50–100 μg/ml (Tiwari et al. 2010). Inhibition of HSV-1 glycoprotein-mediated cell fusion and polykaryocytes formation indicates an additional role of the extract at the viral fusion step, opening new prospectives for the development of bark extract as a novel anti-herpetic microbicide.

4.3 Fungal Diseases

Nimbidin inhibits the growth of *Tinea rubrum*, while gedunin can treat a range of fungal infections including *Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus niger*, *Candida albicans*, *Microsporum gypseum*, *Microsporum canis*, *Trichophyton rubrum*, *Trichophyton mentagrophytes*, *Fusarium oxysporum*, *Cladosporium* sp., *Penicillium notatum* and *Penicillium citrinum* (SaiRam et al. 2000; Asif 2012; Al-Samarrai et al. 2012). The cyclic trisulfides and tetrasulfides of steam distillate of mature leaves can be used against *Trichophyton mentagrophytes* (Pant et al. 1986).

4.4 Ulcer, Oxidative Stress and Inflammation

The aqueous extract from bark is an astringent tonic used for relieving fever, thirst, nausea, vomiting and skin diseases. It also blocks gastric ulcer due to stress, indomethacin and ethanol effectively. Increased lipid peroxidation, increased protein carbonyl content and decreased level of endogenous GSH are the characteristic features of oxidative damage of the gastric mucosa during ulceration (Bandyopadhyay et al. 2000) which are effectively reverted by the bark extract. The antisecretory and antiulcer activity is due to its phenolic glycoside and is superior to that of other natural antioxidants such as vitamin E and ascorbate and the physiological antioxidant, melatonin (Bandyopadhyay et al. 2000). Nimbidin suppresses basal as well as stimulated gastric acid release along with blockade of histamine H_2 receptors. It shows potent antioxidant activity by directly scavenging free radicals and associated DNA damage, which is observed in apoptotic cell death in gastric mucosal cell injury. It also protects the mucosa by preventing depletion of the mucus adhering to it (Wallace and Grangers 1996). Ethanol leaf extract also inhibits the proliferative phase of inflammation (Chattopadhyay 1998). Methanol extract of bark and leaves is antipyretic at a slightly higher dose. Its ether-soluble fraction is a good analgesic in acute inflammatory pain (Tandan et al. 1990). The bark extract also shows an antithrombotic effect in mice (Olajide 1999).

4.5 Cancer

Neem can prevent or reverse carcinogen-induced accumulation of reactive oxygen metabolites, which play a pivotal role in carcinogenesis (Androutsopoulos et al. 2009). A 5-day pretreatment with leaf extract decreased the formation of lipid peroxides and enhanced the levels of antioxidants and detoxifying enzymes in the stomach, the liver and circulation (Arivazhagan et al. 2000). Azadirachtin and nimbolide show concentration-dependent antiradical scavenging activity and reductive potential in the order: nimbolide > azadirachtin > ascorbate. Furthermore, azadirachtin and nimbolide inhibit the development of DMBA (7,12-dimethylbenz(a)anthracene)-induced hamster buccal pouch carcinomas through prevention of procarcinogen activation and oxidative DNA damage and upregulation of antioxidant and carcinogen detoxification enzymes (Priyadarsini et al. 2009).

The ethanolic extract of neem leaf inhibits the growth of cancerous cells in a dose- and time-dependent manner. It does not affect the viability of lymphocytes significantly indicating its selective cytotoxicity towards the cancer cells and, thus, providing a rationale for development of neem as a biosafe chemopreventive agent (Sharma et al. 2014). This antiproliferative action is associated with the downregulation of cyclin D1expression in cancer cells (Kumar et al. 2010; Priyadarsini et al. 2010; Gunadharini et al. 2011) and upregulation of the proapoptotic genes and proteins including p53, Bax, Bcl-2-associated death promoter protein (Bad) caspases, phosphatase and tensin homolog gene (pTEN) and c-Jun N-terminal kinase (JNK) (Arumugam et al. 2014). Ethanolic extract at EC_{50} doses causes a significant time-dependent increase in the Bax gene expression.

In murine sarcoma, neem leaf glycoprotein causes alteration in cytokine profile in the tumour microenvironment, i.e. from interleukin (IL)-10 and transforming growth factor (TGF)-β. IL-6-rich type 2 characters were switched to type 1 microenvironment with dominance of interferon (IFN) γ secretion (Barik et al. 2013). $CD8^+$ T cell population gets fairly increased with a higher expression of cytotoxicity-related molecules, perforin and granzyme B along with a low expression of $FasR^+$ cells symbolizing prevention from activation induced cell death.

4.6 Reproductive Health and Fertility

Contraceptive activity of neem extracts in male as well as female animals and human beings is well known. It reduces

the weight of the ovaries and uterus along with increase in the incidence of structural changes of metaphase chromosomes. A phytoconstituent of the extract probably interferes with DNA to yield chromosome strand breakage or produced spindle disturbances, inducing genotoxic effects. In a subchronic dose, the leaf powder causes a decrease in total sperm count and in sperm motility in rats with an increase in the relative percentage of abnormal sperm which could be reversed by simultaneous administration of testosterone, suggesting that the effects are due to an androgen deficiency, thereby affecting the physiological maturation of sperm (Aladakatti et al. 2001). Extracts of bark, flower and seed oil induce reversible infertility in male rats such as decrease in spermatid number. The alcohol extract of leaves reduces the sperm count and increases the frequency of spermatozoa with abnormal head morphology (Awasthy 2001).

The volatile fraction of steam-distilled neem oil is spermicidal. A minimum concentration of 0.25 and 25 mg/ml causes dose-dependent inhibition of spermatozoal motility in rat and human semen, respectively, which remains unaffected by the presence of vaginal or cervical mucus. A dose of 15 mg/kg neem extract delays reproduction in male rats up to 60 days (normal reproduction cycle is 20–23 days). At a dose of 25 mg/kg extract, 50% mortality was seen along with reversibly delayed reproduction (up to 3 months) in the remaining animals. Histological studies of testes indicated aberrations in spermatogenesis and sperm production in some of the seminiferous tubules. In vivo vaginal lubrication of neem oil prior to coitus is 100% effective in preventing pregnancy (Jacobson 1995). In rats, disruption of oestrous cycle leading to partial block in ovulation takes place after treatment with alcoholic extract of neem flower (Gbotolorunet al. 2008).

A highly safe and efficacious intravaginal contraceptive formulation has been developed from neem for rabbits and monkeys. Its minimum effective spermicidal concentration is 25% with complete immobilization of sperm within 20 s (Garg et al. 1994). In pregnant rats, the formulation caused complete resorption of the developing embryos on day 15 of pregnancy. The effects were reversible and animals regained fertility.

4.7 Miscellaneous Uses

Neem trees with rich water supply exude a sap which forms the stem tip. The sap is a coolant tonic and particularly useful in skin diseases, indigestion and general debility. The bark exudes a bright and amber-coloured gum which is a powerful stimulant, demulcent and tonic and is used in catarrhal and other infections. Hot water extract of the bark is an oral tonic and emmenagogue for adult female, while flower and leaf if taken orally work as an antihysteric remedy and externally to treat wound. The dried flowers are helpful orally in diabetes, while hot aqueous is used for piles, skin disease and ulcers. The fruit is a tonic, emollient, purgative and an anthelmintic. The dry fruit is mashed in water to treat skin diseases. Leaves are carminative and assist digestion. The tender leaves are used along with *Piper nigrum* Linn. to remove intestinal helminthiasis. The leaves are also effective in treating snake poisons and insect bites due to anti-clotting phytoconstituents. Hot water extract of the entire plant is an efficacious anthelmintic, an insecticide and purgative.

Neem induces radiosensitization radiotherapy (Veeraraghavan et al. 2011). The aqueous leaf preparation has been shown to prevent the cyclophosphamide-, cisplatin- and 5-fluorouracil-induced haematological complications (Ezz-Din et al. 2011). Sodium nimbidinate further produces diuresis facilitating the drug clearance. The aqueous leaf extract normalized gross appearance and histopathological changes of the liver in paracetamol-treated rats along with a reduction in serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and γ-glutamyl transpeptidase (Bhanwra et al. 2000). Subchronic administration of leaf extract is more effective than seed oil in controlling blood sugar levels in normal and diabetic rabbits (Khosla et al. 2000b).

Neem oil contains vitamin E and many other essential amino acids which help to restore moisture and elasticity to the skin. Neem can be used against midges, lice, mites and flies, for minor wounds, scar reduction and hair regrowth. On minor wounds, it promotes healthy, new skin development and reduces scar tissue. To make a rinse solution or spray, neem oil can be diluted by adding a small amount of a mild detergent along with water. A dilution of 1:20 is suggested for a fly spray. Neem oil can be rubbed directly onto the problem area, up to twice a day to soften and remove the scabs (without shampooing), to soothe and to kill the bacteria. The aqueous leaf extract of neem has acaricidal proficiency at par to ivermectin (Seddieket al. 2013), but the antinematodal activity varied with the season and composition (protein content) of diet offered better results in rainy season in comparison to dry season (Chagas et al. 2008).

In vitro both the oil and the aqueous extracts from seeds produce concentration-dependent sterilization and disturbed development of larval ticks, *Boophilus microplus*, *Hyalomma excavatum anatolicum* and *Amblyomma variegatum*. At a concentration of 10,000 ppm, azadirachtin and other limonoids disrupt reproduction of *Rhipicephalus microplus* females. At a dose of 1000 mg/kg body wt, neem extract checks parasitaemia levels, prevents weight loss and extends the lifespan of the host at par to suramin, a well-known trypanocidal drug. It has been suggested that neem leaf extract (125 mg/kg body wt) potentiates, synergizes and increases the half-life of diminazene diaceturate (7 mg/kg) and quickly removes *Trypanosoma brucei brucei* and prevents relapse (Omoja et al. 2011). The methanolic extracts of seed are superior prophylactically.

Azadirachtin, present in neem, is a primary as well as secondary antifeedant for Lepidoptera insects in the concentration of 1–50ppm. It also downregulates haemolymph ecdysteroid level in larval insects by blocking the release of prothoracicotropic hormone from brain-corpus cardiacum complex leading to disordered growth like disrupted moulting, growth inhibition and malformation leading to mortality.

5 Neem as Livestock Feed

5.1 Neem as Animal Feed

A major obstacle to ruminant livestock production in most tropical ecosystems is seasonal fluctuation in forage availability and quality due to the diverse rainfall patterns. For cultivated as well as natural pastures, forage biomass yields and quality decline drastically in the dry season. For example, low crude protein content (5–7%) has been reported for forage legumes during the dry season (Peters et al. 1997). Similarly, decline in crude protein and rise in neutral detergent fibre of leguminous forage crops occur with a change in season (Fujihara et al. 2004). Reproductive losses, together with reduced growth during the arid spell, adversely affect their productivity. This may be overcome by supplementation of ruminant diets, especially during the arid period with high crude protein constituents that can sustain production (Adjorlolo et al. 2016).

Exploitation of tree leaves for animal feeding is an antique practice. The crude protein of grasses becomes a major limiting factor in ruminant diet during the arid weather; tree leaves, with high crude protein content, has given some encouraging results in grazing ruminants (Ansah and Nabilla 2011). Leaves of leguminous plants are of particular significance owing to their higher crude protein content as compared to other vegetation (Adjorlolo et al. 2016). However, their utilization as fodder is also affected by their comparative digestibility, palatability and drought tolerance, which determines its forage biomass availability during the patchy season.

5.1.1 Leaves

Neem ranks high amongst fodder trees in India. A mature neem tree produces 3–4 quintals leaves per annum, which are fed to goats routinely and to cattle during famine. Neem leaves are acceptable to sheep (Chandrawathani et al. 2006) and goats (Seresinhe and Marapana 2011). In the Talensi-Nabdam District of Ghana, approximately one-fifth of farmers use neem leaves and fruits as fodder (Ansah and Nagbila 2011). Neem leaves high in crude protein ranging 17.5–18.7% have been reported (Bhowmik et al. 2008) as compared to 10–15% in leaves of nonleguminous fodder trees. They contain minerals and adequate amount of trace minerals except zinc and alleviate copper deficiency in animals that are stallfed on straw and dry fodder. Neem leaves incorporated into ruminant feeds can facilitate the utility of the plant and help alleviate the severe feed inadequacy experienced in the dryer tropics during the dry season. They have low crude fibre content (11.3%) (Bhowmik et al. 2008) along with 38.0% neutral detergent fibre (NDF) and 27.0% acid detergent fibre (ADF) levels (Ramana et al. 2000). These are quite low compared with NDF and ADF ranges of 27.40–55.23 and 18.87–46.30, respectively, for most of the tropical fodder trees (Kumar and Sharma 2003). Low fibre content of neem, coupled with the reported high nitrogen-free extract up to 53.9% (Bhowmik et al. 2008), makes it an important source of readily fermentable carbohydrates in ruminant feed. Goat and camel enjoy chopped neem leaves even as sole feed. A sole diet of neem leaves to goats increases the voluntary intake of feed up to 3.12% of body weight. Cattle are usually fed twigs and leaves mixed with other feeds.

Several anti-nutritional factors such as tannins, phenolic compounds and oxalates have been identified in neem leaves, but their concentrations are similar to that reported for other ligneous fodder species. The lignin content falls within the range of 4.2–11.7 as reported for Leucaena (Garcia et al. 1996). The bitterness in leaves is conferred by the presence of triterpenoids, mainly azadirachtin, but the concentration varies with season and ecotypes (Dhaliwal et al. 2004). However, ruminants, especially goats, can tolerate bitter taste owing to their ability to detoxify secondary plant compounds through allelochemical-type reactions that take place within them.

Neem leaves as feed supplement, to basal diets of crop residues, improve feed utilization and animal performance in ruminants. Replacement of 30% of mustard straw with neem leaves increases both dry matter and crude protein intakes with a concomitant increases in volatile fatty acid production (Raghuvansi et al. 2007), indicating that neem leaves supply critical nutrients needed to augment ruminal microbial growth and fermentation of feed. Neem leaves can replace up to half of soybean meal in ruminant diets with no negative effects on feed intake, dry matter and fibre digestibility as well as body weight gain (Paengkoum 2010). The improvement in performance of ruminants fed with neem leaves is partially attributable to the antiparasitic effects of the bioactive compounds in the leaves on intestinal parasites like *Haemonchus contortus* (Chandrawathani et al. 2006; Tiwary and Pandey 2010). In addition, hormone-mimicking action of neem extracts causes interference with the parasitic life cycle, thereby inhibiting their nutrition intake as well as the hatching of eggs (Kumar and Navaratnam 2013).

5.1.2 Seed

Several plants seeds have been tried as a protein source in animal feed (Gowda and Sastry 2000; Aruwayo et al. 2011; Ogbuewu et al. 2011a). The seeds can be used as seed meal, seed kernel cake or even as fruit cake. Neem seed is readily available in Northeast India and, perhaps, most parts of Asia and northern part of Nigeria, Australia, Africa and Central and South America, the home to the largest number of ruminants in the country. Neem seed cake, with an approximately 0.9 million tons availability in India (Singh 1993), is an excellent source of protein (30–40%) for livestock. It contains all essential and non-essential amino acids including sulphur-containing amino acids but with small amounts of valine and tryptophan. The sulphur content is 1.07–1.36% which is more than other cakes. The nitrogen content varies from 2 to 3%. Its balanced amino acid and mineral profile (Gowda and Sastry 2000) in respect to other plant seeds carry tremendous potential to compensate protein supplement shortages in the livestock industry. The only concern in this regard is its acceptability owing to its pungent smell and bitterness caused by the presence of bitter and toxic triterpenoids, mainly nimbin, nimbidin, azadirachtin and salannin which impart unpleasant taste or smell to meat (Clausen et al. 1985). Removal of these bitter phytoconstituents through solvent extraction, water washing, alkali soaking and urea ammoniation has achieved appreciable success in improving the livestock acceptability (Gowda and Sastry 2000).

The palatability of the neem seed cake can be improved by removing the bioactive principles or by feeding along with barley, molasses and peanut meal, but reduced consumption (from 79 to 39%) of concentrate mixture may result when neem seed cake levels are increased from 59 to 90% (Bhandari and Joshi 1974). Yearling sheep can completely utilize concentrate mixture having 75 parts of neem seed cake and 25parts of maize, but their feed consumption reduces to one-third if neem seed cake is fed alone (Gupta and Bhaid 1980). Buffalo calves accept readily 5–15 parts of neem seed cake when fed along with 7 parts of molasses and 20 parts of peanut meal, but the overall feed consumption reduces to half following the withdrawal of molasses. In addition, the keeping quality is good with a long shelf life.

5.1.3 Neem Oil

Debitterized neem oil is quite useful as animal feed. It is rich in long chain fatty acids and contains azadirachtin, meliantriol and salannin. Deoiled seeds find use as fertilizer in the agriculture sector. The palatability and utilization of such alternative feedstuffs can be further improved by incorporating them in complete diets and processing into pellets (Reddy and Reddy 1999).

5.2 Neem as Poultry Feed

Neem oil is used in poultry rations. The proximate composition of neem leaf meal indicates it as a good source of protein for poultry as well. Principally, unprocessed neem is not suitable for poultry feeding, because of its toxicity and the presence of bitter compounds that impair feed intake (Gowda and Sastry 2000; Uko and Kamalu 2008). Improvement in performance after supplementing neem leaves is also reported for poultry. Specific properties of these active compounds make it potentially interesting in veterinary medicine, e.g. against external parasites. Similarly, neem seeds or extracts could be used at low incorporation rates in feeds as replacement of antibiotics (Landy et al. 2011). The maximum tolerance levels of neem leaf meal as a protein source described in literature are 10% in starter broilers (Obikaonu et al. 2012*)* and 15% in laying birds (Esonu et al. 2006) and rabbits (Ogbuewu et al. 2010a, b, 2011b), as it contains several bioactive compounds (azadirachtin, nimbin, salannin, limonoids and tannin) that may affect nutrient utilization. The bioactive toxic principles may be reduced by sun-drying (Obikaonu et al. 2012*;* Esonu et al. 2006; Ogbuewu et al. 2010a, b, 2011a).

When used raw, neem seeds or oil meal decreases feed intake and broiler performance (Gowda and Sastry 2000). Soaking, cooking, alkaline or acid treatments and extraction with one or several solvents have been investigated (Gowda and Sastry 2000) to improve the overall acceptability. Some acceptable results at relatively low incorporation rates are also observed. Treatment with alkali or urea maintains reasonable performance at 13% incorporation (Nagalakshmi et al. 1996, 1999). Similarly, toasting or autoclaving alleviates the negative effects of neem (Uko and Kamalu 2008). Overall, neem is not recommended in broiler feeding. However, it could be used with caution at an incorporation rates below 5% with rigorous detoxification protocol.

Raw neem oil meal reduces feed intake and laying performance when used at 15 or 20% in layer diets, while at 10% performance remains unaffected (Gowda et al. 1998). Neem should be used with caution in layers, given the possible long-term effects on health. In male or female breeders, neem should be strictly avoided because of its potential effects on reproduction (Gowda and Sastry 2000). In growing quails, incorporation of 5–10% dehulled oil meal (solvent extracted) decreases slightly growth performance while inducing mild pathological effects (Elangovan et al. 2000b). In laying quails, performance was maintained with 5–10% oil meal in diet but with reduced feed efficiency (Elangovan et al. 2000a).

5.3 Neem as Fish Feed

Neem leaves supplemented diet is an immunostimulant for fish (Talpur and Ikhwanuddin 2013) due to hundreds of bioactive compounds, with proven anti-inflammatory, anti-arthritic, antipyretic, hypoglycaemic, antiulcer, antimicrobial and diuretic properties (Girish and Shankara 2008). It produces both cell-mediated and humoral responses during immunostimulation. There is an increased level of phagocytic activity, which is a module of non-specific immune system in fish (MacArthur and Fletcher 1995). The plant extract is antioxidant and forages superoxide anion which scavenges reactive radicals effectively to provide a possible protection against autotoxicity and fatality (Nya and Austin 2009; Kim et al. 2007).

5.4 Nutritional Quality of Neem Seed Cake

Neem seed cake (NSC) is a nonconventional feed ingredient with great potential for livestock feeding (Bawa et al. 2005). Chemical composition of NSC and neem seed meal (NSM) varies considerably depending on the method of processing. It is a rich protein source with 34–38% crude protein (CP) (Bawa et al. 2007) and 33.20% and 32.90% for alkali-treated neem seed cake (ATNSC) and NSC, respectively (Aruwayo 2011). It is balanced in Ca, P but exceptionally high iron content. Neem cake is a rich source of essential and non-essential amino acids including sulphur-containing ones, with little histidine, lysine and tyrosine (Gowda and Sastry 2000). The undecorticated NSC contains 6.5–11.6% digestible CP (Ananthasubramainiam et al. 1979). The raw neem seed meal has highest CP (23.19%), followed by solvent extracted NSC (23.06%), hydraulic press NSC (22.69%) and expeller NSC (22.5%)(Bawa et al. 2007), and the quality is comparable to that of peanut meal (PNM) (Gowda and Sastry 2000). Much higher CP is present in ATNSC (33.76%) and urea-treated neem seed cake (UANSC) (40.91%) (Katiyar et al. 1993). Therefore, ATNSC can be considered as a wholesome substitute for PNM in terms of performance.

Highest crude fibre content (40.50%) is seen in full fat NSM (FFNSM) (Salawu et al. 1994), while the lowest value is reported in NSC with 11.40% (Reddy et al. 1988). The ether extract (EE) is 0.38% in deoiled NSC (Garg 1989) and 27% in FFNSM (Salawu et al. 1994). The nitrogen-free extract was the lowest in FFNSM (Fajinmi et al. 1989) with 14% and highest in NSC (Bedi et al. 1975) with 52.52%. Amongst all, UANSC seems to be the best because of high crude protein of 40.91% and relatively low crude fibre of 11.43%.

Urea ammoniated neem seed meal (UANSM) has proved to be a satisfactory, economical and wholesome substitute for complete replacement of traditional protein supplement, deoiled groundnut cake (DGNC), in rations of growing goats (Anandan et al. 1996) on the basis of nutritive value, biochemical parameters and feeding economics. Dietary deviation did not significantly influence the efficiency of dry matter (DM) (8.7 vs. 9.1 g), protein (1.2 vs. 1.2 g CP) and metabolic energy (22.9 vs. 22.8 kcal ME) utilization per unit gain. The total body weight gain (kg), average daily gain (g), feed conversion efficiency (feed/gain) and feed cost (Rs.) per kg gain in the UANSM group were 5.6 ± 0.59, 31.0 ± 3.25, 9.1 ± 0.57 and 25.6 ± 1.62, respectively, as compared to the corresponding values as 6.0 ± 0.56, 32.8 ± 3.20, 8.7 ± 0.55 and 29.8 ± 1.83, respectively, in the DGNC group. The haematobiochemical profile (haemoglobin, glucose and urea nitrogen) and the activity of various enzymes (transaminases and alkaline phosphatase) showed insignificant variation, but the feed cost per kg gain of the UANSM diet is cheaper than the DGNC diet by 14.2%. The feeding cost per unit weight gain and overall average daily gain were slightly better in males compared to females.

6 Neem as Nutraceutical

The neem leaf accounts for a wide range of pharmacological activities, including antibacterial, anticarcinogenic, antifungal, antihyperglycaemic, anti-inflammatory, antimalarial, antimutagenic, antioxidant, antiulcer, antiviral and immunomodulatory (Subapriya and Nagini 2005), and in addition, chewing fresh leaves acts as a sedative and relaxant. Aqueous extract of neem leaves has noteworthy antiulcer activity and causes reduction in severity of gastric injury and prevents mast cell degranulation and mucus depletion. Neem leaves are quite prosperous in nutritious elements as compared to any other similar vegetation that has been subjected to chemical analysis earlier. Neem leaf chutney was a regular feature of Mahatma Gandhi's everyday diet. A nutraceutical neem tea would indisputably have been Gandhi's favourite drink. Neem can also be blended with green or black tea. The leaf extract has also been reported to be advantageous in the treatment of carbon tetrachloride-induced liver damage (Mujumdar et al. 1998).

In Ayurveda, neem is always mixed with other herbs to augment its efficacy and to turn the taste to a more favourable side. Herbs good for the pitta dosha such as licorice, honey, sugar, lemon juice and/or spices like cardamom can be used to amplify the efficacy or to reduce the side effects. The bitterness can also be counterbalanced with herbs and spices like cinnamon, orange peel, licorice root and fennel seed. Neem is indicated in contemporary Ayurveda for diabetes mellitus, perhaps, by enhancing the insulin receptor sensitivity. Oral administration of leaf extract significantly reduces insulin requirement for non-insulin-dependent diabetes

patients. Nimbidiol present in root and bark of neem tree can inhibit intestinal glucosidases thus helpful in control of diabetes (Mukherjee and Sengupta 2013). Fresh mature leaves, along with the seeds of *Psoralea corylifolia* and *Cicer arietinum*, are effective in leucoderma. Tender leaves, along with black pepper, are effective in intestinal helminthic infections (Kumar et al. 2016).

With a ban on four commonly used feed antibiotic growth promoter (monensin, salinomycin, avilamycin and flavophospholipol) by European Commission, neem leaf meal (NLM) can be harnesses as an ingredient in diet of broilers owing to its therapeutic and dietary importance (Bonsu et al. 2012), but the inclusion level varies with the age and physiological status. Neem has been shown to exert their antioxidant properties by decreasing tumour necrosis factor-α, increasing interferon-γ and modulating antioxidant enzymes such as glutathione S-transferase (GST) and certain hepatic cytochrome P450-dependent monooxygenases (Manikandan et al. 2008; Kusamran et al. 1998; Schumacher et al. 2011; Vasenwala et al. 2012). It induces apoptosis via both the intrinsic and extrinsic pathways and stimulates cell cycle arrest via p53-dependent p21 buildup and downregulation of the cell cycle regulatory proteins cyclin B, cyclin D1, p53 and propagating cell nuclear antigen (Kumar et al. 2010; Priyadarsini et al. 2010).

Neem, in combination with tulsi leaves extract, activates the cell-mediated immune response and, therefore, creates an enhanced response to any future challenges occurred by disease organisms. So, the feeding neem and tulsi leaves to immunosuppressed poultry birds increase their humoral and cell-mediated immune responses. Low dose of neem leaf extract has an inhibitory action on wide spectrum of microorganisms. Infusion (4%) of neem leaves at a concentration of 50 ml/l of fresh drinking water could be effectively used as a potential natural growth promoter and as immune stimulant contributing to better body weight gain, feed conversion ratio, gross return, lower mortality and higher antibody titre against infectious bursal disease (Durrani et al. 2008; Kumar et al. 2016).

6.1 Efficacy as Nutraceutical

Neem leaves can be eaten on a regular basis. Neem is a potent blood purifier and detoxifier in the Ayurveda. Neem leaves can treat symptoms coupled with viral infections like fever, common cold, herpes, influenza and chicken pox. They contain phospholipase A_2inhibitor which can be used as a snake venom inhibitor (Mukherjee et al. 2008).

Poultry birds supplemented with neem and tulsi leaf extract (1–3 ml/kg poultry ration) show a significant beneficial effect on body weight, weekly gain in weight, feed consumption and feed efficiency (Prasannabalaji et al. 2012). These effects may be due to their antimicrobial and antiprotozoal properties (Kale et al. 2003), which help in reduction of the microbial load of birds and resulted in better absorption of the nutrients present in the gut and finely leading to improvement in feed conversion ratio of the rations.

Fusarium is a filamentous fungus widely distributed in soil and produces mycotoxins such as trichothecenes and zearalenone in cereal crops used as animal and poultry feed and can affect human and animal health once they enter the food chain. Zearalenone triggers reproduction disorders including hyperestrogenic syndromes and tumour production, while trichothecenes are sesquiterpenoid epoxides that act as potent inhibitors of eukaryotic protein synthesis. Neem oil extract decreases zearalenone production at a 0.1–0.5% concentration, but maximum inhibition (59.05%) occurs at 0.1% (Geraldo et al. 2011).

Neem flowers contain compounds capable of inducing monofunctional phase II enzyme and repressing monooxygenases, especially those involved in the metabolic activation of chemical xenobiotics. Feeding diets containing 12.5% neem flowers for 2 weeks strongly enhance GST activity by almost 2.7-fold and a marked reduction in the levels of phase I reactions, thereby increasing the overall xenobiotic toxicity threshold (Kusamran et al. 1998).

Neem is being used profitably in aquaculture systems to control fish predators (Dunkel and Ricilards 1998). Martinez (2002) reported that aqueous extract of neem leaves and other neem-based products are effective alternatives for the control of fish parasites and fish fly predators such as dragonfly larvae in fish farms. Although neem extract is considered for low toxicity towards non-target aquatic life, water extracts of the bark of the neem plant caused respiratory problems in *Tilapia zillii* (Omoregie and Okpanachi 1997), while long exposure to low concentrations of the crude extract delayed the growth of this cichlid fish (Omoregie and Okpanachi 1992).

7 Safety

Neem has attracted worldwide distinction due to its vast range of medicinal properties like antibacterial, antiviral, antifungal, antiprotozoal, hepatoprotective and various other properties without showing any adverse effects (Kale et al. 2003). Its widespread conventional use confirms the safety. Over 75% Ayurvedic remedies contain neem, usually in the form of leaf (or extract), sometimes the bark/fruit/flowers, and almost never the oil. The majority of scientific neem studies have been conducted with neem leaf or leaf extracts. Neem leaves taken internally on a regular or daily basis are considered safe unless any physiological stress is indicated. In millennium indigenous use, no reports of negative side

effects from neem leaves have been acknowledged. Although neem has been found adequately safe for use as an insecticide, animal studies suggest that persistent ingestion of neem oil might generate toxic effects. However, comprehensive safety evaluation of the different formulations of neem has also not been accomplished (Kumar et al. 2016). Formal safety testing has been done only for neem oil, it being an important insecticide product. In addition, whole neem extract may produce genotypic damage on prolonged use or at higher doses (Badam et al. 1999; Awasthy 2001). For all these reasons, use of neem is not advocated in young, pregnant or nursing animals or animals with severe liver or kidney disease. In rats, administration of neem oil during the first few days of pregnancy is abortifacient, and the activity reduces with the advancement of gestation. At a dose of 6ml/kg body wt, even mortality may be seen up to 25% (Lal et al. 1987). Administration of oil increased tail flick reaction time and reduced induced writhing (Khosla et al. 2000a). In normal and hyperglycaemic rats, administration of oil causes a lowering of the blood glucose.

At the higher oral dose (100 mg/kg body wt for 20 days), neem leaf extract decreased serum triiodothyronine (T_3) and increased serum thyroxine (T_4) concentrations but produced no such changes at the lower oral dose (40 mg/kg body wt for 20 days). This indicates that high concentrations of neem extract can be inhibitory to thyroid function, particularly in the conversion of T_4 to T_3, the major source of T_3 generation (Panda and Kar 2000). A concomitant increase in hepatic lipid peroxidation and a decrease in glucose-6-phosphatase activity in the higher dose group also indicated the adverse effect of neem extract despite an augmentation in the activities of defensive enzymes, superoxide dismutase (SOD) and catalase. Thus, it appears that the neem extract in higher dose may show symptoms of thyroid function and lipid peroxidation.

8 Toxicity

The toxicological data of different neem-based preparations have been recently reviewed by Kumar et al. (2016). The daily oral administration of petroleum ether extract of neem whole seed (566 mg/kg body wt) and husk (360 mg/kg body wt) for a period of 60 days produced no alteration in haemoglobin, packed cell volume, leukocyte count and mean corpuscular haemoglobin and blood glucose, but AST and ALT were decreased. Serum protein, serum cholesterol, plasma total lipids and GST were increased, while plasma phospholipids and erythrocyte acetylcholinesterase were decreased (Gupta et al. 2001). Gandhi et al. (1988) reported dose- and time-dependent effects on motor activity, respiration and on the orientation within the cage after ingestion of the neem oil by rats and rabbits. The animals had diarrhoea, tremors and convulsions. The median lethal dose (LD_{50}) was 14 ml/kg body wt for rats and, showing similar symptoms, 24 ml/kg body wt for rabbits.

The tail flick reaction time increased while induced writhing reduced in rats after administration of leaf extract. Naloxone pretreatment partially reversed the effects. The effects of the leaf extract were more pronounced than those of the seed oil (Khosla et al. 2000a). The body weight of goats and guinea pigs was decreased upon addition of leaves to their drinking water. Both acute and chronic toxicities were evident through signs of weakness, loss of condition and depression. Decreases in heart, pulse and respiratory rates were observed, and diarrhoea, tremors and ataxia occurred in some animals. Total erythrocyte count, packed cell volume and haemoglobin decreased slightly, whereas the activities of AST, sorbitol dehydrogenase and the concentrations of cholesterol, urea, creatine and potassium increased. The liver and kidney were most affected (Ali 1987). However, treatment of rats with leaf extract resulted in decreases in total testosterone, total bilirubin and potassium in serum. There was increase in packed cell volume, mean corpuscular haemoglobin concentration, red blood cell, white blood cell and lymphocyte counts, but no cytotoxic effects were observed (Parshad et al. 1994).

The effects of aqueous extracts are ambiguous. Many of the studies do not report dose-effect relationships. Mostly positive effects are mentioned, even after administration of high doses, but toxic effects are observed at concentrations of 200 mg/kg body wt resulting in death of treated goats (Ali 1987). Effects on reproduction are only indirectly mentioned as a decrease in testosterone (Parshad et al. 1994). The most relevant no observed adverse effect level (NOAEL) is 30 mg/kg body wt/day at which there is no modulation of the immune responses (Ray et al. 1996).

The nonaqueous extracts are more repellent than the powders. Acute toxicity of acetone leaf extract caused a decrease in spontaneous activity, respiratory rate and body and limb tone in mice along with decreased responses to the environment, piloerection and a dose-dependent hypothermia (Singh et al. 1987). Two fractions of an acetone leaf extract showed central nervous system depressant activity in mice as evidenced by a reduction in locomotor activity. Both fractions caused reductions in blood pressure and heart rate in rats without showing diuretic activity. Acute toxicity of petroleum ether extract of leaves was evident on the motor activity, on orientation, a reduced reaction to pain and convulsions in mice with an oral LD_{50} of 22g/kg body wt (Koley et al. 1994).

Ethanol leaf extract induced dose-dependent mitotic chromosome abnormalities in bone marrow cells of mice. Gross type abnormalities appeared even at the lowest dose and remained unchanged in frequency at higher doses. The extract caused increase in the incidence of structural changes of metaphase chromosomes. A constituent of the extract

probably interferes with DNA to yield chromosome strand breakage or produced spindle disturbances, inducing genotoxic effects (Awasthy et al. 1999). Ethanol leaf extract in itself had no effect on peripheral utilization of glucose (Chattopadhyay 1996). At doses higher than 50 mg/kg body wt, the extract decreased the blood sugar level. The LD_{50} value in mice was 4.6 g/kg body wt (Chattopadhyay 1999). Ethanol leaf extract did not alter the hepatic glycogen content in normal rats, but in glucose fed rats or in combination with insulin, it reduced the hepatic glycogen content (Chattopadhyay et al. 1993). Examination in rodents previously treated with seed extracts revealed complete resorption of embryos on day 15 of pregnancy (Mukherjee et al. 1996). Hexane seed extract, in contrast to ethanol and water extracts, completely abrogated pregnancy. Restoration of fertility was observed in subsequent cycles, and no further toxic effects were found (Mukherjee et al. 1999).

9 Concluding Remarks and Future Directions

Neem is currently one of the world's most scientifically exploited trees. The domestic, commercial and industrial prospects of neem are unlimited and exciting. It can help the mankind to solve comprehensive health and ecological concerns. It has been widely used in Chinese, Ayurvedic and Unani medicines worldwide especially in Indian subcontinent in the treatment and prevention of various diseases through its role in the scavenging of free radical production and preclusion of initiation of disease pathogenesis. It is drought tolerant with rich forage even during the dry season. Today's reducing growth in animal feed resources is posing a serious concern for livestock health and productivity. In this scenario, neem leaves may be fed as a supplement during the dry season to increase feed intake as well as diet quality. Research on neem leaves as fodder should therefore be given adequate attention to find ways to utilize this abundant resource especially in the low rainfall areas of the subregion where dry season feeding remains a major challenge. Its kernel cake is also quite palatable to adult cattle and buffaloes even without washing, promising future in the feeding of buffalo calves, lambs and rams. For enhanced utilization, decortication of neem seeds can be done effectively at industrial level with maximized oil recovery. The processed proteinaceous kernel by-product carries the potential for a cheaper unconventional protein supplement. However, further research is needed for scientific validation of the multiple effects as well as to explore any other distinct therapeutic potential of the extracts to optimize their utilization.

References

Abbasi MK, Hina M, Tahir MM (2011) Effect of *Azadirachta indica* (neem), sodium thiosulphate and calcium chloride on changes in nitrogen transformations and inhibition of nitrification in soil incubated under laboratory conditions. Chemosphere 82:1629–1635

Adjorlolo LK, Timpong-Jones EC, Boadu S, Adogla-Bessa T (2016) Potential contribution of neem (*Azadirachta indica*) leaves to dry season feeding of ruminants in West Africa. Livestock Res Rural Develop 28:75. http://www.lrrd.org/lrrd28/5/adjo28075.htm. Retrieved 10 Jan 2019

Aladakatti RH, Ahamed RN, Ahmed M et al (2001) Sperm parameters changes induced by *Azadirachta indica* in albino rats. J Basic Clin Physiol Pharmacol 12:69–76

Ali BH (1987) Toxicity of *Azadirachta indica* leaves in goats and guinea pigs. Vet Hum Toxicol 29:16–19

Al-Samarrai G, Singh H, Syarhabil M (2012) Evaluating eco-friendly botanicals (natural plant extracts) as alternatives to synthetic fungicides. Ann Agric Environ Med 19:673–676

Anandan S, Sastry VRB, Musalia LM et al (1996) Growth rate and nutrient efficiency of growing goats fed urea ammoniated neem (Azadirachta indica) seed kernel meal as protein supplement. Small Rumin Res 22:205–212

Ananthasubramainiam CR, Menacherry M, Devasia PA (1979) Studies on the feeding value of neem (*Azadirachta indica*) seed cake for cattle. Kerala J Vet Sci 10:182–185

Androutsopoulos VP, Tsatsakis AM, Spandidos DA (2009) Cytochrome P450 CYP1A1: wider roles in cancer progression and prevention. BMC Cancer 9:187

Ansah T, Nagbila DA (2011) Utilization of local trees and shrubs for sustainable livestock production in the Talensi-Nabdam district of the upper east region of Ghana. Livest Res Rural Develop 23(4):75

Arivazhagan S, Balasenthil S, Nagini S (2000) Modulatory effects of garlic and neem leaf extracts on N-methyl-N$-nitro-N-nitrosoguanidine (MNNG)-induced oxidative stress in Wistar rats. Cell Biochem Funct 18:17–21

Arumugam A, Agullo P, Boopalan T et al (2014) Neem leaf extract inhibits mammary carcinogenesis by altering cell proliferation, apoptosis, and angiogenesis. Cancer Biol Ther 15(1):26–34

Aruwayo A (2011) Effect of evaluation of alkali treated neem kernel cake fed to Uda Sheep in a Semi-Arid zone of Nigeria. Unpublished Ph.D thesis, p 131

Aruwayo A, Maigandi SA, Malami BS et al (2011) Haematological and biochemical parameters of Uda lambs fed graded levels of alkali-treated neem kernel cake. Niger J Basic Appl Sci 19(2):277–284

Asif M (2012) Antimicrobial potential of *Azadirachta indica* against pathogenic bacteria and fungi. J Pharmacog Phytochem 1:78–83

Awasthy KS, Chaurasia OP, Sinha SP (1999) Prolonged murine genotoxic effects of crude extracted from neem. Phytother Res 13:81–83

Awasthy KS (2001) Genotoxicity of a crude leaf extract of neem in male germ cells of mice. Cytobios 106(2):151–164

Badam L, Joshi SP, Bedekar SS (1999) *In vitro* antiviral activity of neem (*Azadirachta indica*, A. Juss) leaf extract against group B *Coxsackie viruses*. J Commun Dis 31:79–90

Bandyopadhyay D, Biswas K, Bandyopadhyay U et al (2000) Melatonin protects against stress-induced gastric lesions by scavenging the hydroxyl radical. J Pineal Res 29:143–151

Barik S, Banerjee S, Sarkar M et al (2013) Normalization of tumor microenvironment by neem leaf glycoprotein potentiates effector T cell functions and therapeutically intervenes in the growth of mouse sarcoma. PLoS One 8(6):e66501

Bawa GS, Orunmuyi M, Onabanjo OA (2005) Effect of dietary inclusion levels of mechanically extracted neem seed cake on performance of young rabbits. Niger J Anim Prod 32:233–239

Bawa GS, Orunmuyi M, Agbaji AS et al (2007) Effect of different methods of processing neem (*Azadirachta indica*) seeds on performance of young rabbits. Pak J Nutr 6(3):213–216

Bedi SPS, Vijjan VK, Ranjhan SK (1975) Utilization of neem (*Azadirachtaindica*) seed cake and its influence on nutrients digestibility in buffaloes. Indian J Sci 28:104–107

Bhandari AS, Joshi MS (1974) The effect of feeding deoiled neem cake on health of sheep. Indian Vet J 51:659–660

Bhanwra S, Singh J, Khosla P (2000) Effect of *Azadirachtaindica* (NEEM) leaf aqueous extract on paracetamol-induced liver damage in rats. Indian J Physiol Pharmacol 44:64–69

Bharitkar YP, Bathini S, Ojha D et al (2014) Antibacterial and antiviral evaluation of sulfonoquinovosyldiacylglyceride: a glycolipid isolated from *Azadirachta indica* leaves. Lett Appl Microbiol 58:184–189

Bhowmik S, Chowdhury SD, Kabir MH et al (2008) Chemical composition of some medicinal plant products of indigenous origin. Bangladesh Vet 25(1):32–39

Bonsu FRK, Kagya-Agyemang JK, Kwenin WKJ et al (2012) Medicinal response of broiler chickens to diets containing neem (*Azadirachta indica*) leaf meal, haematology and meat sensory analysis. World Appl Sci J 19:800–805

Chagas ACS, Vieira LS, Freitas AR et al (2008) Anthelmintic efficacy of neem (*Azadirachta indica* A. Juss) and the homeopathic product Fator Vermes® in Morada Nova sheep. Vet Parasitol 151:68–73

Chandrawathani P, Chang KW, Nurulaini R et al (2006) Daily feeding of fresh Neem leaves (*Azadirachta indica*) for worm control in sheep. Trop Biomed 23(1):23–30

Chattopadhyay RR (1996) Possible mechanism of antihyperglycemic effect of *Azadirachta indica* leaf extract. Part IV. Gen Pharmacol 27:431–434

Chattopadhyay RR (1998) Possible biochemical mode of anti-inflammatory action of *Azadirachta indica* A. Juss. in rats. Indian J Exp Biol 36:418–420

Chattopadhyay RR (1999) A comparative evaluation of some blood sugar lowering agents of plant origin. J Ethnopharmacol 67:367–372

Chattopadhyay RR, Chattopadhyay RN, Maitra SK (1993) Possible mechanism of antihyperglycaemic effect of *Azadirachta indica* leaf extract. Part III. Fitoterapia 64:535–538

Chava VR, Manjunath SM, Rajanikanth AV et al (2012) The efficacy of neem extract on four microorganisms responsible for causing dental caries viz *Streptococcus mutans*, *Streptococcus salivarius*, *Streptococcus mitis* and *Streptococcus sanguis*: an *in vitro* study. J Contemp Dental Pract 13:769–772

Clausen S, Larsen LM, Ploger A et al (1985) Advances in the production and utilization of cruciferous crops. Junk Publications, The Netherlands

Das BK, Mukherjee SC, Sahu BB et al (1999) Neem (*Azadirachta indica*) extract as an antibacterial agent against fish pathogenic bacteria. Indian J Exp Biol 37:1097–1100

Dasgupta T, Banerjee S, Yadava PK et al (2004) Chemopreventive potential of *Azadirachta indica* (Neem) leaf extract in murine carcinogenesis model systems. J Ethnopharmacol 92:23–36

Del Serrone P, Nicoletti M (2013) Antimicrobial activity of a neem cake extract in a broth model meat system. Int J Environ Res Public Health 10:3282–3295

Dhaliwal GS, Arora R, Koul O (2004) Neem research in Asian continent: present status and future outlook. In: Koul O, Wahab S (eds) Neem: today and in the New Millennium. Kluwer Academic, New York

Dhayanithi NB, Ajith Kumar TT, Kathiresan K (2010) Effect of neem extract against the bacteria isolated from marine fish. J Environ Biol 31:409–412

Djenontin TS, Amusant N, Dangou J, KCD et al (2012) Screening of repellent, termiticidal and preventive activities on wood, of *Azadirachta indica* and Carapaprocera (Meliaceae) seeds oils. ISCA J Biol Sci 1(3):2529

Dunkel FV, Ricilards DC (1998) Effect of an Azadirachtin formulation on six non target aquatic macroinvertebrates. Environ Entomol 27:667–673

Durrani FR, Chand N, Jan M et al (2008) Immunomodulatory and growth promoting effects of neem leaves infusion in broiler chicks. Sarhad J Agric 24(4):655–660

Elangovan AV, Verma SVS, Sastry VRB et al (2000a) Laying performance of Japanese quail fed graded levels of neem (*Azadirachta indica*) kernel meal incorporated diets. Anim Feed Sci Technol 88 (1–2):113–120

Elangovan AV, Verma SVS, Sastry VRB et al (2000b) Effect of feeding neem (*Azadirachta indica*) kernel meal on growth, nutrient utilization and physiology of Japanese quails (*Coturnixcoturnix japonica*). Asian-Australas J Anim Sci 13(9):1272–1277

Esonu BO, Opara MN, Okoli IC et al (2006) Physiological responses of laying birds to neem (*Azadirachta indica A. juss*) leaf meal based diets, body weight, organs characteristics and haematology. Online J Health Sci 2:4

Ezz-Din D, Gabry MS, Farrag ARH et al (2011) Physiological and histological impact of *Azadirachtaindica* (neem) leaves extract in a rat model of cisplatin-induced hepato and nephrotoxicity. J Med Plants Res 5(23):5499–5506

Fajinmi AO, Adedeji SK, Hassan WA et al (1989) Inclusion of non-conventional feedstuffs in rabbit concentrate ration, a case of neem seeds. Appl Rabbit Res 13:125–128

Fujihara T, Abdulrazak SA, Ichinohe T et al (2004) Comparative rumen degradability of some legume forages between wet and dry season in West Sumatra, Indonesia. Asian-Australas J Anim Sci 17 (8):1107–1111

Fujiwara T, Sugishita EY, Takeda J et al (1984) Further studies on the structure of polysaccharides from the bark of Melia azadirachta. Chem Pharm Bull 32:1385–1391

Gandhi M, Lal R, Sankaranarayanan A et al (1988) Acute toxicity study of the oil from *Azadirachta indica* seed (neem oil). J Ethnopharmacol 23:39–51

Garcia GW, Ferguson TU, Neckles FA et al (1996) The nutritive value and forage productivity of Leucaenaleucocephala. Anim Feed Sci Technol 60(1-2):29–41

Garg AK (1989) Studies on deoiled neem (*Azadirachta indica*) seed cake as a cattle feed. Unpublished PhD thesis, IVRI, Izatnagar, India, pp 129–175

Garg S, Doncel G, Chabra S et al (1994) Synergistic spermicidal activity of neem seed extract, reetha saponins and quinine hydrochloride. Contraception 50:185–190

Gbotolorun SC, Osinubi AA, Noronha CC et al (2008) Antifertility potential of *neem* flower extract on adult female Sprague-Dawley rats. Afr Health Sci 8:168–173

Geraldo MR, Da Costa CL, Arrotéia CC, Kemmelmeier C (2011) The neem [*Azadirachta indica* A. juss (*meliaceae*)] oil reduction in the *in vitro* production of zearalenone by *Fusarium graminearum*. Braz J Microbiol 42:707–710

Girish K, Shanakara BS (2008) Neem—a green treasure. Electron J Biol 4(3):102–111

Gowda SK, Sastry VRB (2000) Neem (*Azadirachta indica*) seed cake in animal feeding-scope and limitations: review. Asian-Australas J Anim Sci 13(5):720–728

Gowda SK, Verma SVS, Elangovan AV et al (1998) Neem (*Azadirachta indica*) kernel meal in the diet of White Leghorn layers. Br Poult Sci 39(5):648–652

Gunadharini DN, Elumalai P, Arunkumar R et al (2011) Induction of apoptosis and inhibition of PI3K/Akt pathway in PC-3 and LNCaP prostate cancer cells by ethanolic neem leaf extract. J Ethnopharmacol 134(3):644–650

Gupta RS, Bhaid MV (1980) A study on the effect of feeding different levels of deoiled neem fruitcake in the concentrate mixture on water consumption in lambs. Rev Agro Anim Sci Health 121

Gupta S, Kataria M, Gupta PK et al (2001) Effect of petroleum ether extracts of different parts of neem seed (*Azadirachta indica*) on haematological and biochemical parameters in rats. Indian J Anim Res 35:21–26

Harjai K, Bala A, Gupta RK et al (2013) Leaf extract of *Azadirachta indica* (neem): a potential antibiofilm agent for *Pseudomonas aeruginosa*. Pathog Dis 69:62–65

Jacobson M (1995) Antifertility effects and population control. In: Schummtterer H (ed) The neem tree. VCH Publication, Hoshenheim, pp 526–530

Kale BP, KothekarMA THP et al (2003) Effect of aqueous extract of *Azadirachtaindica* leaves on hepatotoxicity induced by antitubercular drugs in rats. Indian J Pharmacol 35:177–180

Kartikar KR, Basu BD (1935) Azadirachta indica, vol 1 (2nd ed). Indian medicinal plants. Lalitha Mohan Basu, Allahabad

Katiyar RC, Sastry VRB, Agrawal DK (1993) Nutrient utilization from alkali treated neem seed kernel cake by cattle and buffalo. Indian J Anim Nutr 10(4):223–226

Ketkar AY, Ketkar CM (1995) Various uses of neem product: medicinal uses including pharmacology in Asia. In: Schmutterer H (ed) The neem tree. Weinheim, Federal Republic of Germany, pp 518–525

Khosla P, Sangeeta B, Singh J et al (2000a) Antinociceptive activity of *Azadirachta indica* (Neem) in rats. Indian J Pharmacol 32:372–374

Khosla P, Bhanwra S, Singh J et al (2000b) A study of hypoglycamic effects of *Azadirachta indica* (NEEM) in normal and alloxan diabetic rabbits. Indian J Physiol Pharmacol 44:69–74

Kim JK, Kim Y, Na KM et al (2007) (6)-Gingerol prevents UVB-induced ROS production and COX-2 expression *invitro* and *invivo*. Free Radic Res 41:603e14

Koley KM, Lal J, Tandan SK (1994) Anti-inflammatory activity of *Azadirachta indica* (neem) leaves. Fitoterapia 65:524–528

Kumar VS, Navaratnam V (2013) Neem (*Azadirachta indica*): prehistory to contemporary medicinal uses to humankind. Asian Pac J Trop Biomed 3:505–514

Kumar A, Sharma SD (2003) Nutritive evaluation of some fodder tree leaves for ruminants in Tarai region of Uttaranchal. Indian J Anim Nutr 20(2):161–167

Kumar GH, Vidya Priyadarsini R, Vinothini G et al (2010) The neem limonoids azadirachtin and nimbolide inhibit cell proliferation and induce apoptosis in an animal model of oral oncogenesis. Invest New Drugs 28:392–401

Kumar D, Rahal A, Malik JK (2016) Neem extract. In: Gupta RC (ed) Nutraceuticals: efficacy, safety and toxicity. Academic, Amsterdam, pp 585–597

Kusamran WR, Ratanavila A, Tepsuwan A (1998) Effects of neem flowers, Thai and Chinese bitter gourd fruits and sweet basil leaves on hepatic monooxygenases and glutathione S-transferase activities, and in vitro metabolic activation of chemical carcinogens in rats. Food Chem Toxicol 36(6):475–484

Lal R, Gandhi M, Sankaranarayanan A et al (1987) Antifertility effect of *Azadirachta indica* oil administered per os to female albino rats on selected days of pregnancy. Fitoterapia 58:239

Landy N, Ghalamkari G, Toghyani M (2011) Performance, carcass characteristics, and immunity in broiler chickens fed dietary neem (*Azadirachta indica*) as alternative for an antibiotic growth promoter. Livest Sci 142(1–3):305–309

MacArthur JI, Fletcher TC (1995) Phagocytosis in fish. In: Manning MJ, Tatner MF (eds) Fish immunology. Academic, London, pp 29–46

Mahfuzul HMD, Bari ML, Inatsu Y et al (2007) Antibacterial activity of guava (*Psidium guajava* L.) and neem (*Azadirachta indica* A. Juss.) extracts against foodborne pathogens and spoilage bacteria. Foodborne Pathog Dis 4:481–488

Manikandan P, Letchoumy PV, Gopalakrishnan M et al (2008) Evaluation of *Azadirachta indica* leaf fractions for in vitro antioxidant potential and in vivo modulation of biomarkers of chemoprevention in the hamster buccal pouch carcinogenesis model. Food Chem Toxicol 46(7):2332–2343

Maragathavalli S, Brindha S, Kaviyarasi NS et al (2012) Antimicrobial activity in leaf extract of neem (*Azadirachta indica* Linn.). Int J Sci Nat 3:110–113

Martinez SO (2002) NIM-Azadirachta indica: natureza, usosmúltiploseprodução. InstitutoAgronômico do Paraná (IAPAR), Londrina

Mehrotra S, Srivastava AK, Nandi SP (2010) Comparative antimicrobial activities of neem, amla, aloe, Assam tea and clove extracts against *Vibrio cholera*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. J Med Plants Res 4:2473–2478

Mujumdar AM, Upadhye AS, Pradhan AM (1998) Effect of *Azadirachta indica* leaf extract on CCl_4 induced hepatic damage in albino rats. Indian J Pharm Sci 60:363–367

Mukherjee A, Sengupta S (2013) Characterization of nimbidiol as a potent intestinal disaccharidase and glucoamylase inhibitor present in *Azadirachta indica* (neem) useful for the treatment of diabetes. J Enzyme Inhib Med Chem 28:900–910

Mukherjee S, Garg S, Pal R et al (1996) Effect of neem *Azadirachta indica* seed extracts given orally in on implantations in rodents. Indian J Pharmacol 28:49

Mukherjee S, Garg S, Talwar GP (1999) Early post implantation contraceptive effects of a purified fraction of neem (*Azadirachta indica*) seeds, given orally in rats: possible mechanisms involved. J Ethnopharmacol 67:287–296

Mukherjee AK, Doley R, Saikia D (2008) Isolation of a snake venom phospholipase A_2 (PLA_2) inhibitor (AIPLAI) from leaves of *Azadirachta indica* (neem): mechanism of PLA_2 inhibition by AIPLAI *in vitro* condition. Toxicon 51:1548–1553

Nagalakshmi D, Sastry VRB, Agrawal DK et al (1996) Performance of broiler chicks fed on alkali-treated neem (*Azadirachta indica*) kernel cake as a protein supplement. Br Poult Sci 37(4):809–818

Nagalakshmi D, Sastry VRB, Katiyar RC et al (1999) Performance of broiler chicks fed on diets containing urea ammoniated neem (*Azadirachta indica*) kernel cake. Br Poult Sci 40(1):77–83

NRC (National Research Council) (1992) Neem: a tree for solving global problems. National Academy Press, Washington, DC

Nya EJ, Austin B (2009) Use of garlic, *Allium sativum*, to control *Aeromonas hydrophila* infection in rainbow trout, *Oncorhynchus mykiss* (Walbaum). J Fish Dis 32:963–970

Obikaonu HO, Opara MN, Okoli IC et al (2012) Haematological and serum biochemical indices of starter broilers fed leaf meal of neem (*Azadirachta indica)*. J Agric Technol 8(1):71–79

Ogbuewu IP, Okoli IC, Iloeje MU (2010a) Evaluation of toxicological effects of leaf meal of an ethnomedicinal plant-neem on blood chemistry of puberal Chinchilla rabbit does. Rep Opin 2(2):29–34

Ogbuewu IP, Okoli IC, Iloeje MU (2010b) Evaluation of toxicological effects of leaf meal of an ethnomedicinal plant-neem on blood chemistry of puberal Chinchilla rabbit does. Rep Opin 2(2):54–57

Ogbuewu IP, Odoemenam VU, Obikaonu HO et al (2011a) The growing importance of neem (*Azadirachta indica* A. juss) in Agriculture, industry, medicine and environment: a review. Res J Med Plants 5 (3):230–245

Ogbuewu IP, Okoli IC, Iloeje MU (2011b) Evaluation of dried leaf meal of anethnomedicinal plant neem on linear growths and reproductive tract morphometry of rabbit does. Electron J Environ Agric Food Chem 10(4):*2153*–2159

Olajide OA (1999) Investigation of the effects of selected medicinal plants on experimental thrombosis. Phytother Res 13:231–232

Omoja VU, Anaga AO, Obidike IR et al (2011) The effects of combination of methanolic leaf extract of *Azadirachta indica* and diminazenediaceturate in the treatment of experimental *Trypanosoma brucei brucei* infection in rats. Asian Pac J Trop Med 4(5):337–341

Omoregie E, Okpanachi MA (1992) Growth of *Tilapiazilli* exposed to sublethal concentrations of crude extracts of *Azadirachtaindica*. Acta Hydrobiol 34:281–286

Omoregie E, Okpanachi MA (1997) Acute toxicity of water extracts of bark of the Neem plant, *Azadirachta indica* (Lodd) to the cichlid *Tilapia zillii* (Gervais). Acta Hydrobiol 39:47–51

Paengkoum P (2010) Effect of neem (*Azadirchta indica*) and leucaena (*Leucaenaleucocephala*) fodders on digestibility, rumen fermentation and nitrogen balance of goats fed corn silage. J Anim Vet Adv 9 (5):883–886

Panda S, Kar A (2000) How safe is neem extract with respect to thyroid function in male mice? Pharmacol Res 41:419–422

Pant N, Garg HS, Madhusudanan KP et al (1986) Sulforous compounds from *Azadiracta indica* leaves. Fitoterapia 57:302–304

Parida MM, Upadhyay C, Pandya G et al (2002) Inhibitory potential of neem (*Azadirachta indica* Juss) leaves on Dengue virus type-2- replication. J Ethnopharmacol 79:273–278

Parshad O, Singh P, Gardner M et al (1994) Effects of aqueous neem (*Azadirachta indica*) extract on testosterone and other blood constituents in male rats, a pilot study. West Indian Med J 43:71–74

Peters M, Tarawali SA, Alkamper J (1997) Dry season performance of four tropical pasture legumes in subhumid West Africa as influenced by superphosphate application and weed control. Trop Grassl 31:201–213

Prasannabalaji N, Muralitharan G, Sivanandan RN et al (2012) Antibacterial activities of some Indian traditional plant extracts. Asian Pacific J Trop Dis 2:S291–S295

Prashant GM, Chandu GN, Murulikrishna KS et al (2007) The effect of mango and neem extract on four organisms causing dental caries: *Streptococcus mutans*, *Streptococcus salivarius*, *Streptococcus mitis* and *Streptococcus sanguis*: an *in vitro* study. Indian J Dent Res 18:148–151

Priyadarsini RV, Manikandan P, Kumar GH et al (2009) The neem limonoids azadirachtin and nimbolide inhibit hamster cheek pouch carcinogenesis by modulating xenobiotic metabolizing enzymes, DNA damage, antioxidants, invasion and angiogenesis. Free Radic Res 43(5):492–504

Priyadarsini RV, Murugan RS, Sripriya P et al (2010) The neem limonoids azadirachtin and nimbolide induce cell cycle arrest and mitochondria-mediated apoptosis in human cervical cancer (HeLa) cells. Free Radic Res 44(6):624–634

Raghuvansi SKS, Prasad R, Mishra AS et al (2007) Effect of inclusion of tree leaves in feed on nutrient utilization and rumen fermentation in sheep. Biores Technol 98:511–517

Ramana DBV, Singh S, Solanki KR et al (2000) Nutritive evaluation of some nitrogen and nonnitrogen fixing multipurpose tree species. Anim Feed Sci Technol 88:103–111

Ray A, Banerjee BD, Sen P (1996) Modulation of humoral and cell-mediated immune responses by *Azadirachta indica* (neem) in mice. Indian J Exp Biol 34:698–701

Reddy GVN, Reddy MR (1999) Effect of feeding extruded complete diet containing maize cobs in Ongole bull calves. Indian J Anim Nutri 16:210–214

Reddy VR, Rao PV, Reddy CV (1988) Utilization of chemically treated neem oil in broiler in broiler chicks. Indian J Anim Sci 58:830–834

Rosaline H, Kandaswamy D, Gogulnath D et al (2013) Influence of various herbal irrigants as a final rinse on the adherence of *Enterococcus faecalis* by fluorescence confocal laser scanning microscope. J Conserv Dent 16:352–355

SaiRam M, Ilavazhagan G, Sharma SK et al (2000) Anti-microbial activity of a new vaginal contraceptive NIM-76 from neem oil (*Azadirachta indica*). J Ethnopharmacol 71:377–382

Salawu MB, Adedeji SK, Hassan WH (1994) Performance of broilers and rabbits given diets containing full fat neem (*Azadirachta indica*) seed meal. Anim Prod 58:285–289

Sarmiento WC, Maramba CC, Gonzales MLM (2011) An *in-vitro* study on the antibacterial effect of neem (*Azadirachta indica*) leaf extract on methicillin-sensitive and Methicillin-resistant *Staphylococcus aureus*. PIDSP J 12:40–45

Schmutterer H (1995) The neem tree source of unique national product for IPM, Medine, industry and other purposes. VCH Publication, New York

Schumacher M, Cerella C, Reuter S et al (2011) Anti-inflammatory, pro-apoptotic, and anti-proliferative effects of a methanolic neem (*Azadirachta indica*) leaf extract are mediated via modulation of the nuclear factor-κB pathway. Genes Nutr 6(2):149–160

Seddiek SA, Khater HF, El-Shorbagy MM et al (2013) The acaricidal efficacy of aqueous neem extract and ivermectin against *Sarcoptesscabiei* var. *Cuniculi* in experimentally infested rabbits. Parasitol Res 112:2319–2330

Seresinhe T, Marapana RAUJ (2011) Goat farming systems in the southern province of Sri Lanka: feeding and management strategies. World J Agri Sci 7(4):383–390

Sharma P, Tomar L, Bachwani M et al (2011) Review on neem (*Azadirachtaindica*): thousand problems one solution. Int Res J Pharmacy 2(12):97–102

Sharma C, Vas AJ, Goala P et al (2014) Ethanolic neem (*Azadirachta indica*) leaf extract prevents growth of MCF-7 and HeLa cells and potentiates the therapeutic index of cisplatin. J Oncol 2014:321754

Singh K (1993) Livestock production and health. In: Neem research and development, Publication No. 3, Soc Pesticide Sci India, pp 187–198

Singh PP, Junnarkar AY, Reddi GS, Singh KV (1987) *Azadirachta indica*: neuro-psychopharmacological antimicrobial studies. Fitoterapia 58:235–238

Subapriya R, Nagini S (2005) Medicinal properties of neem leaves: a review. Curr Med Chem Anticancer Agents 5:149–146

Subramanian MS, Lakshmanan KK (1996) *Azadirachta indica* A. Juss. Stem bark as an anti-leprosy source. In: Singh RP, Chari MS, Raheja AK, Kraus W (eds) Neem and environment, vol 2. Oxford & IBH Publishing, New Delhi, pp 1143–1150

Talpur AD, Ikhwanuddin M (2013) *Azadirachta indica* (neem) leaf dietary effects on the immunity response and disease resistance of Asian seabass, *Latescalcarifer* challenged with *Vibrio harveyi*. Fish Shellfish Immunol 34:254–264

Tandan SK, Chandra S, Gupta S et al (1990) Pharmacological effects of *Azadirachtaindica* leaves. Fitoterapia 61:75–78

Tiwari V, Darmani NA, Yue BY et al (2010) *In vitro* antiviral activity of neem (*Azardirachta indica* L.) bark extract against herpes simplex virus type-1 infection. Phytother Res 24:1132–1140

Tiwary MK, Pandey A (2010) Feeding neem (*Azadirachtaindica*) products to small ruminants as anthelmintics. Food Sci Tech Lett 1 (1):10

Uko OJ, Kamalu TN (2008) Trend of feed consumption and efficiency of broiler production with raw or heat-treated neem kernels. Arch Zootec 57(220):489–496

Vasenwala SM, Seth R, Haider N et al (2012) A study on antioxidant and apoptotic effect of *Azadirachta indica* (neem) in cases of cervical cancer. Arch Gynecol Obstet 286(5):1255–1259

Veeraraghavan J, Natarajan M, Lagisetty P et al (2011) Impact of curcumin, raspberry extract, and neem leaf extract on rel protein-regulated cell death/radiosensitization in pancreatic cancer cells. Pancreas 40(7):1107–1119

Verkerk RHJ, Wright DJ (1993) Biological activity of neem seed kernel extract and synthetic azadirachtin against larvae of *Plutellaxylostellal*. Pesticide Sci 37:83–91

Vinoth B, Manivasagaperumal R, Rajaravindran M (2012) Phytochemical analysis and antibacterial activity of *Azadirachta indica* A juss. Int J Res Plant Sci 2:50–55

Wallace JL, Grangers DN (1996) The cellular and molecular basis of gastric mucosal defense. FASEB J 10:731–740

Nutraceutical Potential of Ginger

Krishnamoorthy Srinivasan, Pratik Adhya, and Shyam Sunder Sharma

Abstract

In recent years, most people throughout the world have become health conscious, and recently countries have even been ranked on the basis of how health conscious their citizens are. According to the 2018 best countries ranking, Sweden is perceived to be the most health conscious country in the world. People throughout the world would like to live healthy lifestyle and do not want any disease that could should create distress in their life. In order to improve their health, people have started consuming food supplements or nutraceuticals which have many health benefits for the prevention of disease. Dietary fibers, probiotics, prebiotics, polyunsaturated fatty acids (PUFA), antioxidants, vitamins, polyphenols and spices are all being used as nutraceuticals. Ginger is one such spice used all over the world for culinary purposes and also for its health benefits in both animals and humans. The rich phytochemical composition of ginger makes it effective against a wide spectrum of chronic disorders. Phytoconstituents in ginger provide health benefits not only by modulating various intrinsic antioxidant systems of the body but also by acting as free radical scavengers themselves. The nutraceuticals properties of ginger include antioxidant, anti-inflammatory, gastroprotective, immunomodulatory, neuroprotective, cardioprotective to chemopreventive, and antidiabetic. In fact, the beneficial effects of ginger have propelled its study in various clinical trials in different countries to test its efficacy in various disease conditions. Even different formulations with a ginger extract or its constituents, like gingerol, are commercially available for improving the health of both humans and animals. In this chapter, we have reviewed evidence of the beneficial effects of ginger as a dietary supplement, together with scientific studies in animals and humans proving its benefits in the improvement of health.

Keywords

Veterinary nutraceuticals · Ginger

1 Introduction

Ginger (*Zingiber officinale*, Family: Zingiberaceae) is a tropical, flowering plant, and its roots or rhizome have been widely used as a spice worldwide for culinary purposes especially for its characteristic aroma and pungency (Kubra and Rao 2012). The generic name, *Zingiber*, is derived from the Greek word "zingiberis" which itself has come from the Sanskrit name of the spice "*singabera*". The Sanskrit name, singabera, means "shaped like a horn" because of its root's resemblance to a deer's antler (Sharma 2017). Specifically, ginger is widely regarded as a plant of potent medicinal value in many ancient systems of medicine including Chinese medicine, Tibb-Unani herbal medicine, and Ayurvedic medicine. In fact, ginger is depicted as *Mahaoushadha* (great medicine) in Indian traditional medicine because of its wide medical benefits. Traditional medicinal systems have used the rhizome of ginger in its fresh or dried form for the treatment of various disease conditions like nausea, vomiting, loss of appetite, stomach cramps, heartburn, flatulence, indigestion, common cold, influenza, cough, catarrh, nervous diseases, gingivitis, toothache, asthma, stroke, constipation, diabetes, arthritis, rheumatism, migraines, headaches, cardiac palpitations, hypertension, and impotence (Ali et al. 2008; Kubra and Rao 2012). It may also aid in preventing numerous ailments like coronary artery disease, ulcers, cancer, and various inflammatory disorders. The lifestyle of human beings has changed tremendously over the last few decades because of industrialization and change in work culture which has led to a culture of fast-food eating. These meals

K. Srinivasan · P. Adhya · S. S. Sharma (✉)
Department of Pharmacology and Toxicology, National Institute of Pharmaceutical Education and Research (NIPER), Mohali, Punjab, India
e-mail: sssharma@niper.ac.in

R. C. Gupta et al. (eds.), *Nutraceuticals in Veterinary Medicine*, https://doi.org/10.1007/978-3-030-04624-8_4

are instant and more tasty, but they have little nutritional value. This has led to increase in the incidence of various chronic and lifestyle diseases like diabetes, obesity, cancer, cardiovascular, and neurodegenerative diseases and immune dysfunction. Hence, there has been an increasing global interest for health-promoting food products, so-called nutraceuticals, by consumers all over the world. Nutraceutical is the new hybrid term between nutrient and pharmaceutical as coined by Dr. Stephen L. DeFelice in 1989 (Prabu et al. 2012). Numerous in vitro, in vivo experimental and clinical validation studies have substantiated the various pharmacological effects of ginger and its safety (Semwal et al. 2015; Srinivasan 2017). Certain bioactive molecules and active principles have also been identified as being responsible for its activity. Apart from its therapeutic indications, ginger and its phytochemical constituents can be used for multiple health-promoting benefits encompassing prevention and treatment of diseases in humans as well as in animals (Srinivasan 2017). This book chapter describes the evidence of the health-promoting benefits of dietary ginger on a pharmacological basis and its potential to be labeled a nutraceutical in the near future.

2 Phytochemical Composition of Ginger

The occurrence, cultivation, harvest, morphological characteristics, and chemical constituents of ginger have recently been described (Sharma 2017). The chemical constituents of ginger rhizomes are numerous, and the amount of such constituents depends on the place of origin and the condition of rhizomes (fresh or dry) (Ali et al. 2008; Kubra and Rao 2012). Broadly, the chemical constituents in ginger can be classified into four categories as listed in Table 1. It is reported that the rhizome contains 3–6% fatty oil, 9% protein, 60–70% carbohydrates, 3–8% crude fiber, about 8% ash, 9–12% water, and 2–3% volatile oil. The odor of ginger is mainly due to its volatile oil. To date, over 70 components of the oil have been reported and characterized, and these can be classified into two groups: monoterpenoids [β-phellandrene, (+)-camphene, cineole, geraniol, curcumene, citral, terpineol, borneol] and sesquiterpenoids [α-zingiberene (30–70%), β-sesquiphellandrene (15–20%), β-bisabolene (10–15%), (E-E)-α-farnesene, arcurcumene, zingiberol]. Among which zingiberol is principally responsible for the distinct aroma of the ginger rhizome (Ali et al. 2008; Baliga et al. 2011).

Table 1 Phytochemical constituents of ginger

Category	Components
Monoterpenoids	Geraniol, curcumene, β-phellandrene, (+)-camphene, 1,8-cineole, citral, terpineol, borneol, linalool, neral
Sesquiterpenoids	Zerumbone, α-zingiberene, β-sesquiphellandrene, β-bisabolene, (E, E)-α-farnesene, arcurcumene, zingiberol
Nonvolatile pungent components	Gingerols, shogaols, paradols, zingerone, dehydrozingerone
Miscellaneous	Zingibain

Ginger also has nonvolatile pungent components, including gingerols, shogaols, paradols, zingerone, and dehydrozingerone, which have potent biological activities as shown in Fig. 1.

Of these components, gingerols (1-(30-methoxy-40-hydroxyphenyl)-5-hydroxyalkan-3-ones) are the most important phytoconstituents and are responsible for its spicy flavor and various pharmacological properties. Among the gingerols, 6-gingerol ([5]-hydroxy-1-(4-hydroxy-3-methoxy phenyl) decan-3-one) is the most abundant (Baliga et al. 2012; Kubra and Rao 2012). Other nonvolatile more pungent components present in lower concentrations in ginger are the shogaols (phenylalkanones) which have wide pharmacological utility. Shogaols come from dehydration of gingerols, and their concentration increases during drying and storage (Baliga et al. 2012; Haniadka et al. 2013). Gingerols are thermally labile because of the presence of a β-hydroxy keto group and readily undergo dehydration to form the corresponding shogaols (Semwal et al. 2015). These shogaols can be further converted to paradols by hydrogenation. Other compounds, such as gingediols, gingediacetates, gingerdione, and gingerenones, are also reported to be present in the pungent fraction of ginger in varied concentrations. Some other constituents reported to be present in the ginger rhizome in minor quantities include zingibain (potent proteolytic enzyme), capsaicin, gingediol, galanolactone, gingesulfonic acid, galactosylglycerols, gingerglycolipids, diarylheptanoids, phytosterols, vitamins, and minerals (Baliga et al. 2011; Haniadka et al. 2013). Nutraceutical compounds which claim to have medicinal value include gingerols, shogaol, gingerdiols, eugenol, paradols, and zingerone. Of these, gingerols are thought to be the most pharmacologically active components (Mekuriya and Mekibib 2018).

3 Pharmacological Profile of Ginger

3.1 Potential Benefits in Gastrointestinal Disorders

Ginger has been widely used since ancient times to ameliorate certain gastrointestinal symptoms such as dyspepsia and gastrointestinal hemorrhage; it is mainly used in indigestion as it adsorbs and neutralizes certain toxins in the stomach and improves the production and secretion of bile from the liver and gall bladder. Bile aids in the digestion of fats which in turn helps to lower cholesterol levels. Ginger has been used

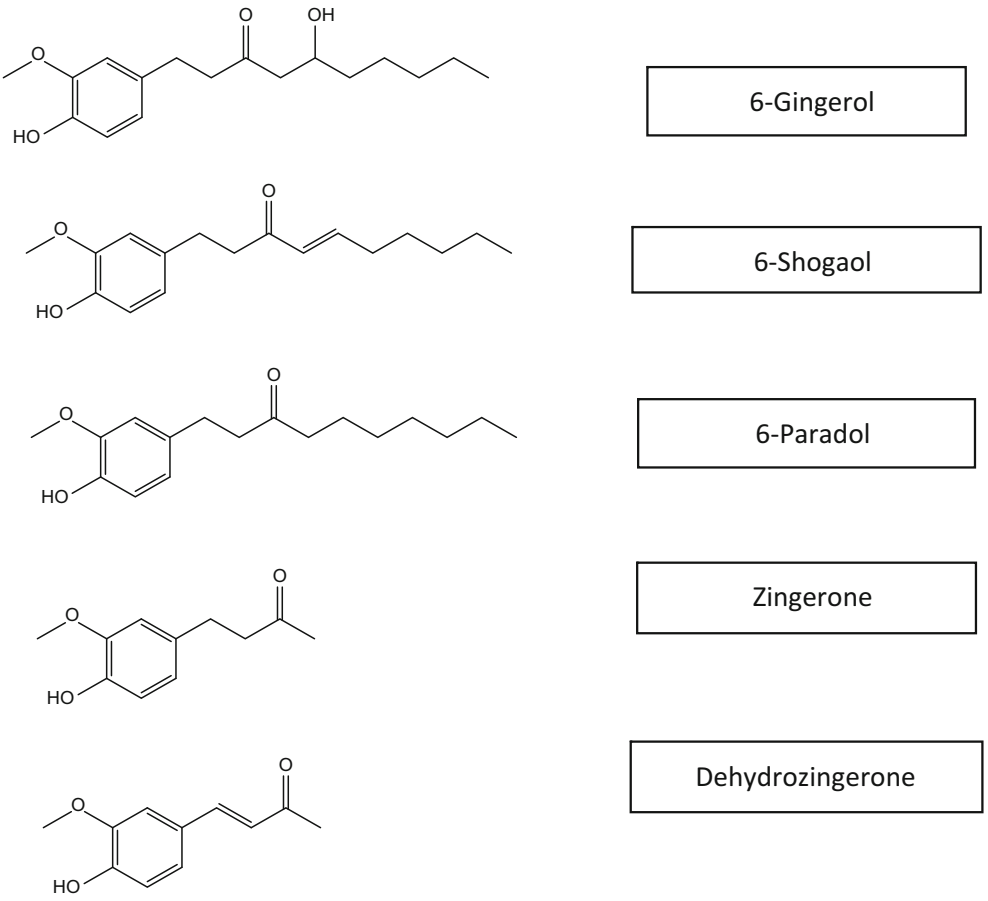


Fig 1 Phytochemical constituents of ginger with pharmacological properties

as carminative to enhance digestion and reduce gastrointestinal gas and flatulence (Qin and Xu 2008; Sharma 2017). Gastric complications like constipation, dyspepsia, belching, bloating, gastritis, epigastric discomfort, gastric ulcer, indigestion, nausea, and vomiting have been effectively mitigated with ginger extract in various preclinical and clinical studies (Haniadka et al. 2013; Keng-Liang et al. 2008). Ginger extract, [6]-gingerol, and zingerone have been shown to inhibit ACh-induced contractions of rat intestine and hypermotility-induced diarrhea, supporting its clinical application of ginger in gastrointestinal motility disorders (Chatturong et al. 2018; Iwami et al. 2011). Mechanistically, the effects of zingerone as a prokinetic agent have recently been attributed to inhibition of pacemaker potentials of interstitial cells of Cajal (ICCs) (responsible for slow waves in the gastrointestinal (GI) tract) via nitric oxide/cyclic guanosine monophosphate (NO/cGMP)-dependent adenosine triphosphate (ATP)-sensitive K^+ channels through mitogen-activated protein kinase (MAPK)-dependent pathways in vitro (Kim et al. 2018).

Ginger is also widely reported to possess powerful antiemetic properties in hyperemesis gravidarum, motion sickness, cancer chemotherapy, postoperative nausea, and vomiting and pregnancy-associated vomiting (Ali et al. 2008). Researchers also reported the beneficial effect of ginger extract in cisplatin (a chemotherapeutic agent)-induced emesis and delayed gastric emptying (Sharma et al. 1997). Notably, it was determined that the acetone alcoholic extract of ginger is effective rather than the aqueous extract (Sharma and Gupta 1998; Sharma et al. 1997). However, in clinical studies conducted in cancer patients receiving chemotherapeutic agents (cisplatin, doxorubicin), ginger powder extract showed no significant efficacy, which may be due to the use of a water extract in such patients (Arslan and Ozdemir 2015; Li et al. 2018a; Lua et al. 2015). Ginger extract and its essence is reported to be effective in pregnant women undergoing cesarean section (Zeraati et al. 2016), with postoperative nausea and vomiting associated with nephrectomies (Hosseini and Adib-Hajbaghery 2015) and with antiretroviral-induced nausea and vomiting (Dabaghzadeh et al. 2014) signifying a different mechanism for vomiting stimuli in different settings. Some clinical trials over efficacy of ginger on GI disorders have been and are being undertaken in different countries as shown in Table 2. The extracts of ginger have been reported to increase digestion efficiency by enhancing the activity of pancreatic lipases and amylases. Ginger also improves the overall health of the gastrointestinal tract via its gastroprotective actions and by

Table 2 Clinical trials on ginger (2011–2018)

Condition/ disease	Title of study	Sponsor	Study design	Outcome	References
Gastrointestinal disorders					
Nausea and vomiting	Efficacy of ginger on intraoperative and postoperative nausea and vomiting in elective cesarean section patients (2013)	New York Methodist Hospital, Brooklyn, NY 11215, USA	239 women were divided into 2 groups: dry powder ginger ($n = 116$) and placebo group ($n = 123$). Each were given 1 g capsule before induction of spinal anesthesia	Dry ginger powder group reported reduced number of episodes of intraoperative nausea but not postoperative nausea as compared to placebo group	Kalava et al. (2013) ClinicalTrials.gov Identifier: NCT01733212)
	The effect of ginger on the incidence of postoperative nausea and vomiting (2017)	The University of The West Indies, Kingston, Kng 7, Jamaica	110 female patients were divided into 2 groups : placebo and 0.5 g ginger powder, given 2 h before gynecological surgery	Results not published	ClinicalTrials.gov Identifier: NCT03626441
	Effect of ginger on nausea and vomiting during acute gastroenteritis in children (2016)	University of Naples Federico II, Italy, 80131	150 children, below the age of 10, were administered ginger extract in acute gastroenteritis	Results not published	ClinicalTrials.gov Identifier: NCT02701491
	Efficacy of ginger as an adjunctive prophylaxis for chemotherapy-induced nausea and vomiting (2015)	Mahidol University, Thailand	34 participants were divided into 2 groups: placebo capsule and ginger capsule (500 mg) taken orally twice a day during the first 5 days of chemotherapy cycle	Results not published	ClinicalTrials.gov Identifier: NCT02390648
	A study to assess the antiemetic efficacy of ginger in children and adolescents receiving chemotherapy (2009–2011)	All India Institute of Medical Sciences, New Delhi, India	32 children receiving highly emetogenic chemotherapeutic agents were administered ginger root powder capsule of different dose at 1 h, 3 h, and 8 h after chemotherapy. Their effect was compared to placebo capsule	The ginger root powder was reported to be effective in reducing the severity of acute and delayed chemotherapeutic-agent-induced nausea and vomiting, but it did not eliminate completely	Pillai et al. (2011) ClinicalTrials.gov Identifier: NCT00940368
	Efficacy study of ginger (*Zingiber officinale*) extract "Ginpax" to manage nausea in cancer patients receiving high emetogenic treatments and standard anti-emetogenic therapy (2013)	Istituto Nazionale dei Tumori, Milan, Italy	250 participants were randomized to receive 2 soft gel capsules of ginger extract per day during two cycles of cisplatin within 28 days	Results not published	ClinicalTrials.gov Identifier: NCT01887314
Abdominal distention	Efficacy of ginger in the prevention of abdominal distention in post-cesarean section patient (2016)	Chulalongkorn University, Thailand	178 participants were divided into 2 groups: placebo capsule and ginger capsule (500 mg) taken orally, 2 capsules after each of three meals for 3 days	Results not published	ClinicalTrials.gov Identifier: NCT02809027
Non-alcoholic fatty liver disease	The effects of ginger on nonalcoholic fatty liver disease (2014)	Shiraz University of Medical Sciences, Iran	90 adult patients were divided into 2 groups, viz., placebo and ginger group (500 mg capsule) and were administered this capsule for 3 months	Results not published	ClinicalTrials.gov Identifier: NCT02289235
	Effect of ginger supplement on nonalcoholic fatty liver (2015)	National Nutrition and Food Technology Institute, Iran	60 adult patients were randomly divided into 2 groups: placebo capsule and ginger capsule (500 mg) three times daily for 3 weeks	Results not published	ClinicalTrials.gov Identifier: NCT02535195

(continued)

Table 2 (continued)

Condition/ disease	Title of study	Sponsor	Study design	Outcome	References
CNS Disorders					
Migraine	Double-blind placebo-controlled randomized clinical trial of ginger (*Zingiber officinale* Rosc.) addition in migraine acute treatment (2018)	Federal University of Minas Gerais, Brazil	60 patients were given 400 mg ginger extract capsule, in addition to an intravenous drug (100 mg of ketoprofen), and result was compared to placebo-controlled group	The study showed that the addition of ginger extract to intravenous ketoprofen may contribute to the treatment of migraine attack	Martins et al. (2018) ClinicalTrials.gov Identifier: NCT02568644

Condition/ disease	Title of study	Sponsor	Study design	Outcome	References
CVS disorders					
Antiplatelet activity	The effect of drinking ginger daily on platelet function in the Saudi population (2013)	King Abdullah International Medical Research Center, Saudi Arabia	40 individuals were randomly divided into 2 groups and administered 4 g of ginger powder once or twice for 5 days	Results not published	ClinicalTrials.gov Identifier: NCT02882776
Cardiovascular disease markers	Acute effects of ginger extract consumption on risk markers of cardiovascular disease (2014)	University of Reading, UK	22 male volunteers were divided into 2 groups: placebo drink group and ginger drink group (300 mL) with breakfast on 2 visits separated by 2 weeks	Results not published	ClinicalTrials.gov Identifier: NCT02735486

Condition/ disease	Title of study (year)	Sponsor	Study design	Outcome	References
Inflammatory diseases					
Inflammation	The effects of ginger supplementation on inflammation in exercising individuals (2017)	Loma Linda, California, USA	12 individuals were divided in 2 groups: exercising group and non-exercising group. Both group received 3 g of ginger extract three times weekly, for 8 weeks	Results not published	ClinicalTrials.gov Identifier: NCT03219463

Disease	Title of study (year)	Location	Study design	Outcome of study	References
Cancer					
Colorectal cancer	Pilot clinical study of the effects of ginger root extract on eicosanoids in colonic mucosa of subjects at increased risk for colorectal cancer (2015)	University of Michigan Medical School, USA	21 patients who are at increased risk of colorectal cancer were administered 2 g of encapsulated ginger root extract daily for 28 days, and effect was compared with placebo	Ginger is reported to have chemopreventive effects and is both tolerable and safe	Zick et al. (2015) ClinicalTrials.gov Identifier: NCT01344538

enhancing the growth of beneficial bacteria in the intestines (Butt and Sultan 2011; Haniadka et al. 2013). Ginger exhibits anti-ulcerogenic properties in the animal models of gastric ulcers induced by ethanol, nonsteroidal anti-inflammatory drugs (NSAIDs), and hydrochloric acid (Liju et al. 2015; Salah Khalil 2015). In addition, it also inhibits the growth of *Helicobacter pylori*, a major ulcerogenic, and the associated inflammatory lesions, thereby protecting the gut (Gaus et al. 2009; Mahady et al. 2003). In addition, ginger volatile oil and gingerols were shown to be effective in different animal models of colitis or ulcerative colitis (Fig. 2) (Rashidian et al. 2014; Zhang et al. 2017).

Ginger not only improves functionality of the gastrointestinal tract, but it also gives a major boost to liver health as well

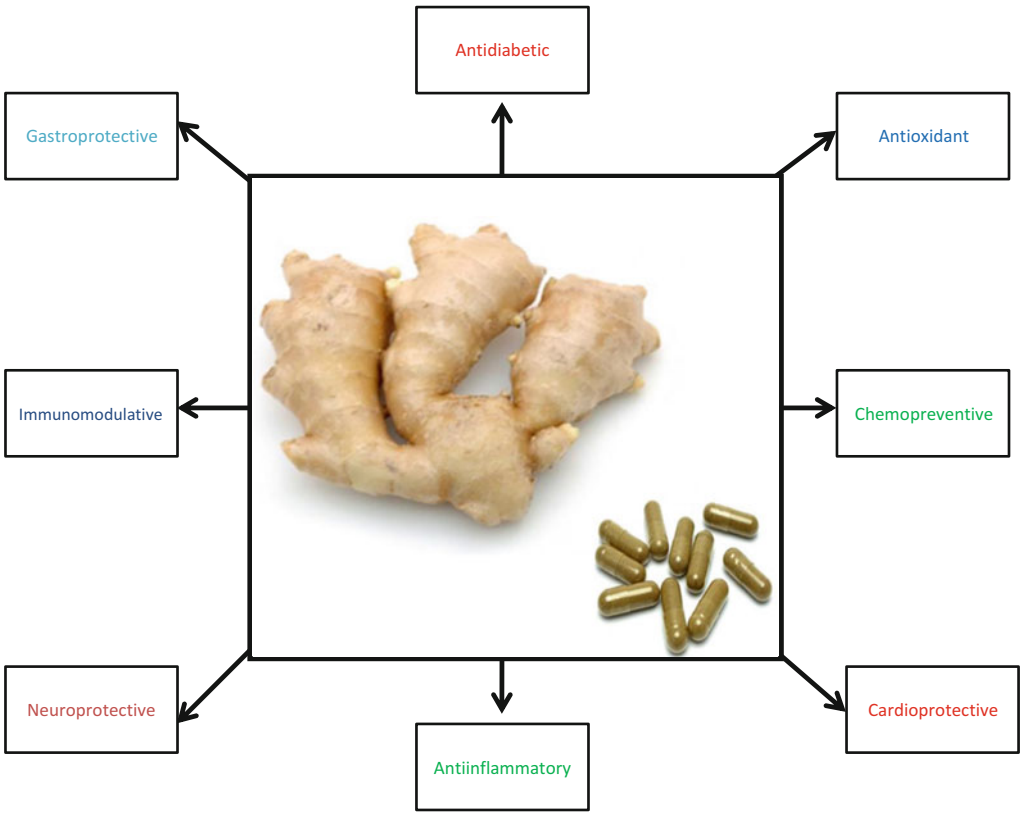


Fig. 2 Various pharmacological effects of ginger

(Abdulaziz Bardi et al. 2013; El-Sharaky et al. 2009; Emrani et al. 2016). It is reported to possess nutraceutical value in preventing hepatic fibrosis (Motawi et al. 2011), nonalcoholic fatty liver disease (Lai et al. 2016; Sahebkar 2011), and alcohol induced liver cirrhosis (Zhuang et al. 2015). It has also been reported to possess an anti-hepatotoxic property in various chemical induced-hepatotoxicity preclinical models (Abdulaziz Bardi et al. 2013; Baiomy and Mansour 2016; Sabina et al. 2011).

3.2 Potential Benefits as Antioxidant

Redox reactions are constantly going on inside each and every living tissue to provide energy. Consequently, such reactions are liberating free radicals like reactive oxygen species and (ROS) reactive nitrogen species (RNS). It is reported that increased generation of such free radicals gives rise to a condition known as oxidative stress, which is cytotoxic and mutagenic to the cells. In normal circumstances antioxidant enzymes of the cells like superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) help in maintaining normal oxidant homeostasis. During increased free radical generation and oxidative stress, however, such enzyme activity is reduced, requiring antioxidant supplementation through the diet (Ali et al. 2008; Chandel and Schieber 2014).

Various studies have reported the strong antioxidant properties of ginger extract and its phytochemical constituents (Ahmad et al. 2015; Mashhadi et al. 2013). Ginger exerts antioxidant effects against all oxidative stress induced by the different agents, ranging from chemical to biological agents and to radiation (Jeena et al. 2016; Saberi et al. 2017). For example, ginger has been reported to alleviate oxidative stress and organ damage induced by chemical agents like carbon tetrachloride (Ali et al. 2008), malathion, lindane (Butt and Sultan 2011), lambda cyhalothrin (Al-Amoudi 2018), hydrogen peroxide (Peng et al. 2015), chlorpyrifos (Abolaji et al. 2017), mercuric chloride (Al Hroob et al. 2018; Joshi et al. 2017), sodium arsenite (Chakraborty et al. 2012), sulfite (Afkhami Fathabad et al. 2018), lead acetate (Mohamed et al. 2016), carbendazim (Salihu et al. 2017), and aflatoxin B_1 (Vipin et al. 2017). It also has antioxidant properties against drug-induced toxicities like acetaminophen (Abdel-Azeem et al. 2013; Sabina et al. 2011), vancomycin (Kandemir et al. 2018), piroxicam (Badawi 2018), gentamicin (Hegazy et al. 2016), and cisplatin (Ali et al. 2008; Alibakhshi et al. 2018) in

various organs of experimental animals. Hyperglycemia (Al Hroob et al. 2018)-, ethanol (Akbari et al. 2017; Heshmati et al. 2018; Shirpoor et al. 2017, 2018)-, lipopolysaccharide (Li et al. 2012a)-, lipids (Si et al. 2018)-, interleukin-1β (Hosseinzadeh et al. 2017)-, and ischemia (Jittiwat and Wattanathorn, 2012; Li et al. 2017)-associated oxidative damages are also ameliorated by ginger extracts. Therefore ginger has antioxidant properties in several organs from the brain to the testes in experimental animals, signifying its nutraceutical value in combating oxidative stress induced by several agents. In fact, the oxidative stress-reducing property of ginger is comparable to that of chemical antioxidants and free radical scavengers like butylated hydroxyl toluene (BHT) and butylated hydroxyl anisole (BHA) (Kubra and Rao 2012). The antioxidant property of ginger extract and its phytoconstituents is attributed to the enhancement of antioxidant enzymes (SOD, catalase, GPx), free radical scavenging action, and reduction of lipid peroxidation (Butt and Sultan 2011; Kubra and Rao 2012; Zhuang et al. 2015). The other molecular mechanisms underlying the antioxidant properties of ginger include the activation of nuclear factor erythroid 2-related factor 2 (Nrf-2) and heme oxygenase-1 (HO-1) pathway (Peng et al. 2015; Zhu et al. 2016).

3.3 Potential Benefits as Anti-inflammatory and Immunomodulatory

Inflammatory disorders and related diseases like rheumatic conditions have been treated with ginger or ginger-derived formulations for ages worldwide because of its broad anti-inflammatory actions. Experimentally, ginger and its constituents exhibited anti-inflammatory effects in various models of acute and chronic inflammation (Banji et al. 2014; Mashhadi et al. 2013; Song et al. 2016; Xie et al. 2014). For instance, zerumbone is reported to inhibit endotoxin-induced lung inflammation in mice (Ho et al. 2017), while 6-shogaol reduces neuroinflammation associated with cognitive impairment (Moon et al. 2014) and Parkinson's disease by inhibiting the plasma level of tumor necrosis factor-α (TNF-α) (Park et al. 2013) (Luettig et al. 2016). Ginger suppresses prostaglandin synthesis through the inhibition of cyclooxygenase (COX)-1 and COX-2. It also suppresses leukotriene biosynthesis by inhibiting 5-lipoxygenase (5-LOX). This pharmacological property distinguishes ginger as a dual inhibitor of COX and 5-LOX from nonsteroidal anti-inflammatory drugs which may have a better therapeutic profile with fewer side effects. Ginger extracts have been shown to inhibit the induction of several genes encoding for cytokines, chemokines, and the inducible COX-2 involved in the inflammatory response (Grzanna et al. 2005). Immunomodulation refers to a process and a course of action in which an immune response is altered to a desired level. Herbs exhibit an array of diverse biological activity such as anti-stress, adaptogenic, antiaging, and immunomodulatory activity (Mahima et al. 2012). Ginger has an excellent immunomodulatory property in many experimental settings induced by infectious agents, allergies, drugs, inflammation, and cancer (Carrasco et al. 2009). It was indicated that the alcohol extract of *Z. officinale* also improved the immunological functions in the tumorous mice of immune inadequacy. In contrast, it was also reported that ginger inhibited lymphocyte proliferation and suppressed interleukins (IL-2 and IL-10) production in human lymphocytes (Wilasrusmee et al. 2002b). Moreover, ginger may inhibit both mitogen- and alloantigen-stimulated lymphocyte proliferations in mice (Wilasrusmee et al. 2002a). The effects of the volatile oil of ginger on the immune response were also evaluated in vitro and in vivo in mice, and the underlying mechanism of its anti-inflammatory activity was explored. Ginger extract reduces the expression of IL-17 and IL-23 and also modulates the expression of chemokines CCL20 and CCL22 and their receptors (CCR6 and CCR4) in the central nervous system (CNS) of mice with experimental autoimmune encephalomyelitis (Jafarzadeh et al. 2015, 2017b). [6]-Gingerol and [6]-shogaol, active ingredients of the traditional Japanese medicine hangeshashinto, relieve oral ulcerative mucositis-induced pain via action on Na^+ channels (Hitomi et al. 2017).

3.4 Potential Benefits in Diabetes

Diabetes mellitus (DM) is one of the major chronic metabolic disorders, and there are currently more than 420 million people living with diabetes (IDF 2017). Presently available allopathic medicines for diabetes are limited and further associated with adverse effects. The antidiabetic activity of ginger powder has been recently promoted, with the recommendation that it be included as one of the dietary supplements for diabetic patients (Bi et al. 2017; Medagama and Bandara 2014). It may be helpful in reducing the dosages of antidiabetic medications and associated side effects via its antidiabetic and antioxidant effects. Data from in vitro, in vivo, and clinical trials have demonstrated the antihyperglycemic effect of ginger. Preventive and protective properties of ginger in diabetes mellitus, diabetic complications, and associated lipid and other metabolic disorders were also reported (Li et al. 2012c). Multiple mechanisms have been proposed underlying the antidiabetic effects of ginger which are due to its insulinotropic, insulin-sensitizing actions including enhancing glucose uptake via cell surface glucose transporter 4 (GLUT4), and restoration of altered carbohydrate and lipid metabolism. It also includes the inhibition of several transcriptional pathways at the molecular level, lipid peroxidation, carbohydrate-metabolizing enzymes, increase in adenosine monophosphate kinase

(AMPK) phosphorylation and 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase, and the activation of antioxidant enzyme capacity and low-density lipoprotein receptors (Akash et al. 2015; Li et al. 2012b; Rani et al. 2012).

Aqueous ginger extract administered orally (daily) in three different doses (100, 300, 500 mg/kg body weight) for a period of 30 days improved carbohydrate metabolism in STZ-induced type 1 diabetic rats through its effects on the glycolytic enzyme activities (Abdulrazaq et al. 2012). Ginger exhibited inhibitory actions on gut α-glucosidase and α-amylase that help in ameliorating hyperglycemia (Rani et al. 2011, 2012). Administration of ginger extracts significantly improved hyperglycemia and hyperlipidemia, as well as the impaired kidney function and hemogram in alloxan-induced type 1 diabetic rabbits (Elkirdasy et al. 2015). Polyphenol extracts of ginger rhizome have exhibited amelioration of pancreatic and renal derangements in STZ-induced diabetic rats (Kazeem et al. 2015). Since oxidative stress is involved in the pathophysiology of diabetes, the effect of ginger on oxidative stress markers was investigated on STZ diabetic rats. Ginger significantly increased antioxidant effects as compared to glibenclamide (Ahmadi et al. 2013). In another study, the combination of gelam honey and ginger significantly ameliorated oxidative stress and metabolic profile in STZ type 1 diabetic rats (Sani et al. 2014).

[6]-Gingerol, a major constituent of ginger, has been reported to ameliorate hyperglycemia in Lepr (db/db) type 2 diabetic mice. Endocrine signaling involved in insulin secretion is perturbed in these diabetic mice. Four-week treatment of [6]-gingerol led to significant increase in glucose-stimulated insulin secretion and improved glucose tolerance involving glucagon-like peptide (GLP)-1-mediated insulin secretion pathway (Samad et al. 2017). 6-Paradol and [6]-gingerol supplementation significantly reduced plasma glucose, alanine aminotransferase, aspartate aminotransferase, advanced glycation end products (AGEs), and insulin levels in mice on high-fat diet (Sampath et al. 2017; Wei et al. 2017).

3.5 Potential Benefits in Diabetic Complications

Ginger has been reported to offer health benefits in certain micro- and macro-vascular diabetic complications related to the kidney, liver, peripheral nerve, and cardiovascular systems (Li et al. 2012c). Recent investigations have revealed that ginger alleviates hyperglycemia-induced oxidative stress, inflammation, and apoptosis and protects rats against STZ-induced diabetic nephropathy (Al Hroob et al. 2018). Ginger extract and zingerone were shown to exert nephroprotective effects by ameliorating various biochemical parameters and pathological injuries of the kidneys in different animal models of diabetic nephropathy (Ramudu et al. 2011; Rehman et al. 2018; Cui et al. 2018). Chronic treatment with 50 mg daily dose of ginger extract intragastrically for 6 weeks significantly reduced heart structural abnormalities in diabetic rats, and this suggested that these effects might be associated with improvements in serum apo, leptin, cathepsin G, and homocysteine levels (Ilkhanizadeh et al. 2016). Ginger hydroalcoholic extract and zerumbone, a phytochemical of subtropical ginger, alleviated diabetic retinopathy through reducing arginase I activity, blocking the AGEs/RAGE/NF-κB (nuclear factor- κB) pathway in the retina, and also through anti-inflammatory and antiangiogenic actions (Dongare et al. 2016; Lamuchi-Deli et al. 2017; Tzeng et al. 2016). Antiglycating potential of ginger and delay of diabetic cataract in rats have also been reported (Saraswat et al. 2010). Zingerone ameliorates enhanced vascular contraction in diabetic aorta which may be mediated by its vasodilator effect through NO- and guanylate cyclase stimulation (Ghareib et al. 2016). This is of significant importance for therapeutic interventions in complementary treatment/management of diabetes-related complications. Treatment with ginger ingredients like zingerone, geraniol, and 6-gingerol in doses of 20, 200, and 75 mg/kg, respectively, alleviate diabetic prostatic complications through suppressing oxidative stress and tissue fibrosis (Eid et al. 2017). The combination of ginger and cinnamon has significant beneficial effects on sperm viability and motility, serum total testosterone, luteinizing hormone (LH), follicle-stimulating hormone (FSH), and serum antioxidant levels in diabetic animals (Khaki et al. 2014). Treatment with ginger extracts caused alleviation of the testicular lesions in diabetic mice suggesting that the intake of ginger roots as a drink may be useful for diabetic patients who suffer from sexual impotency (Shalaby and Hamowieh 2010).

3.6 Clinical Studies

Some clinical studies have reported and substantiated the health benefits of ginger in diabetic patients. A randomized, double-blind, placebo-controlled trial demonstrated that daily consumption of three 1 gram capsules of ginger powder for 8 weeks is useful for patients with type 2 diabetes due to fasting blood sugar (FBS) and HbA1c reduction and improvement of insulin resistance indices such as QUICKI index (Mozaffari-Khosravi et al. 2014). Ginger improved insulin sensitivity and some fractions of the lipid profile and reduced CRP and PGE_2 in type 2 diabetic patients (Arablou et al. 2014). Ginger exhibited ameliorative effects on glucose control, insulin sensitivity, and lipid profile and proved to be a promising adjuvant therapy for T2DM and metabolic syndrome (Makhdoomi Arzati et al. 2017; Zhu et al. 2018). Three-month supplementation of ginger improved glycemic indices, total antioxidant capacity, malondialdehyde (MDA), C-reactive protein (CRP), and serum paraoxonase (PON-1)

activity in patients with T2DM (Shidfar et al. 2015). Daily administration of 1000 mg ginger reduces serum fasting glucose, which is a risk factor for hyperinsulinemia, dyslipidemia, peritoneal membrane fibrosis, and cardiovascular disease in patients on peritoneal dialysis (Imani et al. 2015). Patients with type 2 diabetes are prone to cardiovascular disease (CVD) due to inflammation process and oxidative stress. ADMA (asymmetric dimethylarginine) and ICAM-1 (intercellular adhesion molecule-1) play an important role in CVD pathogenesis. Ginger as an antioxidant and anti-inflammatory agent has been shown to have an effect on these biomarkers (Zarezadeh et al. 2018). Ginger supplementation improved insulin sensitivity and some fractions of lipid profile in T2DM patients. Therefore, it may be considered as a useful remedy to reduce the secondary complications of T2DM (Mahluji et al. 2013). Functional foods like ginger can be effective in the prevention of metabolic syndrome and subsequently the onset of cardiovascular diseases and T2DM as seen from human interventional trials (van den Driessche et al. 2018). The study also revealed the effect of ginger herbal spray on reducing xerostomia in patients with T2DM (Mardani et al. 2017).

3.6.1 Potential Benefits in Obesity

Obesity is a complex, multifactorial, and largely preventable disease, affecting over a third of the world's population today. If secular trends continue, by 2030, an estimated 38% of the world's adult population will be overweight and another 20% will be obese (Hruby and Hu 2016). Obesity, which describes the condition of an abnormal accumulation of body fat mass, is directly related to an increased risk of several chronic diseases, including glucose intolerance, cardiovascular disease hypertension, hyperlipidemia, hemostatic variables, and increased insulin resistance. Ginger provides anti-obesity effects by accelerating weight loss and metabolism. The beneficial effects of ginger on obesity and metabolic syndrome have been recently reviewed (Ebrahimzadeh Attari et al. 2018; Wang et al. 2017). Most preclinical studies have supported the weight-lowering effect of ginger extract or powder in obese animal models. Ginger may modulate obesity through various mechanisms including increasing thermogenesis, increasing lipolysis, suppression of lipogenesis, inhibition of intestinal fat absorption, and by controlling appetite (Ebrahimzadeh Attari et al. 2018). Rats fed a high-fat diet supplemented with 5% ginger powder exhibited a significantly greater ability to reduce body weight without inhibiting pancreatic lipase level, or affecting bilirubin concentration, with positive effect on increasing peroxisomal catalase level and HDL cholesterol as compared to orlistat supplementation (200 mg/kg diet) (Mahmoud and Elnour 2013). de Las Heras et al. (2017) recently investigated the molecular factors involved in the anti-obesity and hypolipidemic effects of a hydroethanolic ginger extract in HFD-fed rats. The hypolipidemic and insulin-sensitizing effects of ginger extract were associated with higher liver expression of transcription factors peroxisome proliferator-activated receptor (PPAR)α, PPARγ, GLUT-2, and collagen at molecular levels and the enhancement of plasma adiponectin levels (de Las Heras et al. 2017). The ameliorative potential of gingerol via modulating inflammatory factors and enzymes involved in cholesterol metabolism and targeting of the AMPK-NF-κB pathway through elevation in sirutin (SIRT)-6 and reduction in resistin levels has been demonstrated in HFD-induced obese rats (Brahma Naidu et al. 2016; Hashem et al. 2017). The plasma cholesterol-lowering activity of gingerol- and shogaol-enriched extract was also demonstrated in a hamster model which was mediated by enhancing the excretion of fecal cholesterol and bile acids via upregulation of hepatic CYP7A1 and downregulation of mRNA of intestinal Niemann-Pick C1-like 1 protein (NPC1L1), acyl CoA:cholesterol acyltransferase 2 (ACAT2), and microsomal triacylglycerol transport protein (MTP) (Lei et al. 2014). Gingerenone A, yet another polyphenol present in ginger, has been shown to mitigate obesity and adipose tissue inflammation in HFD-fed mice (Suk et al. 2017).

3.6.2 Potential Benefits in Cancer

Despite tremendous advancement in the last two decades in cancer research, cancer remains the second leading cause of death globally after heart ailments and has been responsible for an estimated 9.6 million deaths so far in 2018. Globally, about one in six deaths is due to cancer. Epidemiological studies report that Asians have a lower incidence of cancer than Western countries, which may be due to consumption of a diet rich in plants among the Asian population (Prasad and Tyagi 2015). A wide variety of phenolic compounds derived from spices possess potent antioxidant, anti-inflammatory, antimutagenic, and anticarcinogenic activities. The protective and therapeutic potential of ginger extract and various other constituents of ginger against different types of cancer have been reviewed and are shown in Table 3 (de Lima et al. 2018; Poltronieri et al. 2014; Prasad and Tyagi 2015; Shukla and Singh 2007).

The anticancer properties of ginger and its constituents have been reported to be associated with antioxidant, anti-inflammatory, and antimutagenic properties, as well as other biological activities as shown in Table 3. [6]-Gingerol is the major pungent principle of ginger, with numerous pharmacological properties. The proposed mode of molecular actions of gingerol include but is not limited to: (1) decrease in iNOS and TNF-α expression, (2) NF-kB nuclear translocation, (3) the release of cytochrome c, (4) caspases activation, and (5) increase in apoptotic protease-activating factor-1 (Apaf-1) as a mechanism of apoptosis induction. Further, 6-gingerol also stimulates apoptosis through upregulation of NSAID-activated gene-1 (NAG-1) and by modulating β-catenin, protein kinase C (PKCζ), and GSK-3β pathways (Lee et al.

Table 3 Anticancer property of ginger

Cancer type	Models	Compounds	Outcome	References
Colorectal cancer	HT-29 cell line	6-Shogaol	6-Shogaol chemo-preventive potential for colorectal cancer	Li and Chiang (2017)
	HCT-116, SW-480 cell lines	6-Shogaol	6-Shogaol induces death of colon cancer cells by inducing apoptosis and G2/M cell cycle arrest	Yogosawa et al. (2012)
	HCT116, SW480, and LoVo cell lines	Ginger leaf extract	Colon cancer chemopreventive properties	Park et al. (2014)
Gastric cancer	Human gastric adenocarcinoma cells (AGS)	6-Gingerol	6-Gingerol is reported to be effective in gastric cancer by inducing apoptosis	Mansingh et al. (2018)
	MKN1, MKN28, MKN45, MKN74, NUGC4, and AGS cell line	Zerumbone	Zerumbone is reported to be effective for the treatment of gastric cancer	Tsuboi et al. (2014)
Glioblastoma	U87 glioblastoma cells	Gingerols	Effective in preventing glioblastoma by inducing apoptosis	Lee et al. (2014)
Lung cancer	Urethane induced lung cancer in female mice and the Lewis lung carcinoma (LLC) cells	6-Gingerol	6-Gingerol prevents lung carcinogenesis by acting as an arginase inhibitor	Yao et al. (2018)
Non-small cell lung cancer (NSCLC)	A549 cell line	6-Shogaol	6-Shogaol is found to be effective by modulating the activity of mPGES-1 enzyme	Eren and Betul (2016)
	A549 cell line	Zerumbone	Zerumbone prevents metastasis of NSCLC	Kang et al. (2016)
	NCI-H1650 cells and nude mice	6-Shogaol	6-Shogaol is reported to prevent NSCLC	Kim et al. (2014)
Prostate cancer	HRPC cell lines, PC-3, and DU-145	Zerumbone	Zerumbone inhibits hormone refractory prostate cancer cell, by inducing apoptosis and autophagy	Chan et al. (2015)
Liver cancer	SNU182 cell line	Zingerone	Zingerone prevents metastasis of hepatocellular carcinoma cell line	Kim et al. (2017)
	SMMC-7721 cells in vitro and in vivo in xenograft mice model	6-Shogaol	6-Shogaol is effective in human hepatocellular carcinoma cells by inducing ER stress and apoptosis	Hu et al. (2012)
	Human hepatoma Hep3B cells	6-Shogaol and 6-gingerol	6-Shogaol and 6-gingerol is reported to prevent cancer by antiangiogenic mechanism	Weng et al. (2010)
Breast cancer	MDA-MB-231 and MDA-MB-468 cells	10-Gingerol	10-Gingerol is reported to be effective in triple negative breast cancer by causing cell-cycle arrest	Bernard et al. (2017)
	MCF-7 and MDA-MB-231 cell lines	6-Shogaol	It inhibits breast cancer cells by activating Notch pathway	Ray et al. (2015)
Leukemia	Human leukemia cells in vitro (U 937 cells) and in vivo (U937 xenograft mice model)	6-Shogaol	It is reported to be effective in hematologic malignancies	Liu et al. (2013b)
Renal carcinoma	Caki cells	6-Shogaol	6-Shoagol has been reported to possess anticancer activity against renal cancer by promoting apoptosis	Han et al. (2015)
Pancreatic cancer	Panc-1, AsPC-1, BxPC-3, CAPAN-2, CFPAC-1, MIAPaCa-2 and SW1990, and mouse pancreatic cancer cells, Panc02	Ginger extract	Effective in pancreatic cancer through oxidative stress-induced apoptotic cell death	Akimoto et al. (2015)
	BxPC-3 and MIA PaCa-2 cell lines	Zerumbone	It is reported to exhibit anticancer activity by inhibiting angiogenesis	Shamoto et al. (2014)
	PANC-1 and BxPC-3 cell lines	6-Shogaol	It acts as an important adjuvant by inhibiting the growth of human	Zhou et al. (2014)

(continued)

Table 3 (continued)

Cancer type	Models	Compounds	Outcome	References
			pancreatic tumors and chemo-sensitize them to gemcitabine treatment	
Cervical cancer	HeLa, CaSki, and SiHa cells	6-Gingerol	It is shown to inhibit proliferation of the HPV positive cervical cancer cells through proteasome activation	Rastogi et al. (2015)
	HeLa cell line	Essential oils of ginger	Along with mitomycin C has reported enhanced cytotoxicity and apoptosis in cervical cancer	Al-Otaibi et al. (2018)
Oral cancer	Oral squamous cell carcinoma (OSCC) cell lines	Zerumbone	Effective in OSCC by inhibiting cell proliferation, migration, and invasion	Zainal et al. (2018)
	Golden Syrian hamsters age 8–10 weeks. Cancer has been induced by paint with 7, 12-dimethylbenz[a] anthracene (0.5%) in liquid paraffin using a No. 4 sable brush, three times per week for 16 weeks on right buccal pouches of hamsters	6-Shogaol	Ameliorated DMBA-induced inflammation and cell proliferation-mediated tumorigenesis	Annamalai et al. (2016)
Skin cancer	B16F10 mouse melanoma cells	6-Shogaol	Inhibited melanogenesis in melanoma cells by ERK activation	Yao et al. (2013)

2008). Recently, the new gingerol derivatives were evaluated for their cytotoxic activities against human cancer cells (Li et al. 2018b). Taken together, the chemopreventive potentials of [6]-gingerol present a promising future alternative to therapeutic agents that are expensive, toxic, and possibly even carcinogenic (Oyagbemi et al. 2010). Recently, [6]-gingerol aspirinate as a novel chemopreventive prodrug of aspirin for colon cancer was investigated in vitro and in vivo that showed enhanced anticancer properties along with gastroprotective effects (Zhu et al. 2017). The use of nanotechnology for drug delivery has shown great promise for improving cancer treatment. However, potential toxicity, hazardous environmental effects, issues with large-scale production, and potential excessive costs are challenges that confront their further clinical applications. Edible ginger-derived nanoparticles have been reported as a novel therapeutic approach for the prevention and treatment of inflammatory bowel disease and colitis-associated cancer. Further, a nanovector made from ginger-derived lipids as a delivery platform for the therapeutic agent doxorubicin to treat colon cancer has also been reported (Zhang et al. 2016a, b).

3.7 Potential Benefits in CNS Disorders

Ginger extract is also reported to be effective in various CNS disorders like age-related neurodegenerative disorders (Alzheimer's disease, Parkinson's disease) (Choi et al. 2018), multiple sclerosis (Sapkota et al. 2018), experimental autoimmune encephalomyelitis (Jafarzadeh et al. 2015, 2017a), cognitive dysfunction (Wattanthorn and Sutalangka 2017), neuroinflammation (Ho et al. 2013), and migraine (Choi et al. 2018; Maghbooli Mehdi et al. 2013), as well as diabetes-induced CNS changes (Shanmugam et al. 2011). It has been experimentally demonstrated that ginger and its constituents, such as 6-gingerol, 6-shogaol, 6-paradol, zingerone, and dehydrozingerone, are effective for ameliorating the neurological symptoms and pathological conditions of age-related neurodegenerative disorders through modulating cell death or cell survival signaling molecules (Choi et al. 2018). [6]-shogaol has significant protective effects in various neuronal and astrocyte cell culture models via induction of heat shock protein 70 (HSP70), histone deacetylase (HDAC) inhibition (Shim et al. 2011) and upregulation of neurotropic factors (Kim and Kwon 2013) in vitro. Apart from its anti-inflammatory and antioxidant actions, 6-shogaol is also shown to have anti-amyloidogenic activity. This activity of 6-shagaol ameliorates Alzheimer's disease via cysteinyl leukotriene 1 receptor (CysLT1R)-mediated inhibition of cathepsin B and activation of sortilin-related receptor 1, resulting in enhanced neuronal cell survival through the inhibition of Aβ production (Na et al. 2016a, 2017).

6-shogaol has been shown to attenuate brain damage in ischemic stroke after middle cerebral artery occlusion in mice (Na et al. 2016b). Delayed administration of zingerone mitigates the behavioral and histological alteration via repression of oxidative stress and intrinsic programmed cell death in focal transient ischemic rats (Vaibhav et al. 2013). Ginger extract and its contituents have displayed neuroprotective properties and improved cognitive functions in different preclinical models of cognitive impairment (Lim et al. 2014;

Moon et al. 2014). Recently, ginger fermented with *Schizosaccharomyces pombe* alleviates memory impairment via protection of the hippocampal neuronal cells in amyloid β_{1-42} plaque injected mice (Huh et al. 2018). Ginger extract also has an anti-addictive property against morphine in preclinical settings. In migraine, the efficacy profile of ginger extract is comparable to that of the standard prescribed drug sumatriptan, although it had less side effects that sumatriptan in 100 Iranian patients. However, the chemical constituent responsible for its anti-neuroinflammatory property is still debatable. Some researchers attribute it to 6-shogaol (Sapkota et al. 2018; Ha et al. 2012), but others attribute it to 10-gingerol (Ho et al. 2013). Thus, ginger extract has the potential to be used for various CNS disorders; however, much research still needs to be undertaken before it can be used.

3.8 Potential Benefits in Cardiovascular Disorders

The beneficial effect of ginger as a nutraceutical is also reported in cardiovascular complications. Cardiovascular disease is reported to be the leading cause of death in the world (Aimin Shi et al. 2016). Ginger extract is reported to halt and treat such events together with that of lifestyle and dietary modifications. The aqueous extract of ginger has an antihypertensive effect (Akinyemi et al. 2014; Liu et al. 2013a). Ginger also has antiplatelet (Lee et al. 2017; Nicoll and Henein 2009), antithrombotic (Nicoll and Henein 2009), and lipid-lowering properties in dyslipidemic patients (El-Seweidy et al. 2015; Khosravani et al. 2016; Pourmasoumi et al. 2018). Ginger confers significant protection from stroke and heart attack due to its ability to help prevent blood clotting. It has also been reported to possess a positive inotropic effect (Nicoll and Henein 2009) and therefore may be very effective in mitigating congestive heart failure in preclinical studies.

Ginger extract's beneficial role in the heart, combined with that of its potent anti-inflammatory and antioxidant properties, make it a very useful nutraceuticals in alleviating cardiovascular complications (Nicoll and Henein 2009). [6]-shogaol exerts its antiproliferative effect of vascular smooth muscle cells through accumulation of cells in the G0/G1 cell-cycle phase associated with activation of the Nrf2/HO-1 pathway which helps in alleviating the pathogenesis of certain cardiovascular disease (Liu et al. 2015). In fact, recent clinical studies conducted in Iran have shown that dietary ginger supplementation has been able to lower the plasma level of triglycerides and low-density lipoprotein cholesterol (LDL-C), thereby reducing the risk of atherosclerosis (Pourmasoumi et al. 2018). Indeed, such reports are well aligned to previous preclinical findings in various animal models (El-Seweidy et al. 2015; Elseweidy et al. 2015; Khosravani et al. 2016). Moreover, ginger extract also provided protection from myocardial damage induced by isoproterenol in rats (Amran et al. 2015).

The cardioprotective effect of ginger extract in cardiovascular complications is reported to be attributed to different phytochemical constituents. For example, its antiplatelet activity is reported to be due to zingerone (Lee et al. 2017), while its blood pressure-lowering effect is attributed to 6-gingerols and 6-shogaols (Akinyemi et al. 2014; Liu et al. 2013a). In fact, 6-gingerol is also depicted to possess potent (angiotensin) AT_1 receptor blocking activity in in vitro cell lines (Liu et al. 2013a). Furthermore, the lipid-lowering activity of ginger extract is considered to be due to 10-dehydrogingerdione (El-Seweidy et al. 2015). A cross-sectional study revealed that daily ginger consumption has a potential preventive property against some chronic diseases, especially hypertension and coronary heart diseases, as well as an ability to reduce the probability of illness (Wang et al. 2017). Recent systematic review and meta-analysis suggests that ginger has a favorable effect on triacylglycerol and LDL-C. This review also revealed that a low dose of ginger ($\leq$2 g/day) had a greater lowering impact on triacylglycerol and total cholesterol (Pourmasoumi et al. 2018). Thus, ginger supplementation has beneficial effects in almost all facets of cardiovascular complications.

4 Veterinary Use of Ginger

Ginger has been used in veterinary medicine for thousands of years for various GI disorders of animals. It has been used in treatment of constipation, food poisoning, diarrhea, eye diseases, hematuria, to improve stamina, indigestion, tympany, dysentery, stomachache, and skin diseases (Tiwari and Pande 2010). Some clinical investigations have experimentally validated its use.

Cattle Ginger products are used in veterinary medicine for the treatment of digestive problems in cattle. The beneficial effect of ginger is likely due to an elevated synthesis of bile acids in the liver and their excretion in bile resulting in an increase in digestion and absorption of lipids. It also helps to increase the absorption of essential nutrients and to increase stability of feed and beneficially influence the gastrointestinal ecosystem through inhibition of pathogenic microorganisms growth (Mekuriya and Mekibib 2018). In the last 10 years, researchers in the field of animal science have used alternative natural materials such as medical herbs in feeding dairy cattle to substitute or minimize the use of chemical compounds like antibiotics. These chemical compounds may cause unfavorable side effects and be hazardous to animals and humans (Al-dain and Jarjeis 2015). Apart from

the prokinetic action of ginger demonstrated in laboratory animals and humans, the effect of a hydroalcoholic extract of ginger was also evaluated on contraction and motility of the reticulum and rumen of ruminants. The results of an in vitro study indicated that hydroalcoholic extract of ginger contained spasmogenic and spasmolytic constituents where an in vivo study presented evidence that the extract may have a stimulant effect on reticulorumen motility in a 40 mg/kg concentration (Mamaghani et al. 2013). Recently, the ameliorative effect of ginger powder was demonstrated against experimentally induced arsenic toxicity in calves (Biswas et al. 2017). Ginger provided in vivo anthelmintic activity in sheep, thus justifying the age-old traditional use of this plant in helminth infestation (Iqbal et al. 2006).

Equine The effects of ginger extract were studied on the physiological response to exercise as well as markers of muscle damage and mRNA expression for the inflammatory cytokines TNF-α, interferon(IFN)-γ, and IL-6 after an exhaustive bout of exercise in horses. Ginger extract was shown to reduce cardiovascular recovery time in horses completing a short, strenuous bout of exercise. Ginger extract's positive effect on recovery time may make it a useful tool for horses competing in endurance, jumping or racing events. However, further analysis of the compounds present in ginger extract is needed to determine whether those compounds would be picked up by drug tests often done at competition (Liburt et al. 2009). The anti-inflammatory effect of a single dose of ginger has also been demonstrated in the horse post-exercise (Williams and Lamprecht 2008). In current veterinary practice, many professionals and horse owners prefer herbal preparations, including ginger, to treat ulcer cases as compared to allopathic medicine.

Dog The use of nutraceuticals is gaining interest in veterinary medicine for pets. Nearly 30% of pet owners have used or considered the use of novel ingredients like nutraceuticals and herbs/botanicals in their animals (Boothe 2014). Like in humans, the use of ginger (e.g., gingersnap cookies, ginger ale) appears to have some benefit against motion sickness in the dog (Mowrey and Clayson 1982; Plumb 2015).

Poultry Nutritionists have found herbs to be one of the alternatives following the European Union ban on the use of antibiotic feed additives as growth promoters. Powdered rhizome of ginger is one such potential herb with a wide range of medicinal effects. In broilers and layers, this plant has been used in different forms, doses, and durations. Feeding of ginger promoted growth performance and weight gain in broilers and egg-laying characteristics in hens and also enhanced gut function and anti-oxidation in poultry. There were improvements in semen quality, sperm fatty acids, and reproductive performance in aged Cobb 500 breeder roosters fed diets containing dried ginger rhizomes (Akhlaghi et al. 2014). Dietary supplementation of ginger powder exhibited improved laying performance, improved serum and egg yolk antioxidant status, and lowered egg cholesterol in a dose-dependent manner. The optimum supplementation rate of ginger powder in the diet of laying hens is 10–15 g/kg of diet (Akbarian et al. 2011; Zhao et al. 2011). Dietary supplementation with either ginger or probiotics showed a significant influence on birds' immune response, probably because ginger has a strong antioxidant activity and the probiotics stimulate the production of natural antibodies (Qorbanpour et al. 2018).

Fish In order to avoid emergence of antibiotic-resistant bacteria and the generation of toxicants following the extensive use of antibiotics in aquaculture, the use of natural alternative feed additives like ginger has gained attention for disease control strategies. The application of ginger in aquaculture is advocated as an innovative approach to enhance the health of fish and to prevent diseases. A dose of 0.50 g/kg feed significantly reduced mortality associated with enhancement of growth rate, feed conversion, and protein efficiency (Mekuriya and Mekibib 2018). It provides protection against invading microorganisms, including *E. coli* and *Staphylococcus aureus* (a common cause of skin infections), and fungi, including *Candida albicans*. Ginger is a strong antioxidant substance and may either mitigate or prevent generation of free radicals that alleviate putrefaction/rancidity of fish and increase its consumability and marketability.

5 Safety Profile of Ginger

Recently, there has been increased usage of medicinal plants for the prevention and treatment of various diseases. Though their efficacy is proven in preclinical studies, a thorough scientific profile emphasizing the risk and benefit ratio is needed (Talalay and Talalay 2001). Currently, there is a growing interest among people to take ginger not only as a spice but also as a dietary supplement for preventing chronic diseases (Ali et al. 2008). Although ginger is considered to be safe with an LD_{50} of 10.25 g/kg and 11.75 g/kg of its methanolic and aqueous extract, respectively, in mice administered orally (Li et al. 2012c), still some countries do not recommend its use for the prevention of nausea and vomiting associated with pregnancy. A study conducted in France has reported that ginger root is safe and efficacious at a dose of 1 g/day for 4 days and has no risk to either fetus or mother (Stanisiere et al. 2018). In fact, the recommended safe value of ginger metabolites like 6-gingerol and 6-shogaol in

Table 4 Some of the available commercial products of ginger

Product name	Manufacturer	Nutraceutical value
Focalgin B	Method Pharmaceuticals, LLC, USA	Alleviates nausea and vomiting
B-Nexa	Upsher-Smith Laboratories, USA	Nausea and vomiting
Ginger Root	Gaia Herbs, India	Circulatory tonic and warming agent
Ginger Supreme	Gaia Herbs, India	Aids in digestion, supports heart health, and alleviates nausea
Macao Boost-Cacao-Ginger	Gaia Herbs, India	Aids in digestion
Jarrow Formulas Ginger	Jarrow Formulas, USA	Improves gastrointestinal health and antioxidant property
Now Ginger Essential Oils	Now Foods, USA	Nausea and vomiting
Sunthi(Ginger)	The Himalaya Drug Company, India	Anti-nausea in humans
Dabur Honey-Ginger	Dabur India Ltd.	Cough and cold in humans
Turmeric-Curcumin and Ginger with Bioperine	Vimerson Health, USA	Improves skin, brain, digestion, and immune health
Sea-Band Anti-Nausea Ginger Gum	Sea-Band, USA	Relief of nausea
Daily digestion (Ginger and Mint)	Animal Essentials, USA	Improves digestion in dogs and cats

healthy humans is up to 2000 mg (Zick et al. 2008), and its LD_{50} values are 250 and 687 mg/kg, respectively (Li et al. 2012c). However, high dose of ginger extract can cause various gastrointestinal disturbances, central nervous system depression, cardiac arrhythmias, and bleeding due to its potent antiplatelet property (Gunathilake and Rupasinghe 2015). Furthermore, there is also the risk of herb-drug interactions in clinical settings (Agbabiaka et al. 2017). Thus, a detailed scientific study verifying ginger's mechanism of action and safety is needed before it can be safely taken to prevent or protect against pathologic conditions (Bode and Dong 2011).

6 Concluding Remarks and Future Directions

Ginger powder, ginger root extract, and standardized ginger extract (5–7% gingerols) available in various formulations (tablet, capsule, syrup) and strengths (30, 280, 500, and 1000 mg or more) is available as a dietary supplement on the market for improving various disease conditions (Table 4). A considerable number of the aforementioned scientific studies suggest its nutraceutical value. Apart from gastrointestinal disorders, ginger is shown to have health benefits in cancer, diabetes, coronary heart disease, obesity, osteoporosis, and neurodegenerative diseases. The beneficial pharmacological effects of ginger in these disease conditions may be attributed to activation of antioxidant defenses, signal transduction pathways, cell survival-associated gene expression, cell proliferation and differentiation, and preservation of mitochondrial integrity. It appears that these properties play a pivotal role in the protection and providing health benefits. The majority of preclinical studies in disease models indicate the beneficial role of ginger. Several clinical studies have also been carried out on healthy volunteers and in patients using ginger and its formulations. Although several clinical studies have shown beneficial effects, some have shown mixed results, and results of some clinical studies have yet to be published. To enhance the health benefit of ginger, there is a need for further investigation of the beneficial effect of ginger with other nutraceuticals. More focused scientific efforts are required to explore the true nutraceutical value of ginger.

References

Abdel-Azeem AS, Hegazy AM, Ibrahim KS et al (2013) Hepatoprotective, antioxidant, and ameliorative effects of ginger (*Zingiber officinale* Roscoe) and vitamin E in acetaminophen treated rats. J Diet Suppl 10:195–209

Abdulaziz Bardi D, Halabi MF, Abdullah NA et al (2013) In vivo evaluation of ethanolic extract of *Zingiber officinale* rhizomes for its protective effect against liver cirrhosis. Biomed Res Int 2013:918460. https://doi.org/10.1155/2013/918460

Abdulrazaq NB, Cho MM, Win NN et al (2012) Beneficial effects of ginger (*Zingiber officinale*) on carbohydrate metabolism in streptozotocin-induced diabetic rats. Br J Nutr 108:1194–1201

Abolaji AO, Ojo M, Afolabi TT et al (2017) Protective properties of 6-gingerol-rich fraction from *Zingiber officinale* (Ginger) on chlorpyrifos-induced oxidative damage and inflammation in the brain, ovary and uterus of rats. Chem Biol Interact 270:15–23

Afkhami Fathabad A, Shekarforoush S, Hoseini M et al (2018) Attenuation of sulfite-induced testicular injury in rats by *Zingiber officinale* Roscoe. J Diet Suppl 15:398–409

Agbabiaka T, Wider B, Watson L et al (2017) Concurrent use of prescription drugs and herbal medicinal products in older adults: a systematic review. Drugs Aging 34:891–905. https://doi.org/10.1007/s40266-40017-40501-40267

Ahmad B, Rehman MU, Amin I et al (2015) A review on pharmacological properties of zingerone (4-(4-hydroxy-3-methoxyphenyl)-2-

butanone). Sci World J 2015:816364. https://doi.org/10.1155/2015/816364
Ahmadi R, Pishghadam S, Mollaamine F et al (2013) Comparing the effects of ginger and glibenclamide on dihydroxybenzoic metabolites produced in stz-induced diabetic rats. Int J Endocrinol Metab 11:e10266
Aimin Shi ZT, Wei P, Zhao J (2016) Epidemiological aspects of heart diseases. Exp Ther Med 12:1645–1650. https://doi.org/10.3892/etm.2016.3541
Akash MS, Rehman K, Tariq M et al (2015) *Zingiber officinale* and Type 2 diabetes mellitus: evidence from experimental studies. Crit Rev Eukaryot Gene Expr 25:91–112
Akbari A, Nasiri K, Heydari M et al (2017) The protective effect of hydroalcoholic extract of *Zingiber officinale* Roscoe (Ginger) on ethanol-induced reproductive toxicity in male rats. J Evid Based Complement Altern Med 22:609–617
Akbarian A, Golian A, Sheikh Ahmadi A et al (2011) Effects of ginger root (*Zingiber officinale*) on egg yolk cholesterol, antioxidant status and performance of laying hens. J Appl Anim Res 39:19–21
Akhlaghi A, Ahangari YJ, Navidshad B et al (2014) Improvements in semen quality, sperm fatty acids, and reproductive performance in aged Cobb 500 breeder roosters fed diets containing dried ginger rhizomes (*Zingiber officinale*). Poult Sci 93:1236–1244
Akimoto M, Iizuka M, Kanematsu R et al (2015) Anticancer effect of ginger extract against pancreatic cancer cells mainly through reactive oxygen species-mediated autotic cell death. PLoS One 10:e0126605
Akinyemi AJ, Ademiluyi AO, Oboh G (2014) Inhibition of angiotensin-1-converting enzyme activity by two varieties of ginger (*Zingiber officinale*) in rats fed a high cholesterol diet. J Med Food 17:317–323
Al Hroob AM, Abukhalil MH, Alghonmeen RD et al (2018) Ginger alleviates hyperglycemia-induced oxidative stress, inflammation and apoptosis and protects rats against diabetic nephropathy. Biomed Pharmacother 106:381–389
Al-Amoudi WM (2018) Toxic effects of Lambda-cyhalothrin, on the rat thyroid: involvement of oxidative stress and ameliorative effect of ginger extract. Toxicol Rep 5:728–736
Al-Dain QZS, Jarjeis EA (2015) Vital impact of using ginger roots powder as feed additive to the rations of local Friesian dairy cows and its effect on production & economic efficiency of milk and physiological of blood. Kufa J Vet Med Sci 6:154–165
Ali BH, Blunden G, Tanira MO et al (2008) Some phytochemical, pharmacological and toxicological properties of ginger (*Zingiber officinale* Roscoe): a review of recent research. Food Chem Toxicol 46:409–420
Alibakhshi T, Khodayar MJ, Khorsandi L et al (2018) Protective effects of zingerone on oxidative stress and inflammation in cisplatin-induced rat nephrotoxicity. Biomed Pharmacother 105:225–232
Al-Otaibi WA, Alkhatib MH et al (2018) Cytotoxicity and apoptosis enhancement in breast and cervical cancer cells upon coadministration of mitomycin C and essential oils in nanoemulsion formulations. Biomed Pharmacother 106:946–955
Amran AZ, Jantan I, Dianita R et al (2015) Protective effects of the standardized extract of *Zingiber officinale* on myocardium against isoproterenol-induced biochemical and histopathological alterations in rats. Pharm Biol 53:1795–1802
Annamalai G, Kathiresan S, Kannappan N (2016) [6]-Shogaol, a dietary phenolic compound, induces oxidative stress mediated mitochondrial dependant apoptosis through activation of proapoptotic factors in Hep-2 cells. Biomed Pharmacother 82:226–236
Arablou T, Aryaeian N, Valizadeh M, Sharifi F, Hosseini A, Djalali M (2014) The effect of ginger consumption on glycemic status, lipid profile and some inflammatory markers in patients with type 2 diabetes mellitus. Int J Food Sci Nutr 65:515–520
Arslan M, Ozdemir L (2015) Oral intake of ginger for chemotherapy-induced nausea and vomiting among women with breast cancer. Clin J Oncol Nurs 19:E92–E97
Badawi MS (2018) Histological study of the protective role of ginger on piroxicam-induced liver toxicity in mice. J Chin Med Assoc. https://doi.org/10.1016/j.jcma.2018.06.006
Baiomy AA, Mansour AA (2016) Genetic and histopathological responses to cadmium toxicity in rabbit's kidney and liver: protection by ginger (*Zingiber officinale*). Biol Trace Elem Res 170:320–329
Baliga MS, Haniadka R, Pereira MM et al (2011) Update on the chemopreventive effects of ginger and its phytochemicals. Crit Rev Food Sci Nutr 51:499–523
Baliga MS, Haniadka R, Pereira MM et al (2012) Radioprotective effects of *Zingiber officinale* Roscoe (ginger): past, present and future. Food Funct 3:714–723
Banji D, Banji OJ, Pavani B et al (2014) Zingerone regulates intestinal transit, attenuates behavioral and oxidative perturbations in irritable bowel disorder in rats. Phytomedicine 21:423–429
Bernard MM, McConnery JR, Hoskin DW (2017) [10]-Gingerol, a major phenolic constituent of ginger root, induces cell cycle arrest and apoptosis in triple-negative breast cancer cells. Exp Mol Pathol 102:370–376
Bi X, Lim J, Henry CJ (2017) Spices in the management of diabetes mellitus. Food Chem 217:281–293
Biswas S, Maji C, Sarkar PK et al (2017) Ameliorative effect of two ayurvedic herbs on experimentally induced arsenic toxicity in calves. J Ethnopharmacol 197:266–273
Bode AM, Dong Z (2011) The amazing and mighty ginger. In: Benzie IFF, Wachtel-Galor S (eds) Herbal medicine: biomolecular and clinical aspects. CRC Press, Boca Raton, FL
Boothe D (2014) Medical and nutritional issues of nutraceuticals in veterinary medicine: a focus on quality and safety. World Small Animal Veterinary Association World Congress Proceedings. pp 1–4
Brahma Naidu P, Uddandrao VV, Ravindar Naik R et al (2016) Ameliorative potential of gingerol: promising modulation of inflammatory factors and lipid marker enzymes expressions in HFD induced obesity in rats. Mol Cell Endocrinol 419:139–147
Butt MS, Sultan MT (2011) Ginger and its health claims: molecular aspects. Crit Rev Food Sci Nutr 51:383–393
Carrasco FR, Schmidt G, Romero AL, Sartoretto JL, Caparroz-Assef SM, Bersani-Amado CA, Cuman RK (2009) Immunomodulatory activity of *Zingiber officinale* Roscoe, *Salvia officinalis* L. and *Syzygium aromaticum* L. essential oils: evidence for humor- and cell-mediated responses. J Pharm Pharmacol 61:961–967
Chakraborty D, Mukherjee A, Sikdar S et al (2012) [6]-Gingerol isolated from ginger attenuates sodium arsenite induced oxidative stress and plays a corrective role in improving insulin signaling in mice. Toxicol Lett 210:34–43
Chan ML, Liang JW, Hsu LC et al (2015) Zerumbone, a ginger sesquiterpene, induces apoptosis and autophagy in human hormone-refractory prostate cancers through tubulin binding and crosstalk between endoplasmic reticulum stress and mitochondrial insult. Naunyn Schmiedebergs Arch Pharmacol 388:1223–1236
Chandel NS, Schieber M (2014) ROS function in redox signaling and oxidative stress. Curr Biol 24:R453–R462. https://doi.org/10.1016/j.cub.2014.1003.1034
Chatturong U, Kajsongkram T, Tunsophon S et al (2018) Ginger extract and [6]-gingerol inhibit contraction of rat entire small intestine. J Evid Based Integr Med 23. https://doi.org/10.1177/2515690X18774273
Choi JG, Kim SY, Jeong M et al (2018) Pharmacotherapeutic potential of ginger and its compounds in age-related neurological disorders. Pharmacol Ther 182:56–69
Cui Y, Shi Y, Bao Y et al (2018) Zingerone attenuates diabetic nephropathy through inhibition of nicotinamide adenine dinucleotide phosphate oxidase 4. Biomed Pharmacother 99:422–430
Dabaghzadeh F, Khalili H, Dsahti-Khavidaki S, Abbasian L et al (2014) Ginger for prevention of antiretroviral-induced nausea and

vomiting: a randomized clinical trial. Expert Opin Drug Saf 13:859–866. https://doi.org/10.1517/14740338.14742014.14914170

de Las Heras N, Valero-Munoz M, Martin-Fernandez B et al (2017) Molecular factors involved in the hypolipidemic- and insulin-sensitizing effects of a ginger (*Zingiber officinale* Roscoe) extract in rats fed a high-fat diet. Appl Physiol Nutr Metab 42:209–215

de Lima RMT, Dos Reis AC, de Menezes APM et al (2018) Protective and therapeutic potential of ginger (*Zingiber officinale*) extract and [6]-gingerol in cancer: a comprehensive review. Phytother Res 32 (10):1885–1907

Dongare S, Gupta SK, Mathur R et al (2016) *Zingiber officinale* attenuates retinal microvascular changes in diabetic rats via anti-inflammatory and antiangiogenic mechanisms. Mol Vis 22:599–609

Ebrahimzadeh Attari V, Malek Mahdavi A, Javadivala Z et al (2018) A systematic review of the anti-obesity and weight lowering effect of ginger (*Zingiber officinale* Roscoe) and its mechanisms of action. Phytother Res 32:577–585

Eid BG, Mosli H, Hasan A et al (2017) Ginger ingredients alleviate diabetic prostatic complications: effect on oxidative stress and fibrosis. Evid Based Complement Alternat Med 2017:6090269. https://doi.org/10.1155/2017/6090269

Elkirdasy A, Shousha S, Alrohaimi AH et al (2015) Hematological and immunobiochemical study of green tea and ginger extracts in experimentally induced diabetic rabbits. Acta Pol Pharm 72:497–506

Elseweidy MM, Younis NN, Elswefy SE et al (2015) Atheroprotective potentials of curcuminoids against ginger extract in hypercholesterolaemic rabbits. Nat Prod Res 29:961–965

El-Seweidy MM, Asker Mel S, Eldahmy SI et al (2015) Haemostatic risk factors in dyslipidemic rabbits: role of 10-dehydrogingerdione as a new hypolipemic agent. J Thromb Thrombolysis 39:196–202

El-Sharaky AS, Newairy AA, Kamel MA et al (2009) Protective effect of ginger extract against bromobenzene-induced hepatotoxicity in male rats. Food Chem Toxicol 47:1584–1590

Emrani Z, Shojaei E, Khalili H (2016) Ginger for prevention of antituberculosis-induced gastrointestinal adverse reactions including hepatotoxicity: a randomized pilot clinical trial. Phytother Res 30:1003–1009

Eren D, Betul YM (2016) Revealing the effect of 6-gingerol, 6-shogaol and curcumin on mPGES-1, GSK-3beta and beta-catenin pathway in A549 cell line. Chem Biol Interact 258:257–265

Gaus K, Huang Y, Israel DA et al (2009) Standardized ginger (*Zingiber officinale*) extract reduces bacterial load and suppresses acute and chronic inflammation in Mongolian gerbils infected with cagA Helicobacter pylori. Pharm Biol 47:92–98

Ghareib SA, El-Bassossy HM, Elberry AA et al (2016) Protective effect of zingerone on increased vascular contractility in diabetic rat aorta. Eur J Pharmacol 780:174–179

Grzanna R, Lindmark L, Frondoza CG (2005) Ginger-an herbal medicinal product with broad anti-inflammatory actions. J Med Food 8:125–132

Gunathilake K, Rupasinghe V (2015) Recent perspectives on the medicinal potential of ginger. Bot Targets Ther 5:55–63

Ha SK, Moon E, Ju MS et al (2012) 6-Shogaol, a ginger product, modulates neuroinflammation: a new approach to neuroprotection. Neuropharmacology 63:211–223

Han MA, Woo SM, Min KJ et al (2015) 6-Shogaol enhances renal carcinoma Caki cells to TRAIL-induced apoptosis through reactive oxygen species-mediated cytochrome c release and down-regulation of c-FLIP(L) expression. Chem Biol Interact 228:69–78

Haniadka R, Saldanha E, Sunita V et al (2013) A review of the gastroprotective effects of ginger (*Zingiber officinale* Roscoe). Food Funct 4:845–855

Hashem RM, Rashed LA, Hassanin KMA et al (2017) Effect of 6-gingerol on AMPK- NF-kappaB axis in high fat diet fed rats. Biomed Pharmacother 88:293–301

Hegazy AM, Mosaed MM, Elshafey SH et al (2016) 6-gingerol ameliorates gentamicin induced renal cortex oxidative stress and apoptosis in adult male albino rats. Tissue Cell 48:208–216

Heshmati E, Shirpoor A, Kheradmand F et al (2018) Chronic ethanol increases calcium/calmodulin-dependent protein kinase II delta gene expression and decreases monoamine oxidase amount in rat heart muscles: rescue effect of *Zingiber officinale* (ginger) extract. Anatol J Cardiol 19:19–26

Hitomi S, Ono K, Terawaki K et al (2017) [6]-gingerol and [6]-shogaol, active ingredients of the traditional Japanese medicine hangeshashinto, relief oral ulcerative mucositis-induced pain via action on Na(+) channels. Pharmacol Res 117:288–302

Ho SC, Chang KS, Lin CC (2013) Anti-neuroinflammatory capacity of fresh ginger is attributed mainly to 10-gingerol. Food Chem 141:3183–3191

Ho YC, Lee SS, Yang ML et al (2017) Zerumbone reduced the inflammatory response of acute lung injury in endotoxin-treated mice via Akt-NFkappaB pathway. Chem Biol Interact 271:9–14

Hosseini F, Adib-Hajbaghery M (2015) Ginger essence effect on nausea and vomiting after open and laparoscopic nephrectomies. Nurs Midwifery Stud 4:e28625. https://doi.org/10.17795/nmsjournal28625

Hosseinzadeh A, Bahrampour Juybari K, Fatemi MJ et al (2017) Protective effect of ginger (*Zingiber officinale* Roscoe) extract against oxidative stress and mitochondrial apoptosis induced by Interleukin-1beta in cultured chondrocytes. Cells Tissues Organs 204:241–250

Hruby A, Hu FB (2016) The epidemiology of obesity: a big picture. Pharmacoeconomics 33:673–689

Hu R, Zhou P, Peng YB et al (2012) 6-Shogaol induces apoptosis in human hepatocellular carcinoma cells and exhibits anti-tumor activity in vivo through endoplasmic reticulum stress. PLoS One 7: e39664

Huh E, Lim S, Kim HG et al (2018) Ginger fermented with *Schizosaccharomyces pombe* alleviates memory impairment via protecting hippocampal neuronal cells in amyloid beta1-42 plaque injected mice. Food Funct 9:171–178

IDF (2017) Diabetes atlas, 8th edn. IDF, Brussels

Ilkhanizadeh B, Shirpoor A, Khadem Ansari MH et al (2016) Protective effects of ginger (*Zingiber officinale*) extract against diabetes-induced heart abnormality in rats. Diabetes Metab J 40:46–53

Imani H, Tabibi H, Najafi I et al (2015) Effects of ginger on serum glucose, advanced glycation end products, and inflammation in peritoneal dialysis patients. Nutrition 31:703–707

Iqbal Z, Lateef M, Akhtar MS et al (2006) In vivo anthelmintic activity of ginger against gastrointestinal nematodes of sheep. J Ethnopharmacol 106:285–287

Iwami M, Shiina T, Hirayama H et al (2011) Inhibitory effects of zingerone, a pungent component of *Zingiber officinale* Roscoe, on colonic motility in rats. J Nat Med 65:89–94

Jafarzadeh A, Azizi SV, Nemati M et al (2015) Ginger extract reduces the expression of IL-17 and IL-23 in the sera and central nervous system of EAE mice. Iran J Immunol 12:288–301

Jafarzadeh A, Arabi Z, Ahangar-Parvin R et al (2017a) Ginger extract modulates the expression of chemokines CCL20 and CCL22 and their receptors (CCR6 and CCR4) in the central nervous system of mice with experimental autoimmune encephalomyelitis. Drug Res (Stuttg) 67:632–639

Jafarzadeh A, Arabi Z, Ahangar-Parvin R et al (2017b) Ginger extract modulates the expression of chemokines CCL20 and CCL22 and their receptors (CCR6 and CCR4) in the central nervous system of mice with experimental autoimmune encephalomyelitis. Drug Res (Stuttg) 67:632–639

Jeena K, Liju VB, Ramanath V et al (2016) Protection against whole body gamma-irradiation induced oxidative stress and clastogenic damage in mice by ginger essential oil. Asian Pac J Cancer Prev 17:1325–1332

Jittiwat J, Wattanathorn J (2012) Ginger pharmacopuncture improves cognitive impairment and oxidative stress following cerebral ischemia. J Acupunct Meridian Stud 5:295–300

Joshi D, Srivastav SK, Belemkar S et al (2017) *Zingiber officinale* and 6-gingerol alleviate liver and kidney dysfunctions and oxidative stress induced by mercuric chloride in male rats: a protective approach. Biomed Pharmacother 91:645–655

Kalava A, Darji SJ, Kalstein A et al (2013) Efficacy of ginger on intraoperative and postoperative nausea and vomiting in elective cesarean section patients. Eur J Obstet Gynecol Reprod Biol 169:184–188

Kandemir FM, Yildirim S, Kucukler S et al (2018) Therapeutic efficacy of zingerone against vancomycin-induced oxidative stress, inflammation, apoptosis and aquaporin 1 permeability in rat kidney. Biomed Pharmacother 105:981–991

Kang CG, Lee HJ, Kim SH et al (2016) Zerumbone suppresses osteopontin-induced cell invasion through inhibiting the FAK/AKT/ROCK pathway in human non-small cell lung cancer A549 cells. J Nat Prod 79:156–160

Kazeem MI, Akanji MA, Yakubu MT (2015) Amelioration of pancreatic and renal derangements in streptozotocin-induced diabetic rats by polyphenol extracts of Ginger (*Zingiber officinale*) rhizome. Pathophysiology 22:203–209

Keng-Liang CKR, Chuah S-K, Chi-Sin C et al (2008) Effects of ginger on gastric emptying and motility in healthy humans. Eur J Gastroenterol Hepatol 20:436–440

Khaki A, Khaki AA, Hajhosseini L et al (2014) The anti-oxidant effects of ginger and cinnamon on spermatogenesis dys-function of diabetes rats. Afr J Tradit Complement Altern Med 11:1–8

Khosravani M, Azarbayjani MA, Abolmaesoomi M et al (2016) Ginger extract and aerobic training reduces lipid profile in high-fat fed diet rats. Eur Rev Med Pharmacol Sci 20:1617–1622

Kim S, Kwon J (2013) [6]-shogaol attenuates neuronal apoptosis in hydrogen peroxide-treated astrocytes through the up-regulation of neurotrophic factors. Phytother Res 27:1795–1799

Kim MO, Lee MH, Oi N et al (2014) [6]-shogaol inhibits growth and induces apoptosis of non-small cell lung cancer cells by directly regulating Akt1/2. Carcinogenesis 35:683–691

Kim YJ, Jeon Y, Kim T et al (2017) Combined treatment with zingerone and its novel derivative synergistically inhibits TGF-beta1 induced epithelial-mesenchymal transition, migration and invasion of human hepatocellular carcinoma cells. Bioorg Med Chem Lett 27:1081–1088

Kim JN, Kim HJ, Kim I et al (2018) The mechanism of action of zingerone in the pacemaker potentials of interstitial cells of cajal isolated from murine small intestine. Cell Physiol Biochem 46:2127–2137

Kubra IR, Rao LJ (2012) An impression on current developments in the technology, chemistry, and biological activities of ginger (*Zingiber officinale* Roscoe). Crit Rev Food Sci Nutr 52:651–688

Lai YS, Lee WC, Lin YE et al (2016) Ginger essential oil ameliorates hepatic injury and lipid accumulation in high fat diet-induced nonalcoholic fatty liver disease. J Agric Food Chem 64:2062–2071

Lamuchi-Deli N, Aberomand M, Babaahmadi-Rezaei H et al (2017) Effects of the hydroalcoholic extract of *Zingiber officinale* on arginase I activity and expression in the retina of streptozotocin-induced diabetic rats. Int J Endocrinol Metab 15:e42161

Lee SH, Cekanova M, Baek SJ (2008) Multiple mechanisms are involved in 6-gingerol-induced cell growth arrest and apoptosis in human colorectal cancer cells. Mol Carcinog 47:197–208

Lee DH, Kim DW, Jung CH et al (2014) Gingerol sensitizes TRAIL-induced apoptotic cell death of glioblastoma cells. Toxicol Appl Pharmacol 279:253–265

Lee W, Ku SK, Kim MA et al (2017) Anti-factor Xa activities of zingerone with anti-platelet aggregation activity. Food Chem Toxicol 105:186–193

Lei L, Liu Y, Wang X et al (2014) Plasma cholesterol-lowering activity of gingerol- and shogaol-enriched extract is mediated by increasing sterol excretion. J Agric Food Chem 62:10515–10521

Li TY, Chiang BH (2017) 6-shogaol induces autophagic cell death then triggered apoptosis in colorectal adenocarcinoma HT-29 cells. Biomed Pharmacother 93:208–217

Li F, Nitteranon V, Tang X et al (2012a) In vitro antioxidant and anti-inflammatory activities of 1-dehydro-[6]-gingerdione, 6-shogaol, 6-dehydroshogaol and hexahydrocurcumin. Food Chem 135:332–337

Li Y, Tran VH, Duke CC et al (2012b) Gingerols of *Zingiber officinale* enhance glucose uptake by increasing cell surface GLUT4 in cultured L6 myotubes. Planta Med 78:1549–1555

Li Y, Tran VH, Duke CC et al (2012c) Preventive and protective properties of *Zingiber officinale* (Ginger) in diabetes mellitus, diabetic complications, and associated lipid and other metabolic disorders: a brief review. Evid Based Complement Alternat Med 2012:516870

Li Y, Xu B, Xu M et al (2017) 6-Gingerol protects intestinal barrier from ischemia/reperfusion-induced damage via inhibition of p38 MAPK to NF-kappaB signalling. Pharmacol Res 119:137–148

Li X, Qin Y, Liu W et al (2018a) Efficacy of ginger in ameliorating acute and delayed chemotherapy-induced nausea and vomiting among patients with lung cancer receiving cisplatin-based regimens: a randomized controlled trial. Integr Cancer Ther 17:747–754

Li Z, Wang Y, Gao M et al (2018b) Nine new gingerols from the Rhizoma of *Zingiber officinale* and their cytotoxic activities. Molecules 23. https://doi.org/10.3390/molecules23020315

Liburt NR, McKeever KH, Streltsova JM et al (2009) Effects of ginger and cranberry extracts on the physiological response to exercise and markers of inflammation in horses. Comp Exercise Physiol 6:157–169

Liju VB, Jeena K, Kuttan R (2015) Gastroprotective activity of essential oils from turmeric and ginger. J Basic Clin Physiol Pharmacol 26:95–103

Lim S, Moon M, Oh H et al (2014) Ginger improves cognitive function via NGF-induced ERK/CREB activation in the hippocampus of the mouse. J Nutr Biochem 25:1058–1065

Liu Q, Liu J, Guo H et al (2013a) [6]-gingerol: a novel AT(1) antagonist for the treatment of cardiovascular disease. Planta Med 79:322–326

Liu Q, Peng YB, Zhou P et al (2013b) 6-Shogaol induces apoptosis in human leukemia cells through a process involving caspase-mediated cleavage of eIF2alpha. Mol Cancer 12:135

Liu R, Heiss EH, Sider N et al (2015) Identification and characterization of [6]-shogaol from ginger as inhibitor of vascular smooth muscle cell proliferation. Mol Nutr Food Res 59:843–852

Lua PL, Salihah N, Mazlan N (2015) Effects of inhaled ginger aromatherapy on chemotherapy-induced nausea and vomiting and health-related quality of life in women with breast cancer. Complement Ther Med 23:396–404

Luettig J, Rosenthal R, Lee IM et al (2016) The ginger component 6-shogaol prevents TNF-alpha-induced barrier loss via inhibition of PI3K/Akt and NF-kappaB signaling. Mol Nutr Food Res 60:2576–2586

Maghbooli Mehdi GPF, Moghimi Esfandabadi A, Mehran Y (2013) Comparison between the efficacy of ginger and sumatriptan in the ablative treatment of the common migraine. Phytother Res 28:412–415

Mahady GB, Pendland SL, Yun GS et al (2003) Ginger (*Zingiber officinale* Roscoe) and the gingerols inhibit the growth of Cag A+ strains of Helicobacter pylori. Anticancer Res 23:3699–3702

Mahima, Rahal A, Deb R, Latheef SK et al (2012) Immunomodulatory and therapeutic potentials of herbal, traditional/indigenous and ethnoveterinary medicines. Pak J Biol Sci 15:754–774

Mahluji S, Ostadrahimi A, Mobasseri M et al (2013) Anti-inflammatory effects of *zingiber officinale* in type 2 diabetic patients. Adv Pharm Bull 3:273–276

Mahmoud RH, Elnour WA (2013) Comparative evaluation of the efficacy of ginger and orlistat on obesity management, pancreatic lipase and liver peroxisomal catalase enzyme in male albino rats. Eur Rev Med Pharmacol Sci 17:75–83

Makhdoomi Arzati M, Mohammadzadeh Honarvar N, Saedisomeolia A et al (2017) The effects of ginger on fasting blood sugar, hemoglobin A1c, and lipid profiles in patients with Type 2 diabetes. Int J Endocrinol Metab 15:e57927

Mamaghani A, Maham M, Dalir-Naghadeh B (2013) Effects of ginger extract on smooth muscle activity of sheep reticulum and rumen. Vet Res Forum 4:91–97

Mansingh DP, O JS, Sali VK et al (2018) [6]-Gingerol-induced cell cycle arrest, reactive oxygen species generation, and disruption of mitochondrial membrane potential are associated with apoptosis in human gastric cancer (AGS) cells. J Biochem Mol Toxicol 32(10): e22206

Mardani H, Ghannadi A, Rashnavadi B et al (2017) The effect of ginger herbal spray on reducing xerostomia in patients with type II diabetes. Avicenna J Phytomed 7:308–316

Martins LB, Rodrigues AMDS, Rodrigues DF et al (2018) Double-blind placebo-controlled randomized clinical trial of ginger (*Zingiber officinale* Rosc.) addition in migraine acute treatment. Cephalalgia:333102418776016. https://doi.org/10.1177/0333102418776016

Mashhadi NS, Ghiasvand R, Askari G et al (2013) Anti-oxidative and anti-inflammatory effects of ginger in health and physical activity: review of current evidence. Int J Prev Med 4:S36–S42

Medagama AB, Bandara R (2014) The use of complementary and alternative medicines (CAMs) in the treatment of diabetes mellitus: is continued use safe and effective? Nutr J 13:102

Mekuriya W, Mekibib B (2018) Review on the medicinal values of ginger for human and animal ailments. J Vet Sci Technol 9:2

Mohamed OI, El-Nahas AF, El-Sayed YS et al (2016) Ginger extract modulates Pb-induced hepatic oxidative stress and expression of antioxidant gene transcripts in rat liver. Pharm Biol 54:1164–1172

Moon M, Kim HG, Choi JG et al (2014) 6-Shogaol, an active constituent of ginger, attenuates neuroinflammation and cognitive deficits in animal models of dementia. Biochem Biophys Res Commun 449:8–13

Motawi TK, Hamed MA, Shabana MH et al (2011) *Zingiber officinale* acts as a nutraceutical agent against liver fibrosis. Nutr Metab (Lond) 8:40

Mowrey DB, Clayson DE (1982) Motion sickness, ginger, and psychophysics. Lancet 1:655–657

Mozaffari-Khosravi H, Talaei B, Jalali BA et al (2014) The effect of ginger powder supplementation on insulin resistance and glycemic indices in patients with type 2 diabetes: a randomized, double-blind, placebo-controlled trial. Complement Ther Med 22:9–16

Na JY, Song K, Lee JW et al (2016a) 6-Shogaol has anti-amyloidogenic activity and ameliorates Alzheimer's disease via CysLT1R-mediated inhibition of cathepsin B. Biochem Biophys Res Commun 477:96–102

Na JY, Song K, Lee JW et al (2016b) Pretreatment of 6-shogaol attenuates oxidative stress and inflammation in middle cerebral artery occlusion-induced mice. Eur J Pharmacol 788:241–247

Na JY, Song K, Lee JW et al (2017) Sortilin-related receptor 1 interacts with amyloid precursor protein and is activated by 6-shogaol, leading to inhibition of the amyloidogenic pathway. Biochem Biophys Res Commun 484:890–895

Nicoll R, Henein MY (2009) Ginger (*Zingiber officinale* Roscoe): a hot remedy for cardiovascular disease? Int J Cardiol 131:408–409

Oyagbemi AA, Saba AB, Azeez OI (2010) Molecular targets of [6]-gingerol: its potential roles in cancer chemoprevention. Biofactors 36:169–178

Park G, Kim HG, Ju MS et al (2013) 6-Shogaol, an active compound of ginger, protects dopaminergic neurons in Parkinson's disease models via anti-neuroinflammation. Acta Pharmacol Sin 34:1131–1139

Park GH, Park JH, Song HM et al (2014) Anti-cancer activity of Ginger (*Zingiber officinale*) leaf through the expression of activating transcription factor 3 in human colorectal cancer cells. BMC Complement Altern Med 14:408

Peng S, Yao J, Liu Y, Duan D, Zhang X, Fang J (2015) Activation of Nrf2 target enzymes conferring protection against oxidative stress in PC12 cells by ginger principal constituent 6-shogaol. Food Funct 6:2813–2823

Pillai AK, Sharma KK, Gupta YK et al (2011) Anti-emetic effect of ginger powder versus placebo as an add-on therapy in children and young adults receiving high emetogenic chemotherapy. Pediatr Blood Cancer 56:234–238

Plumb D (2015) Plumb's veterinary drug handbook, 8th edn. Wiley, Hoboken, NJ

Poltronieri J, Becceneri AB, Fuzer AM et al (2014) [6]-gingerol as a cancer chemopreventive agent: a review of its activity on different steps of the metastatic process. Mini Rev Med Chem 14:313–321

Pourmasoumi M, Hadi A, Rafie N et al (2018) The effect of ginger supplementation on lipid profile: a systematic review and meta-analysis of clinical trials. Phytomedicine 43:28–36

Prabu SL, Suriyaprakash TNK, Kumar CD et al (2012) Nutraceuticals and their medicinal importance. Int J Health Allied Sci 1:47

Prasad S, Tyagi AK (2015) Ginger and its constituents: role in prevention and treatment of gastrointestinal cancer. Gastroenterol Res Pract 2015:142979

Qin F-F, Xu H-L (2008) Active compounds in gingers and thier therapeutic use in complimentary medication. Med Aromat Plant Sci Biotechnol 2:72–78

Qorbanpour M, Fahim T, Javandel F et al (2018) Effect of dietary ginger (*Zingiber officinale* Roscoe) and multi-strain probiotic on growth and carcass traits, blood biochemistry, immune responses and intestinal microflora in broiler chickens. Animals (Basel) 8:117

Ramudu SK, Korivi M, Kesireddy N et al (2011) Nephro-protective effects of a ginger extract on cytosolic and mitochondrial enzymes against streptozotocin (STZ)-induced diabetic complications in rats. Chin J Physiol 54:79–86

Rani MP, Padmakumari KP, Sankarikutty B et al (2011) Inhibitory potential of ginger extracts against enzymes linked to type 2 diabetes, inflammation and induced oxidative stress. Int J Food Sci Nutr 62:106–110

Rani MP, Krishna MS, Padmakumari KP et al (2012) *Zingiber officinale* extract exhibits antidiabetic potential via modulating glucose uptake, protein glycation and inhibiting adipocyte differentiation: an in vitro study. J Sci Food Agric 92:1948–1955

Rashidian A, Mehrzadi S, Ghannadi AR et al (2014) Protective effect of ginger volatile oil against acetic acid-induced colitis in rats: a light microscopic evaluation. J Integr Med 12:115–120

Rastogi N, Duggal S, Singh SK et al (2015) Proteasome inhibition mediates p53 reactivation and anti-cancer activity of 6-gingerol in cervical cancer cells. Oncotarget 6:43310–43325

Ray A, Vasudevan S, Sengupta S (2015) 6-Shogaol inhibits breast cancer cells and stem cell-like spheroids by modulation of notch signaling pathway and induction of autophagic cell death. PLoS One 10:e0137614

Rehman MU, Rashid SM, Rasool S et al (2018) Zingerone (4-(4-hydroxy-3-methylphenyl)butan-2-one) ameliorates renal

function via controlling oxidative burst and inflammation in experimental diabetic nephropathy. Arch Physiol Biochem:1–9. https://doi.org/10.1080/13813455.2018.1448422

Saberi H, Keshavarzi B, Shirpoor A et al (2017) Rescue effects of ginger extract on dose dependent radiation-induced histological and biochemical changes in the kidneys of male Wistar rats. Biomed Pharmacother 94:569–576

Sabina EP, Pragasam SJ, Kumar S et al (2011) 6-Gingerol, an active ingredient of ginger, protects acetaminophen-induced hepatotoxicity in mice. Zhong Xi Yi Jie He Xue Bao 9:1264–1269

Sahebkar A (2011) Potential efficacy of ginger as a natural supplement for nonalcoholic fatty liver disease. World J Gastroenterol 17:271–272

Salah Khalil M (2015) The postulated mechanism of the protective effect of ginger on the aspirin induced gastric ulcer: histological and immunohistochemical studies. Histol Histopathol 30:855–864

Salihu M, Ajayi BO, Adedara IA et al (2017) 6-Gingerol-rich fraction from *Zingiber officinale* ameliorates carbendazim-induced endocrine disruption and toxicity in testes and epididymis of rats. Andrologia 49:e12658. https://doi.org/10.1111/and.12658

Samad MB, Mohsin M, Razu BA et al (2017) [6]-Gingerol, from *Zingiber officinale*, potentiates GLP-1 mediated glucose-stimulated insulin secretion pathway in pancreatic beta-cells and increases -RAB8/RAB10-regulated membrane presentation of GLUT4 transporters in skeletal muscle to improve hyperglycemia in Lepr (db/db) type 2 diabetic mice. BMC Complement Altern Med 17:395

Sampath C, Rashid MR, Sang S et al (2017) Specific bioactive compounds in ginger and apple alleviate hyperglycemia in mice with high fat diet-induced obesity via Nrf2 mediated pathway. Food Chem 226:79–88

Sani NF, Belani LK, Sin CP et al (2014) Effect of the combination of gelam honey and ginger on oxidative stress and metabolic profile in streptozotocin-induced diabetic Sprague-Dawley rats. Biomed Res Int 2014:160695

Sapkota A, Park SJ, Choi JW (2018) Neuroprotective effects of 6-shogaol and its metabolite, 6-paradol, in a mouse model of multiple sclerosis. Biomol Ther. https://doi.org/10.4062/biomolther.2018.089

Saraswat M, Suryanarayana P, Reddy PY et al (2010) Antiglycating potential of Zingiber officinalis and delay of diabetic cataract in rats. Mol Vis 16:1525–1537

Semwal RB, Semwal DK, Combrinck S et al (2015) Gingerols and shogaols: important nutraceutical principles from ginger. Phytochemistry 117:554–568

Shalaby MA, Hamowieh AR (2010) Safety and efficacy of *Zingiber officinale* roots on fertility of male diabetic rats. Food Chem Toxicol 48:2920–2924

Shamoto T, Matsuo Y, Shibata T et al (2014) Zerumbone inhibits angiogenesis by blocking NF-kappaB activity in pancreatic cancer. Pancreas 43:396–404

Shanmugam KR, Mallikarjuna K, Kesireddy N et al (2011) Neuroprotective effect of ginger on anti-oxidant enzymes in streptozotocin-induced diabetic rats. Food Chem Toxicol 49:893–897

Sharma Y (2017) Ginger (*Zingiber officinale*)-an elixir of life a review. Pharm Innov 6:22

Sharma SS, Gupta YK (1998) Reversal of cisplatin-induced delay in gastric emptying in rats by ginger (*Zingiber officinale*). J Ethnopharmacol 62:49–55

Sharma SS, Kochupillai V, Gupta SK et al (1997) Antiemetic efficacy of ginger (*Zingiber officinale*) against cisplatin-induced emesis in dogs. J Ethnopharmacol 57:93–96

Shidfar F, Rajab A, Rahideh T et al (2015) The effect of ginger (*Zingiber officinale*) on glycemic markers in patients with type 2 diabetes. J Complement Integr Med 12:165–170

Shim S, Kim S, Choi DS et al (2011) Anti-inflammatory effects of [6]-shogaol: potential roles of HDAC inhibition and HSP70 induction. Food Chem Toxicol 49:2734–2740

Shirpoor A, Zerehpoosh M, Ansari MHK et al (2017) Ginger extract mitigates ethanol-induced changes of alpha and beta – myosin heavy chain isoforms gene expression and oxidative stress in the heart of male wistar rats. DNA Repair (Amst) 57:45–49

Shirpoor A, Heshmati E, Kheradmand F et al (2018) Increased hepatic FAT/CD36, PTP1B and decreased HNF4A expression contributes to dyslipidemia associated with ethanol-induced liver dysfunction: rescue effect of ginger extract. Biomed Pharmacother 105:144–150

Shukla Y, Singh M (2007) Cancer preventive properties of ginger: a brief review. Food Chem Toxicol 45:683–690

Si W, Chen YP, Zhang J et al (2018) Antioxidant activities of ginger extract and its constituents toward lipids. Food Chem 239:1117–1125

Song J, Fan HJ, Li H et al (2016) Zingerone ameliorates lipopolysaccharide-induced acute kidney injury by inhibiting Toll-like receptor 4 signaling pathway. Eur J Pharmacol 772:108–114

Srinivasan K (2017) Ginger rhizomes (Zingiber officinale): A spice with multiple health beneficial potentials. PharmaNutrition 5:18–28

Stanisiere J, Mousset PY, Lafay S (2018) How safe is ginger Rhizome for decreasing nausea and vomiting in women during early pregnancy? Foods 7. https://doi.org/10.3390/foods7040050

Suk S, Kwon GT, Lee E et al (2017) Gingerenone A, a polyphenol present in ginger, suppresses obesity and adipose tissue inflammation in high-fat diet-fed mice. Mol Nutr Food Res 61. https://doi.org/10.1002/mnfr.201700139

Talalay P, Talalay P (2001) The importance of using scientific principles in the development of medicinal agents from plants. Acad Med 76:238–247

Tiwari L, Pande PC (2010) Ethnoveterinary medicines in Indian perspective: reference to Uttarakhand, Himalaya. Indian J Tradit Knowle 9(3):611–617

Tsuboi K, Matsuo Y, Shamoto T et al (2014) Zerumbone inhibits tumor angiogenesis via NF-kappaB in gastric cancer. Oncol Rep 31:57–64

Tzeng TF, Liou SS, Tzeng YC et al (2016) Zerumbone, a phytochemical of subtropical ginger, protects against hyperglycemia-induced retinal damage in experimental diabetic rats. Nutrients 8. https://doi.org/10.3390/nu8080449

Vaibhav K, Shrivastava P, Tabassum R et al (2013) Delayed administration of zingerone mitigates the behavioral and histological alteration via repression of oxidative stress and intrinsic programmed cell death in focal transient ischemic rats. Pharmacol Biochem Behav 113:53–62

van den Driessche JJ, Plat J, Mensink RP (2018) Effects of superfoods on risk factors of metabolic syndrome: a systematic review of human intervention trials. Food Funct 9:1944–1966

Vipin AV, Rao R, Kurrey NK, KA AA (2017) Protective effects of phenolics rich extract of ginger against Aflatoxin B1-induced oxidative stress and hepatotoxicity. Biomed Pharmacother 91:415–424

Wang J, Ke W, Bao R et al (2017) Beneficial effects of ginger *Zingiber officinale* Roscoe on obesity and metabolic syndrome: a review. Ann N Y Acad Sci 1398:83–98

Wattanthorn J, Sutalangka C (2017) Neuroprotective and cognitive-enhancing effects of the combined extract of Cyperus rotundus and *Zingiber officinale*. BMC Complement Altern Med 17:135. https://doi.org/10.1186/s12906-12017-11632-12904

Wei CK, Tsai YH, Korinek M et al (2017) 6-Paradol and 6-Shogaol, the pungent compounds of ginger, promote glucose utilization in adipocytes and myotubes, and 6-Paradol reduces blood glucose in high-fat diet-fed mice. Int J Mol Sci 18(1):168

Weng CJ, Wu CF, Huang HW et al (2010) Anti-invasion effects of 6-shogaol and 6-gingerol, two active components in ginger, on human hepatocarcinoma cells. Mol Nutr Food Res 54:1618–1627

Wilasrusmee C, Kittur S, Siddiqui J et al (2002a) In vitro immunomodulatory effects of ten commonly used herbs on murine lymphocytes. J Altern Complement Med 8:467–475

Wilasrusmee C, Siddiqui J, Bruch D et al (2002b) In vitro immunomodulatory effects of herbal products. Am Surg 68:860–864

Williams CA, Lamprecht ED (2008) Some commonly fed herbs and other functional foods in equine nutrition: a review. Vet J 178:21–31

Xie X, Sun S, Zhong W et al (2014) Zingerone attenuates lipopolysaccharide-induced acute lung injury in mice. Int Immunopharmacol 19:103–109

Yao C, Oh JH, Oh IG et al (2013) [6]-Shogaol inhibits melanogenesis in B16 mouse melanoma cells through activation of the ERK pathway. Acta Pharmacol Sin 34:289–294

Yao J, Du Z, Li Z et al (2018) 6-Gingerol as an arginase inhibitor prevents urethane-induced lung carcinogenesis by reprogramming tumor supporting M2 macrophages to M1 phenotype. Food Funct 9 (9):4611–4620. https://doi.org/10.1039/c1038fo01147h

Yogosawa S, Yamada Y, Yasuda S et al (2012) Dehydrozingerone, a structural analogue of curcumin, induces cell-cycle arrest at the G2/M phase and accumulates intracellular ROS in HT-29 human colon cancer cells. J Nat Prod 75:2088–2093

Zainal NS, Gan CP, Lau BF et al (2018) Zerumbone targets the CXCR4-RhoA and PI3K-mTOR signaling axis to reduce motility and proliferation of oral cancer cells. Phytomedicine 39:33–41

Zarezadeh M, Saedisomeolia A, Khorshidi M et al (2018) Asymmetric dimethylarginine and soluble inter-cellular adhesion molecule-1 serum levels alteration following ginger supplementation in patients with type 2 diabetes: a randomized double-blind, placebo-controlled clinical trial. J Complement Integr Med. https://doi.org/10.1515/jcim-2018-0019

Zeraati H, Shahinfar J, Hesari S et al (2016) The effect of ginger extract on the incidence and severity of nausea and vomiting after cesarean section under spinal anesthesia. Anesth Pain Med 6:e38943. https://doi.org/10.5812/aapm.38943

Zhang M, Viennois E, Prasad M et al (2016a) Edible ginger-derived nanoparticles: a novel therapeutic approach for the prevention and treatment of inflammatory bowel disease and colitis-associated cancer. Biomaterials 101:321–340

Zhang M, Xiao B, Wang H et al (2016b) Edible ginger-derived nanolipids loaded with doxorubicin as a novel drug-delivery approach for colon cancer therapy. Mol Ther 24:1783–1796

Zhang F, Thakur K, Hu F et al (2017) 10-Gingerol, a phytochemical derivative from "tongling white ginger", inhibits cervical cancer: insights into the molecular mechanism and inhibitory targets. J Agric Food Chem 65:2089–2099

Zhao X, Yang ZB, Yang WR et al (2011) Effects of ginger root (*Zingiber officinale*) on laying performance and antioxidant status of laying hens and on dietary oxidation stability. Poult Sci 90:1720–1727

Zhou L, Qi L, Jiang L et al (2014) Antitumor activity of gemcitabine can be potentiated in pancreatic cancer through modulation of TLR4/NF-kappaB signaling by 6-shogaol. AAPS J 16:246–257

Zhu Y, Wang P, Zhao Y et al (2016) Synthesis, evaluation, and metabolism of novel [6]-shogaol derivatives as potent Nrf2 activators. Free Radic Biol Med 95:243–254

Zhu Y, Wang F, Zhao Y et al (2017) Gastroprotective [6]-gingerol aspirinate as a novel chemopreventive prodrug of aspirin for colon cancer. Sci Rep 7:40119

Zhu J, Chen H, Song Z et al (2018) Effects of ginger (*Zingiber officinale* Roscoe) on Type 2 diabetes mellitus and components of the metabolic syndrome: a systematic review and meta-analysis of randomized controlled trials. Evid Based Complement Alternat Med 2018:5692962. https://doi.org/10.1155/2018/5692962

Zhuang X, Deng ZB, Mu J et al (2015) Ginger-derived nanoparticles protect against alcohol-induced liver damage. J Extracell Vesicles 4:28713. https://doi.org/10.3402/jev.v4.28713

Zick SM, Djuric Z, Ruffin MT et al (2008) Pharmacokinetics of 6-gingerol, 8-gingerol, 10-gingerol, and 6-shogaol and conjugate metabolites in healthy human subjects. Cancer Epidemiol Biomarkers Prev 17:1930–1936

Zick SM, Turgeon DK, Ren J et al (2015) Pilot clinical study of the effects of ginger root extract on eicosanoids in colonic mucosa of subjects at increased risk for colorectal cancer. Mol Carcinog 54:908–915

Berberine

Ajay Srivastava, Anita Sinha, Rajiv Lall, and Ramesh C. Gupta

Abstract

Berberine (BBR) is an isoquinoline alkaloid that can be obtained from many plants of genus *Berberis*, *Hydrastis*, *Coptis*, and *Argemone*. Common plants that are sources for BBR include *B. vulgaris* (barberry), *B. aristata* (tree turmeric), *B. aquifolium* (Oregon grape), *B. thunbergii*, *C. chinensis* (*Coptis* or Chinese goldthread), *C. trifolia* (American goldthread), *C. japonica*, *H. canadensis* (goldenseal), *Argemone mexicana* (prickly poppy), and *Thalictrum lucidum*. Following the oral administration, BBR is unstable in the GI tract and poorly bioavailable. It has wide pharmacological and therapeutic applications because of its multiple mechanisms and target effects. The therapeutic use of BBR has been described in obesity, type 2 diabetes, hyperlipidemia, hypertension, congestive heart failure, neurodegenerative diseases, hepatic diseases, chronic trachoma, and cancer. This chapter describes pharmacokinetics, pharmacological and therapeutic applications, and safety and toxicity assessment of BBR and its major metabolites.

Keywords

Berberine · Dihydroberberine · Thalifendine · Jatrorrhizine · Type 2 diabetes · Hypertension · Cognitive disorder syndrome · Obesity · Chronic trachoma · Bacterial diarrhea

1 Introduction

The use of berberine (BBR)-containing plants dates back 3000 years in the Ayurvedic and Chinese medicinal systems. BBR is an isoquinoline alkaloid (2,3-methylenedioxy-9,10-di-methoxyprotoberberine) naturally found in the roots, rhizomes, and stem barks of plants including *Berberis vulgaris* (barberry), *B. aristata* (tree turmeric), *B. aquifolium* (Oregon grape), *Hydrastis canadensis* (goldenseal), *Coptis chinensis* (Chinese goldthread), *C. trifolia* (American goldthread), *C. japonica*, *Argemone mexicana* (prickly poppy), *Thalictrum lucidum*, and others. It has a chemical formula of $C_{20}H_{18}NO_4^+$ with a molecular weight of 336.361. The chemical structure of BBR is shown in Fig. 1.

BBR is used singly or in combination with other supplements/drugs in various ailments, including cognitive impairment, type 2 diabetes, metabolic syndrome, cardiac arrhythmia, congestive heart failure, hyperlipidemia, bacterial diarrhea, obesity, chronic trachoma, and cancer. Since BBR exerts antimicrobial activity, it is considered to be antibiotic. Preclinical studies have well established its potent antimicrobial, antiprotozoal, antifungal, antioxidant, anti-inflammatory, antitumor/anticancer, immunomodulatory, neuroprotective, hepatoprotective, nephroprotective, carminative, uterotonic, and antipyretic properties. Recent in vitro studies suggest the possibility for berberine to be used in osteoarthritis (Liu et al. 2015). BBR exerts its therapeutic effects via multiple pharmacological mechanisms by targeting various receptors, transmitters, enzymes, and other significant biomolecules. Doses for therapeutic efficacy and safety of BBR can vary depending on the disease and animal species. This chapter describes various aspects of BBR including pharmacokinetics, pharmacology, therapeutics, and toxicity and safety assessment.

A. Srivastava · A. Sinha · R. Lall
Vets Plus Inc., Menomonie, WI, USA

R. C. Gupta (✉)
Toxicology Department, Breathitt Veterinary Center, Murray State University, Hopkinsville, KY, USA
e-mail: rgupta@murraystate.edu

R. C. Gupta et al. (eds.), *Nutraceuticals in Veterinary Medicine*, https://doi.org/10.1007/978-3-030-04624-8_5

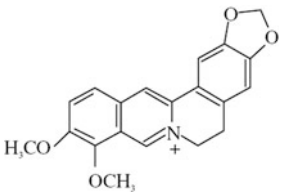


Fig. 1 Chemical structure of berberine

2 Pharmacokinetics and Pharmacodynamics

The oral bioavailability of BBR is low (<1%) due to its poor aqueous solubility, first-pass elimination, interaction with P-glycoprotein, and dissolution (Pan et al. 2002; Chen et al. 2011; Fratter and De Servi 2014; Imenshahidi and Hosseinzadeh 2015; Liu et al. 2015). Kheir et al. (2010) noted a significant difference in bioavailability between the different routes of administration (oral, intravenous, and intraperitoneal). Following oral administration, BBR is usually converted in the gut to dihydroberberine (dhBBR), which is five times more absorbable (Feng et al. 2015). BBR and its 14 metabolites are shown in Fig. 2.

With a single oral dose of BBR (200 mg/kg) in rats, Tan et al. (2013) observed that BBR was quickly distributed in the liver, kidneys, muscle, lungs, brain, heart, pancreas, and fat in descending order of amount. The pharmacokinetic profile indicated that BBR's level in most of the studied tissues was higher than in plasma at 4 h post-administration. Major metabolites of BBR are thalifendine, berberrubine, jatrorrhizine, and demethyleneberberine. In rat plasma, these metabolites can be present as free and glucuronide conjugates (Zuo et al. 2006). Following oral administration of BBR, the highest concentrations of BBR and its metabolites are found in the liver, the site for cholesterol, triglycerides and glucose production and metabolism and pharmacological effects.

In a rat noncompartmental model, unbound BBR is transported to the bile through active transport, and it is metabolized by a cytochrome P450 enzyme system in the liver, with phase I demethylation and phase II glucuronidation (Tsai and Tsai 2003). BBR has four main metabolites identified in rats (berberrubine, thalifendine, demethyleneberberine, and jatrorrhizine), all of which have glucuronide conjugates. Following an oral dose of BBR chloride (0.9 g/day for 3 days) in healthy adults, three sulfate metabolites (jatrorrhizine-3-sulfate, thalifendine-10-sulfate, and demethyleneberberine-2-sulfate) were identified in the urine (Qiu et al. 2008). These authors also identified six additional metabolites (jatrorrhizine-3-*O*-*β*-D-glucuronide, thalifendine-10-*O*-*β*-D-glucuronide, berberrubine-9-*O*-*β*-D-glucuronide, 3,10-demethylpalmatine-10-*O*-sulfate, columbamin-2-*O*-*β*-D-glucuronide, and demethyleneberberine-2,3-di-*O*-*β*-D-glucuronide) following BBR administration in rats (100 mg/kg, p.o.) and humans (300 mg, p.o.). Oral administration of BBR at 200 mg/kg dose in rats resulted in excretion of BBR and its metabolites in the bile, urine, and feces with a total recovery rate of 22.83% BBR (19.07% of prototype and 3.76% of its metabolites) within 48 h (reviewed in Kumar et al. 2015). The maximum amount of BBR (84%) and its metabolites were excreted in the feces. Approximately 83% of BBR was excreted in the bile, and 78% of urinary excretion was thalifendine and berberrubine (Ma et al. 2013).

Pharmacokinetic studies have been conducted with BBR in dogs following oral or intravenous administration (Shen et al. 1993; Feng et al. 2018). Feng et al. (2018a) studied the pharmacokinetics of BBR and its active metabolites in Beagles after single (50 mg/kg) or multiple doses (50 mg/kg/day) for 7 days. These investigators measured dhBBR, butyrate, and BBR, as well as its phase I and phase II metabolites. Results showed that dhBBR was not detected in dog plasma but was excreted in small amounts in the feces on days 3 and 7. Eleven metabolites were detected in plasma and feces after BBR administration, of which eight metabolites were phase I and three metabolites were phase II. The pharmacokinetic profile indicated that the concentration of BBR in the plasma was low, with a C_{max} of 36.88 ± 23.45 ng/mL. The relative content of glucuronic acid conjugates was higher than those of other metabolites in the plasma. In dog feces, BBR was detected with high concentrations on day 3 (2625.04 ± 1726.94 μg/g) and day 7 (2793.43 ± 488.10 μg/g). In rat, hamsters, and dogs, the gut microbiota has been shown to regulate pharmacokinetics of BBR and its metabolites (Zuo et al. 2006; Gu et al. 2015; Feng et al. 2018a).

Intestinal bacterial flora plays a role in enterohepatic circulation of BBR and its conjugated metabolites, while a very small amount of unchanged BBR is eliminated in the urine. Zuo et al. (2006) emphasized that rat intestinal flora may not exert significant metabolic activity against BBR and its metabolites, but it may play a significant role in the enterohepatic circulation of metabolites. The liver and intestinal bacteria participate in the metabolism and disposition of BBR in vivo.

In an in vitro study, Fratter and De Servi (2014) described a novel oral delivery system containing a chitosan-*N*-acetylcysteine salt capable of interacting with *P*-glycoprotein, partially inhibiting the BBR chloride extruding process. D-α-tocopheryl polyethylene glycol 1000 succinate (TPGS) has also been reported to inhibit P-glycoprotein (Dintaman and Silverman 1999). Chen et al. (2011) demonstrated that 2.5% TPGS improved peak concentration and area under the curve of BBR by 2.9 and 1.9 times, respectively, due to its ability to inhibit the activity of P-glycoprotein, thereby reducing the excretion of absorbed BBR into the intestinal lumen of rats.

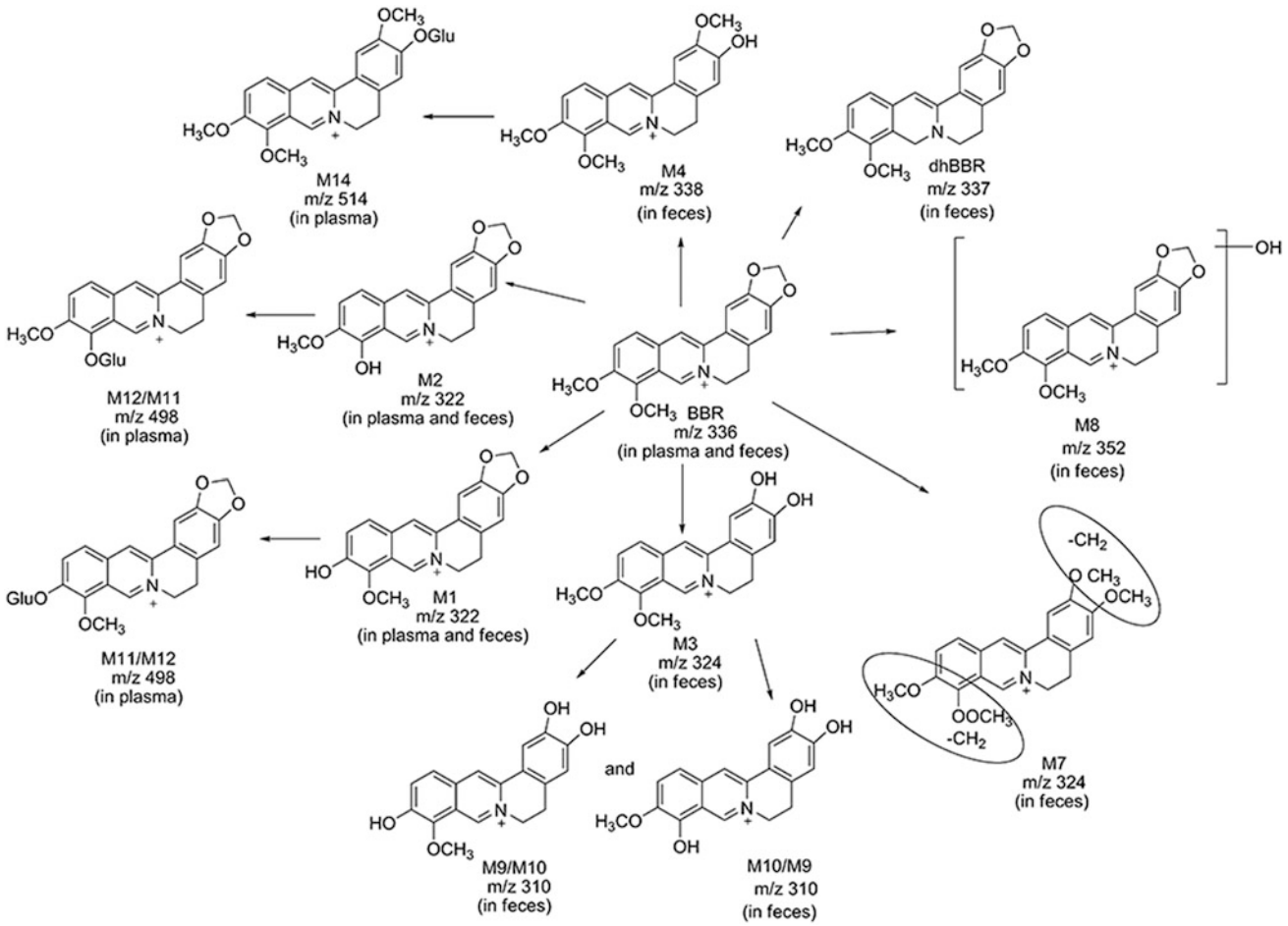


Fig. 2 Berberine and its 14 metabolites in dogs (Courtesy of Feng et al. 2018)

In a number of reports, BBR and its metabolites have exhibited anticancer properties (Li et al. 2015b; Bhattacharyya et al. 2017; Wang et al. 2017). Wang et al. (2017) prepared polyethylene glycol-modified long-circulating BBR liposomes and evaluated their efficacy and safety as potential antitumor agents. Results revealed that BBR liposomes may provide a safe form of intravenous drug therapy for strengthening the antitumor effects of BBR.

3 Pharmacological and Therapeutic Potential of BBR

Berberine is known to exert multiple pharmacological effects by having antioxidative, anti-inflammatory, and microsomal drug-metabolizing enzyme inhibitory properties. Its therapeutic potential has been recognized in a number of diseases and syndromes. The application of berberine in some health conditions has been described in brief.

3.1 Neurodegenerative Diseases

The use of BBR has been implicated in Alzheimer's disease (AD), Parkinson's disease (PD), anxiety, depression, and epilepsy (Peng et al. 2007; Ye et al. 2009; Kumar et al. 2015 also reviewed in chapter on "Cognitive dysfunction"). Although the bioavailability of BBR is poor, it crosses the blood–brain barrier (BBB) in adequate concentrations and accumulates in the hippocampus, where it provides potassium channel blocking, antiapoptotic, and neuroprotective effects (Wang et al. 2004, 2005, 2009; Ye et al. 2009). In addition, BBR exerts other pharmacological and biological actions, such as antioxidant, anti-inflammatory, analgesic, anxiolytic, and immunomodulatory actions, thereby providing preventive and therapeutic effects in neurological conditions (Kumar et al. 2015; Hussein et al. 2018).

BBR has been reported to improve learning and memory and to protect neurons from death by inhibiting AChE (Bhutada et al. 2011; Abd El-Wahab et al. 2013) and

normalizing ChAT, SOD, and MAO-B activities and cytokines, MDA, and neurotransmitters (norepinephrine, dopamine, and 5-HT) in discrete brain regions (Kulkarni and Dhir 2008a, b; Zhang et al. 2009; Kumar et al. 2015). Zhu and Qian (2006) demonstrated that BBR treatment (50 mg/kg, intragastrically once daily) for 14 days ameliorated spatial memory impairment in the rat model of AD. Kong et al. (2001) reported that BBR competitively inhibited MAO A in rat brain mitochondria, with an IC_{50} value of 126 μM. Yoo et al. (2006) found that BBR protects the hippocampal CA1 region from ischemic injury by inhibiting NMDA receptor 1 immunoreactivity in the ischemic gerbil brain.

BBR has also been shown to prevent or delay the process of AD by inhibiting the formation of $A\beta_{1-42}$, in addition to lowering oxidative stress and cholesterol (Abd El-Wahab et al. 2013; Cai et al. 2016). Asai et al. (2007) reported that BBR reduced Aβ levels by modulating amyloid precursor protein (APP) processing in human neuroglioma H4 cells. Additionally, quaternary protoberberine alkaloids, such as stepharanine, cyclanoline, and *N*-methyl stepholidine, from *Stephania venosa* were found to express AChE inhibitory activity (Ingkaninan et al. 2006). In the streptozotocin-induced diabetic rat model, BBR treatment (25–100 mg/kg, po, twice daily for 30 days) improved cognitive performance by normalizing AChE level and attenuating oxidative stress (Bhutada et al. 2011).

BBR appears to be a novel preventive and therapeutic modality for CDS in canine and felines.

3.2 Cardiovascular Effects

BBR has been extensively studied for its effects on the cardiovascular system (Lau et al. 2001; Affuso et al. 2010a; Xia and Luo 2015; Bagade et al. 2017). Xu et al. (1989) investigated the effects of BBR (5 mg/kg, IV) on ventricular tachyarrhythmias and electrophysiologic consequences in normal and ischemic myocardium in open-chest dogs subjected to programmed electrical stimulation (PES). The PES-induced ventricular tachycardia or ventricular fibrillation was prevented in four out of six BBR-treated dogs. BBR lengthened the QTc interval and the effective refractory period. These data suggest that BBR may be effective in preventing the onset of reentrant ventricular tachyarrhythmia and sudden coronary death after myocardial ischemic damage in dogs. In another study, Huang et al. (1992) reported that in dogs with ischemic left ventricular failure, BBR treatment (1 mg/kg IV bolus, followed by a constant infusion of 0.2 mg/kg/min for 30 min) improved left ventricular function by its positive inotropic effect and mild systemic vasodilation. BBR-treated dogs showed increased cardiac output and decreased left ventricular end-diastolic pressure and systemic vascular resistance. BBR increased coronary artery flow in anesthetized open-chest canines and isolated guinea pig hearts. A strong positive inotropic activity has been observed for BBR in dogs (Vik-Mo et al. 1983), rats, rabbits, guinea pigs (Sabir et al. 1978), and humans (Maroko and Ruzyllo 1983; Martin-Neto et al. 1988).

BBR can prolong the duration of ventricular action potential. In an in vitro study, Riccioppo (1993) demonstrated that BBR, in a concentration-dependent manner, increases the action potential (AP) duration in canine Purkinje and ventricular muscle fibers without affecting other parameters of the AP. BBR at 3–30 μM concentration produced a significant prolongation of AP duration in isolated guinea pig ventricular fibers without affecting the resting membrane potential and AP amplitude (Wang and Zheng 1997). Similar findings were observed in rats (Chi et al. 1996), cats (Sanchez-Chapula 1996), rabbits (Riccioppo 1993), and humans (Martin-Neto et al. 1988; Chi et al. 1996). BBR exerts class III antiarrhythmic and proarrhythmic actions in the cardiac muscle of the dog. Furthermore, spasms of isolated swine coronary arterial rings were prevented and treated effectively by BBR (Birdsall and Kelly 1997). BBR may exert its antiarrhythmic effect by acting as a Ca^{2+} agonist and/or a K^+ channel blocker. Cardiac ATP-sensitive K^+ (K_{ATP}) channels are considered potential targets for BBR's action. Lau et al. (2001) further reiterated that some of the cardiovascular effects of BBR and its derivatives are attributed to the blockade of K^+ channels (delayed rectifier and K_{ATP}) and stimulation of Na^+-Ca^{2+} exchanger. Evidence also suggests that BBR, by reducing tyrosine hydroxylase activity, has an inhibitory effect on catecholamine biosynthesis (Lee and Kim 1996).

The vasodilatory and hypotensive actions of BBR can be explained by several mechanisms. Ko and Lim (1980) reported that BBR caused hypotension in rabbits by blocking α-adrenoceptors. However, in dogs this effect appears to be due to the direct vascular action of BBR, which is independent of adrenergic, cholinergic, or histaminergic mechanisms (Sabir and Bhide 1971). BBR reduces blood pressure by competitively inhibiting vascular smooth muscle cells (VSMCs) α_1 receptors, thereby blocking the release of the enzyme adenylyl cyclase, which results in vasodilation and augmented acetylcholine activity (Zhang 2004). The hypotensive effect of BBR has been attributed to its inhibitory effect on acetylcholinesterase (Chun et al. 1979). BBR induces endothelium-dependent smooth muscle relaxation by increasing endothelial NO release (Ko et al. 2000). In a hypertensive rat model, Zhao et al. (2007) demonstrated that BBR at a dose of 5–10 mg/kg improved cardiac contractility, inhibited myocardial fibrosis, and reduced cardiac atrophy.

BBR is widely used for the treatment of congestive heart failure (CHF) (Martin-Neto et al. 1988; Zeng et al. 2003). Martin-Neto et al. (1988) examined the acute hemodynamic effects of intravenous BBR administration (0.2 mg/kg/min

for 30 min) in patients with CHF. A marked reduction in systemic and pulmonary vascular resistance, coupled with increased cardiac output, resulted in significant decreases in both systemic and pulmonary arterial pressures. Further evidence of improvement in cardiovascular function was shown by favorable BBR effects on several hemodynamic and echocardiographic indices of left ventricular performance. In another study, Zeng et al. (2003) treated CHF patients with BBR (1.2–2 g/day), in addition to conventional therapy (including ACE inhibitors, digoxin, diuretics, and nitrates), for 8 weeks. BBR-treated patients showed a significantly greater increase in left ventricular ejection fraction, exercise capacity, improvement of the dyspnea–fatigue index, and a decrease of frequency and complexity of ventricular premature complexes compared with the placebo group.

Hong et al. (2002) investigated the inhibitory effect of BBR on experimental cardiac hypertrophy in rats, which is regarded as a risk factor of chronic heart failure and other heart diseases. Cardiac hypertrophy was induced by suprarenal abdominal data constriction (banding). Rats were treated for 8 weeks with BBR (10 mg/kg body wt, po) and captopril (50 mg/kg body wt, po) beginning from 4 weeks after surgery. The findings revealed that (1) the elevated left ventricular and diastolic pressure was slightly decreased and (2) the maximum rates of contraction and relaxation ($\pm dp/dt_{max}$) were shortened, indicating that functions of the heart, both contraction and relaxation, were improved. Additionally (1) the whole heart and left ventricular weight and (2) cell size of the left ventricular myocardium were decreased compared with the banded rats. These data suggest that BBR can improve abnormal cardiac function and can prevent the development of left ventricular hypertrophy.

In conclusion, BBR exerts positive inotropic, negative chronotropic, antiarrhythmic, and vasodilator properties and can be used as a nutraceutical in hypertension, congestive heart failure, arrhythmias, and heart failure.

3.3 Antihyperlipidemic Effects

BBR has been shown to exert an antihyperlipidemic effect by lowering total cholesterol (TC), triglyceride (TG), and low-density lipoprotein cholesterol (LDL-C) levels in humans and animals (Kong et al. 2004; Bagade et al. 2017). BBR (500 mg) given twice daily for 3 months to hyperlipidemic patients significantly reduced total cholesterol by 29%, triglycerides by 35%, and LDL-C by 25% (Zhang et al. 2008). Dong et al. (2011) reported that BBR attenuates cardiac dysfunction in hypercholesterolemic and hyperglycemic rats. In atherogenic rats, BBR treatment (50–150 mg/kg) reduced total cholesterol by 29–33% and non-high-density lipoprotein (non-HDL) by 31–41%. Huang et al. (1992) found that BBR provided beneficial effects on hemodynamics during acute ischemic left ventricular failure in dogs. BBR, when combined with red yeast rice and policosanol, induced a positive effect on flow-mediated vasodilation in a double-blind, placebo-controlled trial on subjects with hypercholesterolemia (Cicero et al. 2007; Affuso et al. 2010b; Marazzi et al. 2011). Marazzi et al. (2011) observed statistically significant reduction in total cholesterol (−20%) and LDL-C (−31%) in elderly hypercholesterolemic humans or animals and suggested that this combined nutraceuticals is a better strategy for those who are statin-intolerant.

BBR can decrease cholesterol levels by multiple mechanisms, and some of these mechanisms may differ from those that are involved in statins (Kong et al. 2004). Statins upregulate the low-density lipoprotein receptor (LDLR) via inhibiting cholesterol synthesis. Statins inhibit the activity of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase. BBR induces downregulation of LDL-C by upregulation of LDLR expression. BBR stabilizes LDLR by an extracellular signal-regulated kinase (ERK)-dependent pathway and also by increasing transcriptional activity of the LDLR promotor via a c-Jun N-terminal kinase (JNK) pathway (Abidi et al. 2005; Choi et al. 2006; Lee et al. 2007; Tan et al. 2013; Xia and Luo 2015). Choi et al. (2006) reported that in 3T3-L1 cell leptin, transcription factors like sterol regulatory element-binding protein-1c (SREBP-1c) and CCAAT enhancer-binding protein-α (C/EBP-α), peroxisome proliferator-activated receptor-γ (PRAR-γ), fatty acid synthase, acetyl-coenzyme A carboxylase, acyl-CoA synthase, and lipoprotein lipase are reduced by BBR. BBR is also known to activate 5′-adenosine monophosphate (AMP) kinase (AMPK) while blocking the MAPK/ERK pathway, resulting in inhibition of lipid synthesis (Brusq et al. 2006; Xia and Luo 2015; Bagade et al. 2017). BBR is also reported to inhibit the differentiation of preadipocytes, reduce accumulation of lipid droplets, and lower triglyceride levels (Huang et al. 2006; Zhou et al. 2008).

Wang et al. (2014) reported that BBR produced a decrease in cholesterol levels by inhibiting cholesterol absorption (40–51%). BBR interfered with cholesterol micellarization and decreased cholesterol uptake by Caco-2 cells and permeability through Caco-2 monolayer. BBR also inhibited the gene and protein expressions of acyl-coenzyme A cholesterol acyltransferase-2 in the small intestine in rats and Caco-2 cells. In hyperlipidemic hamsters, Wang et al. (2010) found that the combined use of BBR and plant stanols has a greater synergistic effect on inhibiting cholesterol absorption than BBR or stanols alone. Furthermore, Li et al. (2015a, b) reported that hyperlipidemic hamsters treated with BBR (50 or 100 mg/kg) showed gradual decrease in liver

cholesterol levels and an increase in bile cholesterol levels, suggesting that BBR promotes excretion of cholesterol from the liver into the bile. In hamsters, Gu et al. (2015) demonstrated that orally administered BBR modulated the gut microbiota and BBR showed a significant inhibition of the 7α-dehydroxylation conversion of cholic acid to deoxycholic acid, indicating a decreased elimination of bile acids in the gut. In conclusion, BBR can be used as a nutraceutical in hyperlipidemia, hypercholesterolemia, and metabolic diseases.

3.4 Antihyperglycemic and Antidiabetic Effects

Type 2 diabetes mellitus is the most common form of diabetes. Alterations of endothelial homeostasis, due primarily to proinflammatory cytokines and reduced adiponectin secretion, appear to be two key events in insulin resistance and hyperglycemia. These conditions are associated with altered gene expression and cell signaling in the vascular endothelium, thereby affecting the release of endothelium-derived factors, activation of NADPH oxidase, uncoupling of endothelial NOS (eNOS), and expression of endothelin-1 (Bagade et al. 2017). This cascade of events often results in an imbalance between the production of vasodilator and vasoconstrictor mediators and the induction of adhesion molecules (Rask-Madsen and King 2007).

BBR has been found to be effective in type 2 diabetes. Chen and Xie (1986) reported that *Coptis chinensis* and BBR exert a hypoglycemic effect. In a meta-analysis, Dong et al. (2012) confirmed similar results. BBR produces antihyperglycemic effect in diabetes by multiple mechanisms:

1. Decreases glucose absorption by inhibition of α-glucosidase and reduces glucose transport through the intestinal epithelium (Pan et al. 2003)
2. Activates adenosine 5′-monophosphate-activated protein kinase (AMPK) (Lee et al. 2006; Yin et al. 2008a, b; Zhang and Chen 2012)
3. Induces glucose transport by enhancing GLUT1 gene expression (Kim et al. 2007)
4. Increases glucose uptake from blood to target organs, such as the skeletal muscle and adipose tissue (Zhang and Chen 2012)
5. Increases insulin receptor expression and improves insulin secretion and sensitivity and thereby reduces insulin resistance (Kong et al. 2009; Zhang et al. 2010a, b; Pérez-Rubio et al. 2013; Derosa et al. 2014; Xia and Luo 2015)
6. Directly inhibits gluconeogenesis in the liver (Xia et al. 2011; Zhang and Chen 2012)

Furthermore, Yin et al. (2008b) reported that BBR enhanced glucose metabolism by stimulation of glycolysis, which is related to the inhibition of glucose oxidation in mitochondria and consequently the induction of AMPK activation.

In an in vitro study, Wang et al. (2008) reported that BBR enhanced glucose-stimulated insulin secretion in rat pancreatic islets, and both mRNA and protein expressions of hepatic nuclear factor 4 alpha (*HNF4α*) were upregulated by BBR in a dose-dependent manner. Glucokinase activity was found to be increased accordingly. Recently, Bagade et al. (2017) further echoed on the underlying mechanism in BBR's effect on insulin sensitivity by increasing insulin receptor (*InsR*) expression in a dose- and time-dependent manner. This led to the promotion of glucose uptake in the presence of insulin. BBR induces *InsR* gene expression via transcriptional regulation through protein kinase C (PKC) (Zhang et al. 2010a). Additionally, BBR inhibits the intracellular accumulation of ROS, cellular apoptosis, and inflammation that characterize vascular injury, and these are mostly triggered by hyperglycemia (Affuso et al. 2010b; Bagade et al. 2017).

In a clinical study, BBR significantly lowered fasting blood glucose, hemoglobin A_{1c}, triglyceride, and insulin receptor expression in patients with type 2 diabetes (Zhang et al. 2010a, b). In a multicenter clinical trial, Li (2007) also reported that type 2 diabetic patients with dyslipidemia receiving BBR (1 g daily) for 3 months showed significantly reduced levels of blood glucose, hemoglobin A_{1c}, triglyceride, total cholesterol, and LDL-C compared to placebo. In another clinical study, BBR given three times a day at the dose of 500 mg to patients with type 2 diabetes caused significant reduction in hemoglobin A_{1c} (−2%), fasting plasma glucose (−44%), postprandial glucose (−45%), fasting plasma insulin (−28%), and homeostasis model assessment of insulin resistance index (44.7%) (Yin et al. 2008a). In an in vitro study, Hui et al. (1991) shown that BBR acts on both endothelial and underlying vascular smooth muscle cells to induce vasodilation via eNOS leading to NO production.

BBR has positive effects in treating diabetic nephropathy, diabetic neuropathy, and diabetic cardiomyopathy (Pang et al. 2015; Imenshahidi and Hosseinzadeh 2015). Diabetic nephropathy is a progressive kidney disease, which is pathologically characterized by thickened glomerular and tubular basement membranes, accumulation of the cellular matrix, and increased mesangial hypertrophy. The disease is induced by factors, such as dyslipidemia, hyperglycemia, hemodynamic abnormalities, and oxidative stress. Ni et al. (2015) suggested that by lowering blood glucose, regulating blood lipids, and reducing oxidative stress and inflammation, BBR has potential for treating diabetic nephropathy. In systematic reviews and meta-analysis, Zhang and Chen (2012) and Lan et al. (2015) found BBR to be highly effective in type 2 diabetes mellitus and diabetic complications with no serious side effects.

3.5 Hepatoprotective Effects

BBR has been found to be effective against hepatic disease and toxicity. Feng et al. (2010) determined the hepatoprotective effects of BBR on serum and tissue superoxide dismutase (SOD) levels and histopathology in carbon tetrachloride-induced liver injury in rats. The hepatoprotective mechanisms of BBR may be related to the elevation of antioxidative activity and attenuation of oxidative/nitrosative stress and free radical scavenging, as well as to the inhibition of TNF-α, iNOS, and COX-2 activities (Domitrovic et al. 2011). Li et al. (2014) showed that BBR ameliorated liver fibrosis in mice with carbon tetrachloride-induced liver injury and inhibited the proliferation of hepatic stellate cells in a dose- and time-dependent manner. BBR decreased the enzyme release of ALT, AST, and ALP in serum, elevated SOD, and reduced MDA content of liver. BBR treatment activated AMPK activity and decreased the protein expression of Nox4 and TGF-β1 and the phosphorylated Akt. BBR treatment also reduced the expression of smooth muscle actin (α-SMA), the marker of hepatic stellate cell. In another study, Janbaz and Gilani (2000) reported that pretreatment of rats with BBR (4 mg/kg, po, twice daily) for 2 days prevented the acetaminophen- or carbon tetrachloride-induced rise in serum levels of ALT, AST, and ALP, suggesting hepatoprotection. Furthermore, posttreatment with three successive oral doses of BBR (4 mg/kg every 6 h) reduced the hepatic damage induced by acetaminophen, while carbon tetrachloride-induced hepatotoxicity was not modified, suggesting a selective curative effect against acetaminophen.

Methotrexate, a commonly used anticancer and immunosuppressant drug, is known to cause hepatotoxicity. In a recent study, Mehrzadi et al. (2018) pretreated male Wistar rats with BBR (100 mg/kg body wt) for 10 consecutive days and treated rats with methotrexate (20 mg/kg, ip) on the ninth day. Rats were sacrificed on day 11, and the serum and liver were analyzed for various biochemical parameters. Results revealed that BBR might be useful for prevention of the hepatotoxicity induced by methotrexate via ameliorative effects on biochemical, inflammation, and oxidative indices.

Doxorubicin, an anthracycline anticancer drug, is extensively used in chemotherapy due to its efficacy in fighting a wide spectrum of malignancies. In addition to cardiotoxicity, doxorubicin causes hepatotoxicity in approximately 40% of patients. The main toxic effects on hepatocytes include (1) arrest cell cycle of hepatocytes (Kassner et al. 2008), (2) oxidative stress, and (3) disruption of electron transport chain (Zhao et al. 2012). Zhao et al. (2012) pretreated mice with BBR (60 mg/kg, ip) 1 h before doxorubicin (2.5 mg/kg, ip) on alternative days for 7 days. Findings revealed that BBR pretreatment significantly prevented doxorubicin-induced decline in body weight, increase in ALT and AST activities, and attenuated histopathological changes, such as vascular congestion, inflammatory cell infiltration, hepatocellular degeneration, necrosis, and fibrosis in the liver.

Metals such as mercury and lead induce hepatotoxicity by multiple mechanisms, including oxidative stress. Othman et al. (2014) reported that mice treated with mercuric chloride (0.4 mg/kg body wt) for 7 days caused oxidative stress by increasing lipid peroxidation and NO production with a concomitant decrease in glutathione and various antioxidant enzymes (SOD, catalase, glutathione peroxidase, and glutathione reductase). Liver enzymes and bilirubin were also found to be increased in serum. BBR (100 mg/kg body wt) treatment inhibited lipid peroxidation and NO production, restored antioxidant enzymes compared to control, and increased glutathione content. Histopathological examination of the liver revealed the protective effects of BBR against mercury-induced hepatotoxicity.

Laamech et al. (2017) investigated the protective effect of *B. vulgaris* aqueous extract against lead-induced hepatotoxicity in mice. Mice received lead acetate (5 mg/kg body wt) in water for 40 days. In another group, mice also received *B. vulgaris* extract (25, 50, 100, and 150 mg/kg body wt) daily for 30 days from day 11 after the beginning of lead acetate exposure. *B. vulgaris* treatment significantly prevented lead accumulation; increased ALT, AST, total bilirubin, and total cholesterol; inhibited lipid peroxidation and protein carbonyl formation; and normalized antioxidant enzymes (SOD, catalase, and glutathione peroxidase) and architecture of liver tissue.

In patients with nonalcoholic fatty liver disease (NAFLD), Yan et al. (2015) found that BBR (0.5 g three times a day) resulted in a significant reduction of NAFLD and related metabolic disorders. Recently, in an experimental study, Feng et al. (2018b) reported that rats treated with BBR (50 mg/kg body wt) plus curcumin (50 mg/kg body wt) exhibited lower body weight and fat weight compared with those treated with lovastatin (100 mg/kg body wt). Nutraceutical-treated group also showed lower levels of LDL-c, ALT, AST, ALP, MDA, and LSP compared with lovastatin group. Lower expression of SREBP-1c, pERK, TNF-α, and pJNK was also observed in BBR + curcumin group. Authors concluded that the combination of BBR and curcumin exhibited better ameliorative effects in treating NAFLD than lovastatin, and this enhanced effect is associated with oxidative stress, hepatic inflammation, and lipid metabolism.

3.6 Antimicrobial Effects

BBR has demonstrated significant antimicrobial activity against bacteria, fungi, protozoans, helminths, chlamydia, and viruses (Birdsall and Kelly 1997). Since BBR exerts

antimicrobial activity, it is considered to be an antibiotic. Its most common clinical uses have been for bacterial diarrhea, intestinal parasites, and ocular trachoma infections. BBR was shown to inhibit the intestinal secretory response due to cholera toxins. Using a ligated rabbit intestinal loop model, Sack and Froehlich (1982) demonstrated a significant suppression of the intestinal secretory response following exposure to *Vibrio cholerae* crude enterotoxin. It has been suggested that BBR may exert antidiarrheal effects by inhibiting the formation of certain organisms; inhibiting the formation of toxins, direct antagonism of the toxins; inhibiting intestinal ion secretion; and inhibiting smooth muscle contraction (reviewed in Birdsall and Kelly 1997). The antidiarrheal effects of BBR may also be mediated by its ability to delay intestinal transit time due to blockage of muscarinic acetylcholine (ACh) receptors, thereby inhibiting spontaneous intestinal peristalsis (Eaker and Sninsky 1989).

BBR sulfate has been reported to be effective against *Giardia lamblia*, *Trichomonas vaginalis*, and *Entamoeba histolytica* (Kaneda et al. 1991). The crude extract was found to be more effective than the BBR sulfate salt. In both rat and golden hamster models, BBR sulfate provided protection against *E. histolytica*-induced hepatic abscesses (reviewed in Birdsall and Kelly 1997). Ahuja et al. (1993) found BBR sulfate to be very effective against cutaneous leishmaniasis in domestic dogs when BBR sulfate (1%) was inoculated intralesionally. Ghosh et al. (1985) reported that golden hamsters infected with *L. donovani* showed parasite reduction up to 90% in the liver and spleen when treated with BBR (50 or 100 mg/kg i.p./day for 5 days).

3.7 Osteoarthritis

Osteoarthritis (OA) is a chronic debilitating disease affecting the entire joint, and its pathophysiology is very complex (reviewed in Gupta 2016; Gupta et al. 2019; Appleton 2018). One of the early triggers in development and progression of OA is synovial inflammation. Biochemical mediators found in OA synovial fibroblasts (OASFs) affecting the cellular functions of tissues include cytokines, chemokines, growth factors, and matrix metalloproteinases (MMPs). These mediators, such as interleukins (ILs) produced by OASFs, promote inflammation, neovascularization, and cartilage degradation, thereby contributing to joint destruction (Liu et al. 2015; Appleton 2018; Peffers et al. 2018; Watt 2018).

Connective tissue growth factor (CTGF, also known as CCN2) is an inflammatory mediator that is abundantly expressed in OA. IL-1β plays a pivotal role in OA pathogenesis. Liu et al. (2015) reported that CCN2-induced IL-1β expression is mediated by the activation of avβ3/avβ5 integrin-dependent ROS generation and subsequent activation of apoptosis signal-regulating kinase 1 (ASK1), p38/JNK, and NF-κB signaling pathways. The expression of IL-1β in OASFs has been reported to be attenuated by *N*-acetylcysteine (NAC), inhibitors of ASK1, p38, or JNK, or treatment with BBR. BBR has also been reported to reverse cartilage damage in an experimental model of collagenase-induced OA.

In in vitro and in vivo studies in rat OA model, Hu et al. (2011) demonstrated that BBR inhibited the expression of matrix metalloproteinases (MMP-1, MMP-3, and MMP-13) and increased the level of tissue inhibitor of metalloproteinase-1 at the mRNA level in a dose-dependent manner. In IL-1β-induced rat articular chondrocytes, BBR decreased IL-1β-induced glycosaminoglycan release and NO production, suggesting a chondroprotective effect. In high doses, BBR exhibited an anticatabolic effect in an IL-1-β-induced rat OA model. These findings suggest that BBR may play an anti-inflammatory, chondroprotective, and anticatabolic role in the development of OA, and BBR may be useful in the treatment of OA in animals.

4 Safety and Toxicity of BBR

In general, BBR shows very low toxicity and side effects (Pang et al. 2015; Imenshahidi and Hosseinzadeh 2015). Safety data of BBR reports its oral LD_{50} >29,586 and >15,000 mg/kg in mouse and rats, respectively. The LD_{50} of BBR in mice from intravenous (IV) and intraperitoneal (IP) injections is 9.03 and 57.61 mg/kg, respectively (Kheir et al. 2010). Following oral intragastric doses of BBR (10.4, 20.8, 41.6, and 83.2 g/kg), the LD_{50} could not be determined, although a 30% mortality rate was found among mice in the two highest dosage groups. In type 2 diabetics, humans have been given up to 1500 mg BBR (in three divided doses of 500 mg each) daily without any serious adverse effects. In high doses, BBR has been associated with arterial hypotension, dyspnea, flu-like symptoms, mild to moderate gastrointestinal discomfort, constipation, and cardiac damage (Imenshahidi and Hosseinzadeh 2008, 2015).

BBR has not been reported to produce genotoxic, cytotoxic, or mutagenic effects with its clinical doses (Birdsall and Kelly 1997). Berberine has been reported to cross the placenta and cause harm to the developing fetus. It has also been shown to exert a uterine stimulatory effect; therefore its use in pregnancy is cautioned. BBR can be transferred through breast milk; hence caution is required for BBR use while breastfeeding.

5 Concluding Remarks and Future Directions

BBR can be obtained from a number of plants of genus *Berberis*, *Hydrastis*, *Coptis*, *Argemone*, and *Thalictrum*. BBR has a strong potential for ameliorating chronic ailments,

such as neurodegenerative diseases, cardiovascular/metabolic disorders, type 2 diabetes mellitus, bacterial diarrhea, trachoma, gastroenteritis, etc., as it exerts multiple pharmacological actions. Although there is little concern about the side effects from BBR use, safety data from validated preclinical and clinical studies is warranted. Based on acute toxicity data, BBR can be classified as a nontoxic substance. Much of the literature is derived from in vitro studies and human clinical trials, while safety data based on animal studies are lacking. BBR should not be used by pregnant or lactating animals, as it may cause harm to the unborn or newly born.

Acknowledgment The authors would like to thank Ms. Robin B. Doss for her technical assistance in preparation of this chapter.

References

Abd El-Wahab AE, Ghareeb DA, Sarhan EEM et al (2013) In vitro biological assessment of *Berberis vulgaris* and its active constituent, berberine: antioxidant, anti-acetylcholinesterase, anti-diabetic and anticancer effects. BMC Compliment Altern Med 13:218–244

Abidi P, Zhou Y, Jiang JD et al (2005) Extracellular signal regulated kinase-dependent stabilization of hepatic low density lipoprotein receptor mRNA by herbal medicine berberine. Arterioscler Thromb Vasc Biol 25:2170–2176

Affuso F, Mercurio V, Fazio V et al (2010a) Cardiovascular and metabolic effects of berberine. World J Cardiol 2:71–77

Affuso F, Ruvolo A, Micillo F et al (2010b) Effects of a nutraceutical combination (berberine, red yeast rice and policosanols) on lipid levels and endothelial function in randomized, double-blind, placebo-controlled study. Nutr Metab Cardiovasc Dis 20:656–661

Ahuja A, Purohit SK, Yadav JS et al (1993) Cutaneous leishmaniasis in domestic dogs. Indian J Public Health 37:29–31

Appleton FE (2018) Osteoarthritis biomarkers: year in review. Osteoarthr Cart 26:312–318

Asai M, Iwata N, Yoshikawa A et al (2007) Berberine alters the processing of Alzheimer's amyloid precursor protein to decrease Aβ secretion. Biochem Biophys Res Commun 352(2):498–502

Bagade A, Tumbigeremutt V, Pallavi G (2017) Cardiovascular effects of berberine: a review of the literature. J Retroact Med 6:37–45

Bhattacharyya R, Saha B, Tyagi M et al (2017) Differential modes of photosensitization in cancer cells by berberine and coralyne. Free Radic Res 51(7–8):723–738

Bhutada P, Mundhada Y, Bansod K et al (2011) Protection of cholinergic and antioxidant system contributes to the effect of berberine ameliorating memory dysfunction in rat model of streptozotocin-induced diabetes. Behav Brain Res 220(1):30–41

Birdsall TC, Kelly GS (1997) Berberine: therapeutic potential of an alkaloid found in several medicinal plants. Altern Med Rev 2 (2):94–103

Brusq JM, Ancellin N, Grondin P et al (2006) Inhibition of lipid synthesis through activation of AMP kinase: an additional mechanism for the hypolipidemic effects of berberine. J Lipid Res 47:1281–1288

Cai Z, Wang C, Yang W (2016) Role of berberine in Alzheimer's. Neuropsychiatr Dis Treat 12:2509–2520

Chen QM, Xie MZ (1986) Studies on the hypoglycemic effect of *Coptis chinensis* and berberine. Acta Pharm Sin 21:401–406

Chen W, Miao Y-Q, Fan D-J et al (2011) Bioavailability study of berberine and the enhancing effects of TPGS on intestinal absorption in rats. AAPS PharmSciTech 12(2):705–711

Chi JF, Chu SH, Lee CS et al (1996) Mechanical and electrophysiological effects of 8-oxoberberine (JKL1073A) on atrial tissue. Br J Pharmacol 118:503–512

Choi BH, Ahn IS, Kim YH et al (2006) Berberine reduces the expression of adipogenic enzymes and inflammatory molecules of 3T3L1 adipocyte. Exp Mol Med 38:599–605

Chun YT, Yip TT, Lav KL et al (1979) A biochemical study of the hypotensive effect of berberine in rats. Gen Pharmacol 10:177–182

Cicero AF, Rovati LC, Sentikar I (2007) Epidemic effects of berberine administered alone or in combination with other natural cholesterol-lowering agents: a single-blind clinical investigation. Arzneim Forsch 57:26–30

Derosa G, Limas CP, Macías PC et al (2014) Dietary and nutraceutical approach to type 2 diabetes. Arch Med Sci 10:336–344

Dintaman JM, Silverman JA (1999) Inhibition of P-glycoprotein by D-alpha-tocopheryl polyethylene glycol 1000 succinate (TPGS). Pharm Res 16(10):1550–1556

Domitrovic R, Jakovac H, Blagojevic G (2011) Hepatoprotective activity of berberine is mediated by inhibition of TNF-alpha, COX-2, and iNOS expression in CCL_4-intoxicated mice. Toxicology 280:33–43

Dong SF, Hong Y, Liu M et al (2011) Berberine attenuates cardiac dysfunction in hyperglycemic and hypercholesterolemic rats. Eur J Pharmacol 660:368–374

Dong H, Wang N, Zgao L, Lu F (2012) Berberine in the treatment of type 2 diabetes mellitus: a systemic review and meta-analysis. Evid Based Complement Alternat Med 2012:591654

Eaker EY, Sninsky CA (1989) Effect of berberine on myoelectric activity and transit of the small intestine in rats. Gastroenterology 96:1506–1513

Feng Y, Siu KY, Ye X et al (2010) Hepatoprotective effects of berberine on carbon tetrachloride-induced acute hepatotoxicity in rats. Chin Med 5:33

Feng R, Shou JW, Zhao ZX et al (2015) Transforming berberine into its intestine-absorbable form by the gut microbiota. Sci Rep 5:12155

Feng R, Zhao Z-X, Ma S-R et al (2018a) Gut microbiota-regulated pharmacokinetics of berberine and metabolites in beagle dogs after oral administration. Front Pharmacol 9:214

Feng W-W, Kuang S-Y, Tu C et al (2018b) Natural products berberine and curcumin exhibited better ameliorative effects on rats with non-alcohol fatty liver disease than lovastatin. Biomed Pharcother 99:325–333

Fratter A, De Servi B (2014) New oral delivery system to improve absorption of berberine: likely interaction of cationized chitosan with PG-P pump. Int J Drug Deliv Technol 591:33–42

Ghosh AK, Bhattacharyya FK, Ghosh DK (1985) *Leishmania donovani*: amastigote inhibition and mode of action of berberine. Exp Parasitol 60:404–413

Gu S, Cao B, Sun R et al (2015) A metabolomic and pharmacokinetic study on the mechanism underlying the lipid-lowering effect of orally administered berberine. Mol BioSyst 11(2):463–474

Gupta RC (2016) Nutraceuticals in arthritis. In: Gupta RC (ed) Nutraceuticals: efficacy, safety and toxicity. Academic, Amsterdam, pp 161–176

Gupta RC, Srivastava A, Lall R, Sinha A (2019) Osteoarthritis biomarkers. In: Gupta RC (ed) Biomarkers in toxicology, 2nd edn. Academic, Amsterdam, pp 929–943

Hong Y, Hui S-C, Chan T-Y et al (2002) Effect of berberine on regression of pressure-overload induced cardiac hypertrophy in rats. Am J Chin Med 30(4):589–599

Hu P-F, Chen W-P, Tang J-L et al (2011) Protective effects of berberine in an experimental rat osteoarthritis model. Phytother Res 25:878–885

Huang WM, Yan H, Jin JM et al (1992) Beneficial effects of berberine on hemodynamics during acute ischemic left ventricular failure in dogs. Chin Med J 105:1014–1019
Huang C, Zhang Y, Gong Z et al (2006) Berberine inhibits 3T3-L1 adipocyte differentiation through the PPAR gamma pathway. Biochem Biophys Res Commun 348:571–578
Hui KK, Yu JL, Chan WF et al (1991) Interaction of berberine with human platelet alpha 2 adrenoceptors. Life Sci 49:315–324
Hussein HM, Abd-Elmegied A, Ghareeb DA et al (2018) Neuroprotective effect of berberine against environmental heavy metals-induced neurotoxicity and Alzheimer's-like disease in rats. Food Chem Toxicol 111:432–444
Imenshahidi M, Hosseinzadeh H (2008) Pharmacological and therapeutic effects of *Berberis vulgaris* and its active constituent, berberine. Phytother Res 22:999–1012
Imenshahidi M, Hosseinzadeh H (2015) *Berberis vulgaris* and berberine: an update review. Phytother Res 30:1745–1764
Ingkaninan K, Phengpa P, Yuenyongsawad S et al (2006) Acetylcholinesterase inhibitors from *Stephania venosa* tuber. J Pharm Pharmacol 58(5):695–700
Janbaz KH, Gilani AH (2000) Studies on preventive and curative effects of berberine on chemical-induced hepatotoxicity in rodents. Fitoterapia 71:25–33
Kaneda Y, Torii M, Tanaka T (1991) *In vitro* effects of berberine sulfate on the growth and structure of *Entamoeba histolytica*, *Giardia lamblia* and *Trichomonas vaginalis*. Ann Trop Med 15:417–423
Kassner N, Huse K, Martin HJ et al (2008) Carbonyl reductase 1 is a predominant doxorubicin reductase in the human liver. Drug Metab Dispos 36:2113–2120
Kheir MM, Wang Y, Hua L et al (2010) Acute toxicity of berberine and its correlation with the blood concentration in mice. Food Chem Toxicol 48:1105–1010
Kim SH, Shin EJ, Kim ED et al (2007) Berberine activates GLUT1-mediated glucose uptake in 3T3-L1 adipocytes. Biol Pharm Bull 30:2120–2125
Ko ST, Lim DY (1980) Influence of berberine on the blood pressure of rabbits. Arch Pharm Res 3:23–30
Ko WH, Yao XQ, Lau CW et al (2000) Vasorelaxant and antiproliferative effects of berberine. Eur J Pharmacol 399:187–196
Kong LD, Cheng CH, Tan RX (2001) Monoamine oxidase inhibitors from rhizoma of *Coptis chinensis*. Planta Med 67:74–76
Kong W, Wei J, Abidi P et al (2004) Berberine is a novel cholesterol-lowering drug working through a unique mechanism distinct from statins. Nat Med 10:1344–1351
Kong WJ, Zhang H, Song DQ et al (2009) Berberine reduces insulin resistance through protein kinase C-dependent up-regulation of insulin receptor expression. Metabolism 58:109–119
Kulkarni SK, Dhir A (2008a) On the mechanism of anti-depressant like action of berberine chloride. Eur J Pharmacol 589(1–3):163–172
Kulkarni SK, Dhir A (2008b) Berberine: a plant alkaloid with therapeutic potential for central nervous system disorders. Phytother Res 24 (3):317–324
Kumar A, Chopra EK, Mukherjee M et al (2015) Current knowledge and pharmacological profile of berberine: an update. Eur J Pharmacol 761:288–297
Laamech J, El-Hilaly J, Fetoui H et al (2017) *Berberis vulgaris* L. effects on oxidative stress and liver injury in lead-intoxicated mice. J Complement Integr Med. https://doi.org/10.1515/jcim-2015-0079
Lan J, Zhao Y, Dong F et al (2015) Meta-analysis of the effect and safety of berberine in the treatment of type 2 diabetes mellitus, hyperlipidemia and hypertension. J Ethnopharmacol 161:69–81
Lau C-W, Yao X-Q, Chen Z-Y et al (2001) Cardiovascular actions of berberine. Cardiovasc Drug Rev 19(3):234–244
Lee MK, Kim HS (1996) Inhibitory effects of protoberberine alkaloids from the roots of *Coptis japonica* on catecholamine biosynthesis in PC12 cells. Planta Med 62:31–34
Lee YS, Kim WS, Kim KH et al (2006) Berberine, a natural plant product, activates AMP-activated protein kinase with beneficial metabolic effects in diabetic and insulin-resistant states. Diabetes 55:2256–2264
Lee S, Lim HJ, Park JH et al (2007) Berberine induced LDLR up-regulation involves JNK pathway. Biochem Biophys Res Commun 362:853–857
Li X-Y (2007) Efficacy and safety of berberine in the treatment of diabetes with dyslipidemia. US ClinicalTrialsgov
Li J, Pan Y, Kan M et al (2014) Hepatoprotective effects of berberine on liver fibrosis via activation of AMP-activated protein kinase. Life Sci 98(1):24–30
Li W, Hua B, Saud SM et al (2015a) Berberine regulates AMP activated protein kinase signaling pathways and inhibits colon tumorigenesis in mice. Mol Carcinog 54:1096–1109
Li XY, Zhao ZX, Huang M et al (2015b) Effect of berberine on promoting the excretion of cholesterol in high-fat diet-induced hyperlipidemic hamsters. J Transl Med 13:278
Liu S-C, Lee H-P, Hung C-Y et al (2015) Berberine attenuates CCN2-induced IL-1β expression and prevents cartilage degradation in a rat model of osteoarthritis. Toxicol Appl Pharmacol 289:20–29
Ma JY, Feng R, Tan XS et al (2013) Excretion of berberine and its metabolites in oral administration in rats. J Pharm Sci 102 (11):4181–4192
Marazzi G, Cacciotti L, Pelliccia F et al (2011) Long-term effects of nutraceuticals (berberine, red yeast rice, policosanol) in elderly hypercholesterolemic patients. Adv Ther 28(12):1105–1113
Maroko PR, Ruzyllo W (1983) Hemodynamic effects of berberine, a new inotropic drug, in patients with congestive heart failure. Circulation 68:374
Martin-Neto JA, Maciel BC, Secches AL et al (1988) Cardiovascular effects of berberine in patients with severe congestive heart failure. Clin Cardiol 11:253–260
Mehrzadi S, Fatemi I, Esmaeilizadeh M et al (2018) Hepatoprotective effect of berberine against methotrexate induced liver toxicity in rats. Biomed Pharmacother 97:233–239
Ni WJ, Ding HH, Tang LQ (2015) Berberine as a promising anti-diabetic nephropathy drug: an analysis of its effects and mechanisms. Eur J Pharmacol 760:103–112
Othman MS, Safwath G, Aboulkhair M et al (2014) The potential effect of berberine in mercury-induced hepatorenal toxicity in albino rats. Food Chem Toxicol 69:175–181
Pan GY, Wang GJ, Liu XD et al (2002) The involvement of P-glycoprotein in berberine absorption. Pharmacol Toxicol 91:193–197
Pan GY, Huang ZJ, Wang GJ (2003) The antihyperglycemic activity of berberine arises from a decrease of glucose absorption. Planta Med 69:632–636
Pang B, Zhao LH, Zhou Q et al (2015) Application of berberine on treating type 2 diabetes mellitus. Int J Endocrinol 2015:905749
Peffers M, Balaskas P, Smagul A (2018) Osteoarthritis year in review: genetics and epigenetics. Osteoarthr Cart 26:304–311
Peng WH, Lo KL, Le YH et al (2007) Berberine produces anti-depressant-like effects in the forced swim test and in the tail suspension test in mice. Life Sci 81(11):933–938
Pérez-Rubio KG, González-Ortiz M, Martínez-Abundis E et al (2013) Effect of berberine administration on metabolic syndrome, insulin sensitivity, and insulin secretion. Metab Syndr Relat Disord 11 (5):366–369
Qiu F, Zhu Z, Kang N et al (2008) Isolation and identification of urinary metabolites of berberine in rats and humans. Drug Metab Dispos 36 (11):2159–2165
Rask-Madsen C, King GL (2007) Mechanisms of disease: endothelial dysfunction in insulin resistance and diabetes. Nat Clin Pract Endocrinol Metab 3:46–56
Riccioppo NF (1993) Electropharmacological effects of berberine on canine cardiac Purkinje fibers and ventricular muscle and atrial muscle of the rabbit. Br J Pharmacol 108:534–537

Sabir M, Bhide NK (1971) Study of some pharmacological action of berberine. Indian J Physiol Pharmacol 15:111–132

Sabir M, Akhter MH, Bhide NK (1978) Further studies of some pharmacology of berberine. Indian J Physiol Pharmacol 15:111–132

Sack RB, Froehlich JL (1982) Berberine inhibits intestinal secretory response of *Vibrio cholerae* and *Escherichia coli* enterotoxins. Infect Immun 35:471–475

Sanchez-Chapula J (1996) Increase in action potential duration and inhibition of the delayed rectifier outward current I_k by berberine in cat ventricular myocytes. Br J Pharmacol 117:1427–1434

Shen MP, Sun Q, Wang H (1993) Studies on the intravenous pharmacokinetics and oral absorption of berberine HCl in beagle dogs. Chin Pharmacol Bull 9:64–67

Tan XS, Ma JY, Feng R et al (2013) Tissue distribution of berberine and its metabolites after oral administration in rats. PLoS One 8(10): e77969

Tsai PL, Tsai TH (2003) Hepatic excretion of berberine. Drug Metab Dispos 32:405–412

Vik-Mo H, Faria DB, Cheung WM et al (1983) Beneficial effects of berberine on left ventricular function in dogs with congestive heart failure. Clin Res 31:224A

Wang YX, Zheng YM (1997) Ionic mechanism responsible for prolongation of cardiac action-potential duration by berberine. J Cardiovasc Pharmacol 30:214–222

Wang F, Zhao G, Cheng L et al (2004) Effects of berberine on potassium currents in acutely isolated CA1 pyramidal neurons of rat hippocampus. Brain Res 999:91–97

Wang X, Wang R, Xing D et al (2005) Kinetic difference of berberine between hippocampus and plasma in rat after intravenous administration of Coptidis rhizoma extract. Life Sci 77(24):3058–3067

Wang Z-Q, Lu F-R, Leng S-H et al (2008) Facilitating effects of berberine on rat pancreatic islets through modulating hepatic nuclear factor 4 alpha expression and glucokinase activity. World J Gastroenterol 14(39):6004–6011

Wang X, Su B, Zheng L et al (2009) The role of abnormal mitochondrial dynamics in the pathogenesis of Alzheimer's disease. J Neurochem 109:153–159

Wang Y, Jia X, Ghanam K et al (2010) Berberine and plant stanols synergistically inhibit cholesterol absorption in hamsters. Atherosclerosis 209:111–117

Wang Y, Yi X, Ghanam K et al (2014) Berberine decreases cholesterol levels in rats through multiple mechanisms, including inhibition of cholesterol absorption. Metabolism 63:1167–1177

Wang X, Wang Q, Liu Z et al (2017) Preparation, pharmacokinetics and tumor-suppressive activity of berberine liposomes. J Pharm Pharmacol 69:625–632

Watt EF (2018) Osteoarthritis biomarkers: year in review. Osteoarthr Cart 26:312–318

Xia L-M, Luo MH (2015) Study progress of berberine for treating cardiovascular disease. Chronic Dis Transl Med 1:231–235

Xia X, Yan J, Shen Y et al (2011) Berberine improves glucose metabolism in diabetic rats by inhibition of hepatic gluconeogenesis. PLoS One 6:e16556

Xu Z, Cao HY, Li Q (1989) Protective effects of berberine on spontaneous ventricular fibrillation in dogs after myocardial infarction. Acta Pharmacol Sin 10:320–324

Yan HM, Xia MF, Wang Y et al (2015) Efficacy of berberine in patients with non-alcoholic fatty liver disease. PLoS One 10:e0134172

Ye M, Fu S, Pi R et al (2009) Neuropharmacological and pharmacokinetic properties of berberine: a review of recent research. J Pharm Pharmacol 61:831–837

Yin J, Xing H, Ye J (2008a) Efficacy of berberine in patients with type 2 diabetes mellitus. Metabolism 57:712–717

Yin J, Gao Z, Liu D et al (2008b) Berberine improves glucose metabolism through induction of glycolysis. Am J Physiol Endocrinol Metab 294:E148–E156

Yoo KY, Hwang IK, Lim BO et al (2006) Berberry extract reduces neuronal damage and *N*-methyl-D-aspartate receptor 1 immunoreactivity in the gerbil hippocampus after transient forebrain ischemia. Biol Pharm Bull 29:623–628

Zeng X-H, Zeng X-J, Li Y-Y (2003) Efficacy and safety of berberine for heart failure secondary to ischemic or idiopathic dilated cardiomyopathy. Am J Cardiol 92:173–176

Zhang LS (2004) Clinical usage of berberine. Chin Rem Clin 41:78

Zhang M, Chen L (2012) Berberine in type 2 diabetes therapy: a new perspective for an old antidiarrheal drug? Acta Pharm Sin B 2 (4):379–386

Zhang Y, Li X, Zou D et al (2008) Treatment of type 2 diabetes and dyslipidemia with the natural plant alkaloid berberine. J Clin Endocrinol Metab 93:2559–2565

Zhang J, Yang JQ, He BC et al (2009) Berberine and total base from rhizoma *Coptis chinensis* attenuate brain injury in an aluminum-induced rat model of neurodegenerative disease. Saudi Med J 30 (6):760–766

Zhang H, Kong WJ, Shan YQ et al (2010a) Protein kinase D activation stimulates the transcription of the insulin receptor gene. Mol Cell Endocrinol 330:25–32

Zhang H, Wei J, Xue R et al (2010b) Berberine lowers blood glucose in type 2 diabetes mellitus patients through increasing insulin receptor expression. Metabolism 59:285–292

Zhao HP, Hong Y, Xie JD et al (2007) Effect of berberine on left ventricular remodeling in renovascular hypertensive rats. Yao Xue Xue Bao 42:336–341

Zhao X, Zhang J, Tong N et al (2012) Protective effects of berberine on doxorubicin-induced hepatotoxicity in mice. Biol Pharm Bull 35 (5):796–800

Zhou JY, Zhou SW, Zhang KB et al (2008) Chronic effects of berberine on blood, liver glucolipid metabolism and liver PPARs expression in diabetic hyperlipidemic rats. Biol Pharm Bull 31:1169–1176

Zhu F, Qian C (2006) Berberine chloride can ameliorate the spatial memory impairment and increase the expression of interleukin-1 beta and inducible nitric oxide synthase in the rat model of Alzheimer's disease. BMC Neurosci 7:78

Zuo F, Nakamura N, Akao T et al (2006) Pharmacokinetics of berberine and its main metabolites in conventional and pseudo germ-free rats determined by liquid chromatography/ion trap mass spectrometry. Drug Metab Dispos 34:2064–2072

Sea Buckthorn and Apricot Based Nutraceuticals

Vijay K. Bharti, Sahil Kalia, Arup Giri, and Bhuvnesh Kumar

Abstract

A high-altitude environment is characterized by hypobaric hypoxia, extreme temperature variation, low humidity, intense ultraviolet radiation, low rainfall, and high wind velocity. These types of extreme climatic conditions can result in oxidative stress in animals. This stress leads to a marked increase in cellular dysfunction and a decline in the production of antioxidant defense molecules which affects health and productivity of livestock especially at high altitudes. Therefore, the veterinary clinician is required to induce an upregulation of antioxidants and in the immune system to ameliorate the oxidative stress. Currently, nutraceuticals are used in nutritional therapy to manage various disease conditions and to improve productivity of pets and livestock animals. Nutraceuticals refer to natural functional foods or bioactive phytochemicals that have health-promoting and disease-preventing properties. These nutraceuticals in general contain alkaloids, flavonoids, some vitamins, trace minerals, etc. Various studies have revealed that apricot seed cake, sea buckthorn leaves, fruit pomace, and fruit pulp are rich in phytomolecules which modulate the immune system and upregulate the antioxidant defense system in broiler chicken, sheep, and goats. This chapter discusses the value of nutraceuticals and the usefulness of apricot (*Prunus armeniaca*) and sea buckthorn (*Hippophae rhamnoides*) in broiler chicken health management and improvement of weight gain in high-altitude regions.

V. K. Bharti (✉) · S. Kalia
DRDO-Defence Institute of High Altitude Research, Leh-Ladakh, India

A. Giri
Arni University, Kathgarh, Himachal Pradesh, India

B. Kumar
DRDO-Defence Institute of Physiology and Allied Sciences, Timarpur, Delhi, India

Keywords

Antioxidant · Apricot · High altitude · Nutraceuticals · Sea buckthorn

1 High-Altitude Environment

A high-altitude environment is characterized by hypobaric hypoxia, extreme temperature variation, low humidity, intense ultraviolet radiation, low rainfall, and high wind velocity. Physiologically, this environment is not suitable for good health and performance of animals and human beings. The growth performance of livestock animals that are raised in a cold, arid, high altitude is very poor due to the stressful environmental conditions. The climatic adversaries contribute to high-altitude oxidative stress, which ultimately hinders the growth rate of livestock by inducing their catabolic activities, thus producing a low financial return (Biswas et al. 2011; Kalia et al. 2017). The major consequence of oxidative stress is the marked increase in cellular dysfunction and the decline of the antioxidant defense system due to the increased generation of reactive oxygen species (Miller et al. 2013). Oxidative stress results from an excess generation of reactive oxygen species (ROS) like $O_2^{\cdot -}$, H_2O_2, and $ROO^{\cdot}$ that lead to cellular damage due to the interaction of ROS with cellular constituents. To maintain a healthy biological system, it is important to balance the presence of these species with antioxidant defense (Halliwell 1996; Valko et al. 2007). Antioxidants such as polyphenols are on the front line of investigation not only because of their natural origin but also their ability to act as free-radical scavengers, helping the endogenous antioxidant system (Katalinic et al. 2006; Ferreira et al. 2009). A whole range of plant-derived dietary supplements, phytochemicals, and provitamins that assist in maintaining good health and combating disease are now being described as functional foods and nutraceuticals (Bernal et al. 2011). Nutraceutical is a term coined in 1989 by

R. C. Gupta et al. (eds.), *Nutraceuticals in Veterinary Medicine*, https://doi.org/10.1007/978-3-030-04624-8_6

Stephen DeFelice. It is defined as a food or parts of food that provide medical or health benefits, including the prevention and treatment of disease. Nutraceuticals range from isolated nutrients, herbal products, dietary supplements, and diets to genetically engineered "designer" foods and processed products such as cereals, soups, and beverages. A nutraceutical is any nontoxic food extract supplement that has scientifically proven health benefits for both the treatment and prevention of disease. Nutraceutical also refers to natural functional/medical foods or bioactive phytochemicals that have health-promoting, disease-preventing, or medicinal properties. These nutraceuticals, in general, include vitamins, lipids, proteins, carbohydrates, minerals, and other necessary nutrients, depending on their emphases (Zeisel 1999; Whitman 2001). These nutraceuticals are used in nutritional therapy based upon their chemical structures and biological functions (Brower 1998). Phytomolecules such as polyphenols, flavonoids, vitamins, carotenoids, etc. are widely used as prophylactic and therapeutic agents in combating health problems associated with high altitude (Kala 2006). These phytogenic feed additives would be less toxic and ideal to replace antibiotic growth promoters in the broiler chick diet (Kalia et al. 2017, 2018).

This chapter describes the feed supplement of sea buckthorn and apricot plants on poultry for better health management and higher production levels at a high altitude.

2 Constraints and Prospects of Poultry Production at High-Altitude Region

Critical temperature is the temperature at which animals are properly sustained, and if the temperature varies slightly, then the animal may face difficulty. At higher altitudes the temperature significantly fluctuates throughout the year. As altitude rises then the partial pressure of oxygen is very low, and this causes significant effects on animal health as well as farming in the region. Poultry also face other problems related to low humidity, cold stress in winter, and high UV radiation. All these environmental factors are responsible for the poor sustainability of poultry farming in high-altitude regions. Unavailability of feed, lack of proper housing management, and unavailability of suitable germplasm are also critical factors in the regulation of poultry farming (Biswas et al. 2010; Kalia et al. 2017, 2018).

3 Important Diseases and Clinical Conditions of Poultry at High Altitude

The high-altitude environment is not suitable for livestock production, including poultry rearing, due to the harsh climate and the incidence of various physiological conditions, such as ascites, respiratory infection, impaction, coccidiosis, cannibalism, poor feed intake, stunted growth, extremely poor hatchability, cecal hemorrhage, etc. (Biswas et al. 2011).

4 Nonconventional Sources for Poultry Feeding at High-Altitude Cold Desert

High-altitude regions have no economic poultry feed available locally due to extreme climate and limited crop production. Therefore, poultry feeds are brought from other areas which are formulated based on nutrient requirements of chickens reared in specific areas such as the plains. Poultry raised at a high altitude require a special ration formulated specifically for those conditions. Unfortunately, there is very limited availability of this special ration. Therefore, farmers are supplementing poultry ration with antioxidants and other locally available feed resources like lucerne leaves, hydroalcoholic willow leaf extract, crushed oats, sea buckthorn fruit pomace and leaves, and apricot seed cake and seed extract (Biswas et al. 2011). These plant parts can be mixed with the existing poultry diet base as per palatability and acceptance. This feed supplementation with nutraceuticals improves poultry antioxidant defense and immune system requirements for amelioration of high-altitude stress prevalent in the region. Formulation of a nutraceutical-based poultry ration is greatly needed for high-altitude regions considering their nutrient requirements. This will improve health conditions and productivity of poultry in these regions.

5 Sea Buckthorn as a Source of Phytomolecules and Their Therapeutic Value

Hippophae rhamnoides (currently, *Elaeagnus rhamnoides*), also called sea buckthorn (SBT), belongs to the family *Elaeagnaceae* (Fig. 1). It is a significant herbal medicinal plant of the Trans-Himalayan cold desert and is commonly found at an altitude of 3000–4500 m above MSL (Saggu et al. 2007). It is a hardy plant which can tolerate extreme temperatures from −43 to 40 °C, and it contains barriers of different colors. Every part of the SBT plant is a good source of a large number of phytomolecules such as polyphenols, flavonols, flavonoids, proanthocyanidins, vitamins, carotenoids, organic acids, polyunsaturated fatty acids, and amino acids (Beveridge et al. 1999; Saggu et al. 2007; Ma et al. 2017; Puganen et al. 2018). In traditional Ayurvedic medicine, the extract of SBT fruits has been used for treatment of various kinds of health disorders (Saggu et al. 2007). Many bioactive phytomolecules such as *Hippophae*

Fig. 1 Sea buckthorn (*Hippophae rhamnoides*) plant with ripe fruit

cerebroside, vitamin C, vitamin E, gallic acid, kaempferol 3-*O*-sophoroside-7-*O*-rhamnoside, quercetin, etc. were identified in the berries of SBT which are responsible for its pharmacological properties (Chen et al. 2003; Zheng et al. 2009; Upadhyay et al. 2010). The leaves are a rich source of antioxidants including β-carotenoids, vitamin E, catechins, and folic acid and negligible amount of calcium, magnesium, and potassium (Upadhyay et al. 2010).

Various pharmacological activities of SBT, including antioxidative, immunomodulatory, anti-stress, anticancer, hepatoprotective, and radioprotective, have been reported in humans and livestock (Geetha et al. 2002; Goel et al. 2002; Yasukawa et al. 2009; Tulsawani 2010; Maheshwari et al. 2011; Olas et al. 2018). The seeds, leaves, and fruit of SBT are reported to be an ideal feeding material for livestock and poultry in the high-altitude Trans-Himalayan region (Biswas et al. 2011). Supplementation of SBT flavonoids in the diet of broiler chickens has shown a positive influence on their growth performance, fatty acid composition, and lipometabolism in the liver (Ma et al. 2015). Various studies report the usefulness of a sea buckthorn plant-based feed formulation in improving health conditions via modulation of various physio-biochemical indices. These studies are summarized in Table 1.

The potent hepatoprotective activity of SBT berry oils against aflatoxin B1 (AFB1) has been reported in broiler chickens (Solcan et al. 2013). Moreover, supplementation of SBT barriers in broiler chickens at different treatment concentrations improved their humoral and cell-mediated immune response against the adverse effects of T-2 toxin (Lavinia et al. 2009; Ramasamy et al. 2010). Similarly, the increased proliferative activity of chicken peripheral blood lymphocytes with the supplementation of SBT fruit extract has been reported in recent studies (Kalia et al. 2018). Supplementation of SBT as a feed additive in broiler chicken diet elevated the level of free-radical scavenging activity and decreased the level of lipid peroxidation in blood serum at higher altitudes (Kalia et al. 2018). These pharmacological activities of SBT in poultry may be due to the synergistic effect of certain bioactive phytomolecules present in SBT fruit (Table 1).

6 Apricot as Source of Various Phytomolecules and Their Therapeutic Value

Apricot, widely known as *Prunus armeniaca*, is an edible fruit that belongs to the family *Rosaceae* (Fig. 2), and it is grown in climates with very cold winters. Apricot can tolerate temperatures as low as −30 °C (Ahmadi et al. 2008). The major areas of apricot cultivation are in India and include the hilly areas of Himachal Pradesh, northeastern regions of Jammu and Kashmir, and the major growing area of Leh-Ladakh (Wani et al. 2015).

A large number of diverse bioactive phytomolecules such as polyphenols, flavonoids, carotenoids, vitamins, fatty acids, etc. have been found in the apricot, thereby giving this plant pharmacological antioxidative properties (Dragovic-Uzelac et al. 2007; Yigit et al. 2009; Kan et al. 2014). Apricot seeds are an abundant source of dietary proteins along with a significant amount of oil and fibers (Nout et al. 1995). Various pharmacological effects of the apricot have been reported, including antioxidant (Gomaa 2013), antimicrobial (Yigit et al. 2009), antitumor (Gomaa 2013), immunomodulatory (Tian et al. 2016), anti-inflammatory (Minaiyan et al. 2014),

Table 1 Effects of sea buckthorn (SBT) on poultry

No.	Experimental animal	Duration	Dose and route of administration	Effect	References
1.	Broiler chicken	42 days	3% flax oil and 3% dried SBT pomace in feed mixture	↓ Lipid oxidation, ↑ α-tocopherol, ↑ feed intake, ↑ feed conversion ratio (FCR), ↑ body weight, ↓ mortality, ↓ triglycerides, ↓ cholesterol, ↓ glucose, ↓ T3, ↓ T4	Orczewska-Dudek et al. (2018)
2.	Turkey poults	56 days	SBT leaf meal powder at 0.5% in feed mixture	↑ Body weight, ↑ feed conversion ratio (FCR), ↓ plasma uric acid, ↓ alkaline phosphatase, ↑ zinc	Sharma et al. (2018)
3.	Japanese quail	21 days	2% SBT leaf powder in drinking water	↓ Mortality, ↓ alanine aminotransferase (ALT), ↓ uric acid, ↓ cholesterol, ↑ total protein, ↑ albumin	Patial et al. (2015)
4.	Arbor Acres (AA) broilers	28 days	0.25%, 0.5%, 1% SBT powder in feed mixture	↑ Muscle inosine monophosphate, ↑ adenylosuccinate lyase	Zhao et al. (2012)
5.	Broiler chicks	49 days	5% SBT fruit residue in feed mixture	↑ Body weight, ↑ feed conversion ratio (FCR), ↓ mortality	Ben-Mahmoud et al. (2014)
6.	Broiler chicks	56 days	SBT seeds, leaves, and fruit residues in feed mixture	↑ Body weight, ↑ egg laying rate	Biswas et al. (2010)
7.	Broiler chicken	42 days	Group II diet contained 1000 ppm SBT leaf extract, group III contained 400 ppm of SBT pulp, and group IV contained 0.5 mL/kg SBT seed oil in feed	↑ Body weight, no changes in feed conversion ratio (FCR), carcass traits like chilled weight, breast weight, thigh weight, and drumstick weight were improved	Pathak et al. (2011)
8.	Arbor Acres male broilers	42 days	0.05%, 0.10%, and 0.15% SBT fruits in feed mixture	Improved average daily feed intake (ADFI), average daily gain (ADG), and final body weight (BW), ↓ abdominal fat percentage, ↑ intramuscular fat (IMF), ↑ thigh meat, ↑ breast muscle, quadratic effect on the abdominal fat percentage, ↓ levels of triglyceride, cholesterol, and low-density lipoprotein cholesterol	Ma et al. (2015)
9.	Laying hens	21 days	5% and 13% of SBT in feed mixture	↓ Egg productivity and egg weight, color of yolk increased significantly, feed consumption was the highest, no change in feed conversion	Pebriansyah and Silberov (2014)
10.	Isa Brown laying hens	322 days	SBT fruit residues in feed mixture	Total number of eggs laid and egg yolk color were detected, a nonsignificant effect of SBT was found on hen performance or egg quality including egg weight, yolk weight, eggshell strength, and shape index of egg, eggshell thickness, Haugh units, eggshell color and blood spot, albumen weight, proportion	Shaker et al. (2018)
11.	Poultry birds	28 days	Powdered SBT berries were added at 400 and 800 ppm	Significant increase in hemagglutination inhibition titer and total serum Ig	Ramasamy et al. (2010)
12.	RIR cross-bred broiler chickens	42 days	*H. rhamnoides* fruit extract in drinking water	↑ Body weight, ↑ feed conversion ratio (FCR), ↓ mortality, ↑ TAC, ↑ DPPH, ↓ LPO, ↑ total protein, ↑ albumin, ↑ globulin, ↓ cholesterol, ↓ triglyceride, ↑ HDL, ↓ LDL, ↓ glucose, ↑ creatinine, ↓ AST, ↓ ALT	Kalia et al. (2018)
13.	Peripheral blood lymphocyte	24 h	100 ng/mL–400 μg/mL extract	Reduced H_2O_2-induced oxidative stress in lymphocytes, stimulated PBL	Kalia et al. (2018)

↑ upregulation/increase/improvement, ↓ downregulation/decrease/deterioration, *ALP* alkaline phosphatase, *ALT* alanine transaminase, *AST* aspartate transaminase, *Zn* zinc, H_2O_2 hydrogen peroxidase, *LDH* lactate dehydrogenase, *LPO* lipid peroxidase, *MDA* malondialdehyde, *TAC* total antioxidant capacity, *HDL* high-density lipoprotein, *LDL* low-density lipoprotein, *DPPH* 2,2-diphenyl-1-picrylhydrazyl, *Ig* immunoglobulin

hepatoprotective (Yilmaz et al. 2015), radioprotective (Kurus et al. 2013), and cardioprotective (Parlakpinar et al. 2009).

Feeding of apricot seed cake to lambs under the high-altitude climatic conditions of Leh-Ladakh provided adequate nutrition to support normal body growth and had no adverse effects on performance (Jadhav et al. 2011). It has also been reported that supplementation of apricot kernel meal in broiler chicken diet produced a positive effect on the performance and intestinal microbiota (Samli et al. 2014). Furthermore, improved fatty acid composition in broiler meat was reported after administration of apricot without any deleterious effect on performance (Tekeli 2012).

Fig. 2 Apricot (*Prunus armeniaca*) plant at fruiting

Table 2 Effects of apricot on poultry

Experimental animal	Duration	Dose and route of administration	Effect	References
Broiler chickens	21 days	5%, 10%, and 20% apricot kernel meal	↑ Body weight, ↑ intestinal microbiota	Samli et al. (2014)
Broiler chickens	42 days	5, 10, and 15 g/kg apricot kernel oils	Increased the proportions of pentadecanoic acid, heptadecanoic (margaric) acid, heptadecanoic (margoleic) acid, linolenic acid, and eicosanoic acid	Tekeli (2012)
RIR cross-bred broiler chickens	42 days	Seed extract of 100, 150, 200, 300, 400, and 800 mg/kg body weight of chicken administered through drinking water	↑ Body weight, ↑ feed conversion ratio (FCR), ↓ mortality, ↑ TAC, ↑ DPPH, ↓ LPO, ↑ total protein, ↑ albumin, ↑ globulin, ↓ cholesterol, ↓ triglyceride, ↑ HDL, ↓ LDL, ↓ glucose, ↑ creatinine, ↓ AST, ↓ ALT	Kalia et al. (2018)
Peripheral blood lymphocyte	24 h	100, 200, 400, 800 ng/mL extract	Reduced H_2O_2-induced oxidative stress in lymphocytes, stimulated PBL	Kalia et al. (2018)

↑ upregulation/increase/improvement, ↓ downregulation/decrease/deterioration, *ALP* alkaline phosphatase, *ALT* alanine transaminase, *AST* aspartate transaminase, *Zn* zinc, H_2O_2 hydrogen peroxidase, *LDH* lactate dehydrogenase, *LPO* lipid peroxidase, *MDA* malondialdehyde, *TAC* total antioxidant capacity, *HDL* high-density lipoprotein, *LDL* low-density lipoprotein, *DPPH* 2,2-diphenyl-1-picrylhydrazyl, *Ig* immunoglobulin

Supplementation of apricot extract in broiler chicken diet stimulates increased digestion and metabolism of nutrients resulting in improved growth performance of broilers at a higher altitude (Kalia et al. 2017). Increased proliferative activity of chicken peripheral blood lymphocytes following supplementation with apricot extract has been reported (Kalia et al. 2017). Supplementation of apricot as a broiler feed additive also improved immunological, antioxidant, and blood biochemical profiles under high-altitude stress conditions (Kalia et al. 2017). These pharmacological activities in poultry may be due to the synergistic effect of certain bioactive phytomolecules present in the apricot (Table 2).

7 Mechanism of Therapeutic Value of Sea Buckthorn- and Apricot-Based Nutraceuticals

Some of the studies on feed supplements of sea buckthorn and apricot plant products indicate that growth performance, survivability rate, physio-biochemical indices, and economics are positively affected. But, the mechanism of action of these feed supplements has yet to be elucidated. Kalia et al. (2017) found that feeding an aqueous extract of *P. armeniaca* produced significantly higher total antioxidant capacity, free-radical scavenging activity, interleukin-2, total protein, albumin, and globulin levels as well as lower malondialdehyde, interleukin-6, glucose, cholesterol, triglyceride, ALT, and AST levels as compared to the control group. Another study found that *P. armeniaca* extract reduced the level of proinflammatory cytokine IL-6 in treatment groups. This reduction might be due to the anti-inflammatory activity of the polyphenolic compounds of *P. armeniaca* downregulating NF-kB signaling pathway via decreased phosphorylation of NF-kB. Moreover, *P. armeniaca* extracts stimulate the production of IL-2 via activation of the T helper cells 1 (Th1) and also play a central role in cell-mediated immunity. This suggests that *P. armeniaca* extract exerts immunomodulatory effects in broilers through mediating both cellular and humoral immunity. These same findings were also reported in the sea buckthorn feed supplements study on broiler chickens. Therefore, it may be concluded that both feed supplements have an immunomodulatory role along with antioxidative properties which ultimately lead to

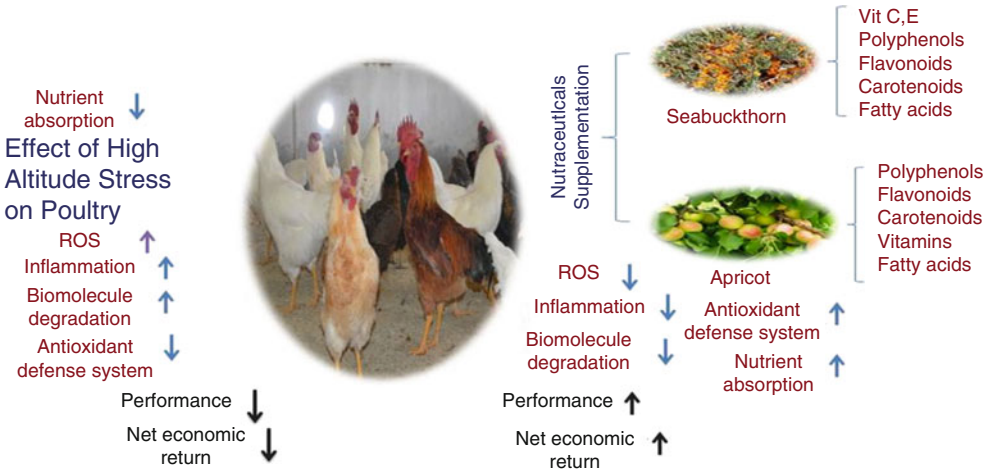


Fig. 3 Probable mechanism of therapeutic action of sea buckthorn- and apricot-based nutraceuticals

Table 3 Feed formulation based on sea buckthorn and apricot

Brand name	Composition	Manufacturer
Monosaturated sea buckthorn berry oil	Linoleic acid 4.7%, oleic acid 66.8%, unsaturated fatty acids 25.2%, phenolic 19%	Natures Natural India
Sea buckthorn oil	Linoleic acid, oleic acid, unsaturated fatty acids, etc.	Arian Enterprises
Sea buckthorn Omega7 oil	Omegas, flavonoids, omega-7	MNC Globle Multitrade
Apricot oil	Mixed fatty acids	Shri Hari Aromatics
Apricot oil	Oleic 64.2%, palmitic 5.0%, linoleic 28.3%, linolenic 0.2%, stearic 1.0%	Bo International
Apricot oil	Arginine, histidine, lysine, phenylalanine, valine, leucine, cystine and tryptophan, and methionine	Natural Cosmetic Supplies (Unit of Mother Herbs)

better growth performance and survivability (Tekeli 2012; Kalia et al. 2017, 2018). The most probable mechanism of action of feed the supplement of sea buckthorn and apricot is represented in Fig. 3.

8 Available Sea Buckthorn and Apricot Plant-Based Feed Formulation

Different types of feed formulations and herbal products have been developed by various institutions and private manufacturers for human and animal use. Scientists also developed a feed bolus (DB-LactoMax, a sea buckthorn and apricot preparation) for dairy cattle (90–100 g or 1 bolus daily/cow). It improves milk yield and body and reproductive condition. A liquid preparation (Immunobooster, 5–10 mL/100 birds) was developed for broiler chickens and layers that is comprised of sea buckthorn, apricot seed, and willow leaf extracts in preferable weight ratio. It enhances cytokines, immune status, and upregulation of antioxidant status in broiler birds under high-altitude stress conditions and thereafter improves overall health, growth performance (150 g in 42 days), and FCR. Bakery products like biscuits, buns, bread, cakes, jellies, health drinks, wine, food colorants, and yogurt have been made for human use also using sea buckthorn pulp and leaves. Some of these formulations are given in Table 3.

9 Concluding Remarks and Future Directions

This chapter describes the value of nutraceuticals and the therapeutic potential of sea buckthorn and apricot in poultry for improvement of their health and productivity in higher-altitude regions. Several research findings suggest the upregulation of cellular and extracellular antioxidant defense and the scavenging of free radicals. Recent findings indicate their immunomodulatory effect which may be beneficial in the control of immunosuppressive disease as well. These plant-based products may be useful in plain areas under different farm conditions. However, further studies are required to establish their dose regime. Moreover, continued research is needed to better understand the mechanisms and specific pathways involved in ROS-induced diseases and to determine the most rational and effective combination of antioxidants in veterinary clinical use for management of various disease conditions.

References

Ahmadi H, Fathollahzadeh H, Mobli H (2008) Some physical and mechanical properties of apricot fruits, pits and kernels (C.V. Tbarzeh). Am – Euras J Agric Environ Sci 3:703–707

Ben-Mahmoud Z, Mohamed MS, Bláha J et al (2014) The effect of sea buckthorn (*Hippophae rhamnoides l.*) Residues in compound feeds on the performance and skin color of broilers. Indian J Anim Res 48:548–555

Bernal J, Mendiola JA, Ibáñez E et al (2011) Advanced analysis of nutraceuticals. J Pharm Biomed Anal 55:758–774

Beveridge T, Li TSC, Oomah BD (1999) Seabuckthorn products: manufacture and composition. J Agric Food Chem 47:3480–3488

Biswas A, Bharti VK, Acharya S et al (2010) Seabuckthorn: new feed opportunity for poultry at cold arid Ladakh region of India. World's Poult Sci J 66:707–714

Biswas A, Bharti VK, Deshmukh PB et al (2011) Commercial poultry farming in cold arid region of Leh-Ladakh. In: Srivastava RB, Selvamurthy W (eds) Innovatives in agro animal technologies. Satish Serial Publishing House, New Delhi, pp 216–233

Brower V (1998) Nutraceuticals: poised for a healthy slice of the healthcare market? Nat Biotechnol 16:728–731

Chen Y, Zhong X, Liu T et al (2003) The study on the effects of the oil from *Hippophae rhamnoides* in hematopoiesis. Zhong Yao Cai 26:572–575

Dragovic-Uzelac V, Levaj B, Mrkic V et al (2007) The content of polyphenol and carotenoids in three apricot cultivars depending on stage of maturity and geographical region. Food Chem 102:966–975

Ferreira ICFR, Barros L, Abreu RMV (2009) Antioxidants in wild mushrooms. Curr Med Chem 16:1543–1560

Geetha S, Ram MS, Singh V et al (2002) Anti-oxidant and immunomodulatory properties of seabuckthorn (*Hippophae rhamnoides*) – an in vitro study. J Ethnopharmacol 79:373–378

Goel HC, Prasad J, Singh S et al (2002) Radioprotection by a herbal preparation of *Hippophae rhamnoides* RH-3, against whole body lethal irradiation in mice. Phytomedicine 9:15–25

Gomaa EZ (2013) *In vitro* antioxidant, antimicrobial and antitumor activities of bitter almond and sweet apricot (*Prunus armeniaca* L.) kernels. Food Sci Biotechnol 22:455–463

Halliwell B (1996) Antioxidants in human health and disease. Annu Rev Nutr 16:33–50

Jadhav SE, Charan G, Raj T, Bharti VK et al (2011) Performance and blood biochemical profile of lambs fed local unconventional feed ingredients at cold and high altitude conditions of Ladakh. Indian J Anim Sci 81:730–734

Kala CP (2006) Medicinal plants of the high altitude cold desert in India: diversity, distribution and traditional uses. Int J Biodiv Sci 2:43–56

Kalia S, Bharti VK, Gogoi D et al (2017) Studies on the growth performance of different broiler strains at high altitude and evaluation of probiotic effect on their survivability. Sci Rep 46074. https://doi.org/10.1038/srep46074

Kalia S, Bharti VK, Giri A et al (2018) *Hippophae rhamnoides* as novel phytogenic feed additive for broiler chickens at high altitude cold desert. Sci Rep 8:5954

Kan T, Gundogdu M, Ercisli S et al (2014) Phenolic compounds and vitamins in wild and cultivated apricot fruit grown in irrigated and dry farming conditions. Biol Res 47:46. https://doi.org/10.1186/0717-6287-47-46

Katalinic V, Milos M, Kulisic T et al (2006) Screening of 70 medicinal plant extracts for antioxidant capacity and total phenols. Food Chem 94:550–557

Kurus M, Ertan C, Celi MR et al (2013) Protective effect of apricot feeding in the pulmonary tissues of rats exposed to low dose X-Ray radiation. Indian J Appl Res 3:1–5

Lavinia S, Gabi D, Daniela M et al (2009) The effect of medicinal plants and plant extracted oils on broiler duodenum morphology and immunological profile. Romania Biotechnol Lett 14:4606–4614

Ma JS, Chang WH, Liu GH et al (2015) Effects of flavones of seabuckthorn fruits on growth performance, carcass quality, fat deposition and lipometabolism for broilers. Poult Sci 94:2641–2649

Ma X, Yang W, Laaksonen O et al (2017) Role of flavonols and proanthocyanidins in the sensory quality of sea buckthorn (*Hippophae rhamnoides* L.) berries. J Agric Food Chem 65:9871–9879

Maheshwari DT, Yogendra K, Verma MS et al (2011) Antioxidant and hepatoprotective activities of phenolic rich fraction of seabuckthorn (*Hippophae rhamnoides*) leaves. Food Chem Toxicol 49:2422–2428

Miller LE, McGinnis GR, Kliszczewicz B et al (2013) Blood oxidative-stress markers during a high-altitude trek. Int J Sport Nutr Exerc Metab 23:65–72

Minaiyan M, Ghannadi A, Asadi M et al (2014) Anti-inflammatory effect of *Prunus armeniaca L.* (apricot) extracts ameliorates TNBS-induced ulcerative colitis in rats. Pharm Res 9:225–231

Nout MJ, Tuncel G, Brimer L (1995) Microbial degradation of amygdalin of bitter apricot seeds (*Prunus armeniaca*). Int J Food Microbiol 24:407–412

Olas B, Skalski B, Ulanowska K (2018) The anticancer activity of sea buckthorn (*Elaeagnus rhamnoides* L., A. Nelson). Front Pharmacol 9:232

Orczewska-Dudek S, Pietras M, Nowak J (2018) The effect of amaranth seeds, sea buckthorn pomace and black chokeberry pomace in feed mixtures for broiler chickens on productive performance, carcass characteristics and selected indicators of meat quality. Ann Anim Sci 18:501–523

Parlakpinar H, Olmez E, Acet A et al (2009) Beneficial effects of apricot-feeding on myocardial ischemia-reperfusion injury in rats. Food Chem Toxicol 47:802–808

Pathak NL, Kasture SB, Bhatt NM et al (2011) Phytopharmacological properties of *Coriandrum sativum* as a potential medicinal tree: an overview. J Appl Pharm Sci 1:20–25

Patial V, Asrani RK, Patil RD et al (2015) Protective effect of sea buckthorn (*Hippophae rhamnoides L.*) leaves on ochratoxin-A induced hepatic injury in Japanese quail. Vet Res Int 3:98–108

Pebriansyah A, Silberov P (2014) The impact of the sea buckthorn (*Hippophae rhamnoides*) supplement in the feed ration on the quality of poultry products. Tropentag, Prague

Puganen A, Kallio HP, Schaich KM et al (2018) Red/green currant and sea buckthorn berry press residues as potential sources of antioxidants for food use. J Agric Food Chem 66(13):3426–3434

Ramasamy T, Varshneya C, Katoch VC (2010) Immunoprotective effect of seabuckthorn (*Hippophae rhamnoides*) and glucomannan on T-2 toxin-induced immunodepression in poultry. Vet Med Int 2010. Article ID: 149373. https://doi.org/10.4061/2010/14973

Saggu S, Divekar HM, Gupta V et al (2007) Adaptogenic and safety evaluation of seabuckthorn leaf extract: a dose dependent study. Food Chem Toxicol 45:609–617

Samli HE, Terzioglu M, Okur AA et al (2014) Effects of sweet apricot kernel meal on performance and intestinal microbiota in broiler chickens. J Teki Agric Fac 11:38–43

Shaker MM, Al-Beitawi NA, Bláha J et al (2018) The effect of sea buckthorn (*Hippophae rhamnoides L.*) fruit residues on performance and egg quality of laying hens. J Appl Anim Res 46:422–426

Sharma A, Shukla PK, Bhattacharyya A et al (2018) Effect of dietary supplementation of sea buckthorn and giloe leaf meal on the body weight gain, feed conversion ratio, biochemical attributes and meat composition of turkey poults. Vet World 11:93–98

Solcan C, Gogu M, Floristean V et al (2013) The hepatoprotective effect of seabuckthorn (*Hippophae rhamnoides*) berries on induced aflatoxin B1 poisoning in chickens. Poult Sci 92:966–974

Tekeli A (2012) Effect of apricot kernel on selected performance and blood parameters and meat fatty acid composition of broilers. J Anim Vet Adv 11:3697–3704

Tian H, Yan H, Tan S et al (2016) Apricot kernel oil ameliorates cyclophosphamide-associated immunosuppression in rats. Lipids 51:931–939

Tulsawani R (2010) Ninety days repeated gavage administration of *Hippophae rhamnoides* extract in rats. Food Chem Toxicol 48:2483–2489

Upadhyay NK, Kumar MSY, Gupta A (2010) Antioxidant, cytoprotective and antibacterial effects of seabuckthorn (*Hippophae rhamnoides*) leaves. Food Chem Toxicol 48:3443–3448

Valko M, Leibfritz D, Moncol J et al (2007) Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol 39:44–84

Wani SM, Masoodi FA, Wani TA et al (2015) Physical characteristics, mineral analysis and antioxidant properties of some apricot varieties grown in North India. Food Sci Technol 1:1–10

Whitman M (2001) Understanding the perceived need for complementary and alternative nutraceuticals: lifestyle issues. Clin J Oncol Nurs 5:190–194

Yasukawa K, Kitanaka S, Kawata K et al (2009) Anti-tumor promoters phenolics and triterpenoid from *Hippophae rhamnoides*. Fitoterapia 80:164–167

Yigit D, Yigit N, Mavi A (2009) Antioxidant and antimicrobial activities of bitter and sweet apricot (*Prunus armeniaca* L.) kernels. Braz J Med Biol Res 42:346–352

Yilmaz I, Cetin A, Bilgic Y (2015) Hepatoprotective effects of apricot against acetaminophen induced acute hepatotoxicity in rats. Am J Pharmacol Sci 3:44–48

Zeisel SH (1999) Regulation of "nutraceuticals". Science 285:185–186

Zhao W, Chen X, Yan C et al (2012) Effect of sea buckthorn leaves on inosine monophosphate and adenylosuccinatelyase gene expression in broilers during heat stress. Asian-Australas J Anim Sci 25:92–97

Zheng RX, Xu XD, Tian Z et al (2009) Chemical constituents from the fruits of *Hippophae rhamnoides*. Nat Prod Res 23:1451–1456

Nigella sativa

Rahul Sharma, Pushpkant Sahu, Amul Jain, Vivek Kumar, Dharmendra Khokhar, Arvind Kumar Geda, and Bhanushree Gupta

Abstract

The wide versatility of medicinal plants has made them of great interest all around the world. *Nigella sativa* (NS, commonly called black seed), a member of *Ranunculaceae* family, is one of the most important medicinal plants. NS is reported in the treatment of a wide variety of medical ailments due to its chemical constituents which may enhance the source of functional and nutritional foods. The constituents of NS have the competence to cure many biological ailments including, asthma, diabetes, digestive diseases, inflammatory diseases, and rheumatoid arthritis. Wide-ranging pharmacological activities including analgesic, antidiabetic, anticancer, anti-inflammatory, antimicrobial, immunostimulatory, bronchodilator, spasmolytic, antihistaminic, and hepatoprotective have been reported for the seeds and oil extract of NS. It affects the reproductive system, central nervous system, and immune system as anticonvulsant and wound-healing mediators. NS can be exploited for producing a series of drugs for the medication of numerous diseases. In the present chapter, we discussed the general introduction of NS and its geographical location followed by chemical constituents. Further, some crucial pharmacological activities and toxicological effects of NS are also described.

Keywords

Nigella sativa · Medicinal plant · Nutraceutical · Thymoquinone · Phytochemical

1 Introduction

Medicinal plants are a major source for treating human ailment throughout the world since ancient times. These plants are invaluable resources, useful in daily life as pigments, food additives, flavors, fragrance, and pharmaceuticals. Today scientists are interested in identification of their main constituents and elucidating their mechanism of action. Many studies have concluded that compounds such as phenolics, flavonoids, alkaloids, terpenoids, saponins, tannins, and anthraquinones have beneficial effects as antioxidant, anti-inflammatory, immunomodulatory, antimicrobial, anticancer, antidiabetic, etc. (Omojate et al. 2014). According to a study (Vuorelaa et al. 2004), in the last 20 years, more than 25% of drugs are directly isolated from plants, and the other 25% are obtained from their chemically derived products. The plant NS, locally known as "kalonji," is used as a traditional medicine to cure many diseases such as diarrhea, asthma, etc. (Tasawar et al. 2011; Gilani et al. 2001; Benhaddou-Andaloussi et al. 2011). The diverse chemical components of NS seeds provide excellent opportunity for development and innovation in the area of medicinal drug.

The seed of *Nigella sativa* (Ranunculaceae) has been used for many years as a spice, food preservative, and medicinal drug to cure many ailments (Abdulelah and Zainal-Abidin 2007; Goreja 2003). They are known as black seed because when they are exposed to air, they turn into black-colored seeds (Goreja 2003). It is an annual herb with an average height of about 20–90 cm. Its leaves are about 2.5–5.0 cm long and linear hastate-shaped. Its flowers are pale blue colored and seeds are black in color, usually flattened,

Author contributed equally with all other contributors. Pushpkant Sahu, Amul Jain and Vivek Kumar

R. Sharma · D. Khokhar · A. K. Geda
Department of Plant Physiology, Agril. Biochemistry, Medicinal and Aromatic Plants, Indira Gandhi Agricultural University, Raipur, Chhattisgarh, India

P. Sahu · A. Jain · V. Kumar · B. Gupta (✉)
Department of Chemistry, Center for Basic Sciences, Pt. Ravishankar Shukla University, Raipur, India

R. C. Gupta et al. (eds.), *Nutraceuticals in Veterinary Medicine*, https://doi.org/10.1007/978-3-030-04624-8_7

oblong, angular, funnel shaped with size of 0.2 cm long and 0.1 cm wide.

Seeds of NS contain various components such as protein (20–27%), fat (34.5–38.7%), carbohydrates (23.5–33.2%), crude fiber (8.4%), and ash (4.8%) (Babyan et al. 1978). These also contain many vitamins and minerals such as Zn, Cu, P, and F. Apart from this NS is also reported to contain carotene, which further converts into vitamin A in the liver (Ahmad et al. 2013). The seeds also contain many active compounds such as nogelleone, thymoquinone, and thymohydroquinone which are reported to provide beneficial activity such as antimicrobial, antitoxic, and other pharmacological activities (Forouzanfar et al. 2014).

NS seeds are reported to have 20% alcohol-soluble extractives, 15% water-soluble extractives, 25–32% total fixed oil, 0.42% volatile oil, and 3.91% organic matter (expressed as w/w) (Sharma et al. 2005).

2 Geographical Location

The origin of *Nigella sativa* is not well established. The plant was certainly under wide cultivation more than 3000 years ago. NS is inherent to Southeast Europe, North Africa, and Southwest Asia. It is cultivated in countries such as the Middle Eastern Mediterranean region, South Europe, India, Pakistan, Oman, Saudi Arabia, Israel, Syria, and Turkey (Fig. 1) (Gilani et al. 2004; Khare 2004).

3 Taxonomical Classification

The taxonomical classification of *Nigella sativa* is as follows: Kingdom, Plantae; subkingdom, Tracheobionta; superdivision, Spermatophyta; phylum, Magnoliophyta; class, Magnoliopsida; order, Ranunculales; family, Ranunculaceae; genus, *Nigella*; species, *sativa* (Fig. 2).

The NS is recognized as black cumin, black caraway, black seed, damascena, devil-in-the-bush, fennel flower, haba-al-barka, kalonji, kalajeera, nutmeg, and many more. It is an annually flowering plant that is characterized with finely divided petals (widely colored as pink, pale blue, purple, white, and yellow) and having 5–10 petals.

4 Chemical Constituents of NS

It has been found that essential oil of NS seed contains numerous chemical constituents, confirmed by GC and GC-MS techniques. Few compounds have been represented in Fig. 3. The extraction of essential oil from NS is done using Clevenger's apparatus. There are two types of extraction technique available using Clevenger's apparatus, one is microwave steam distillation 1 (MSD1) in which seeds are placed inside the oven apparatus, and the second is microwave steam distillation 2 (MSD2) in which seeds have been placed outside the oven apparatus (Akloul et al. 2014). Apart from this cryogenic grinding (CG) method was also used for the oil extraction. In CG methods the process is carried out by either MSD1-CG or MSD2-CG. Akloul et al. (2014) reported that essential oil contains more amount of oxygenated compounds and less amounts of monoterpene hydrocarbons extracted by using MSD1 and MSD2 than MSD1-CG and MSD2-CG. MSD1 oil extract contains comparative amount of ketones and alcohols than MSD2 oil extract, while MSD2 oil extracts contain more monoterpene hydrocarbons than MSD1 oil extracts. The amount of sesquiterpene hydrocarbons has approximately similar quantity in both. By using MSD1-CG and MSD2-CG, similar results have been observed. Some sesquiterpene hydrocarbons like longifolene, α-longipinene, and Z-γ-bisabolene have been reported in essential oil using MSD1 and MSD1-CG methods.

A qualitative assessment of NS seeds revealed the presence of triterpenes, sterols, flavonoids, tannins, alkaloids, cardiac glycosides, coumarins, saponins, volatile oils, volatile bases, anthraquinones, and glucosinolates (Al-Yahya 1986). It has been reported that seeds of NS contain more than 30% of fixed oil and 0.40–0.50% of volatile oil (w/w). Qualitative estimation of essential oil of NS seed by gas chromatography-mass spectrometry (GC-MS) technique has showed around 65–67 different chemical compounds. When

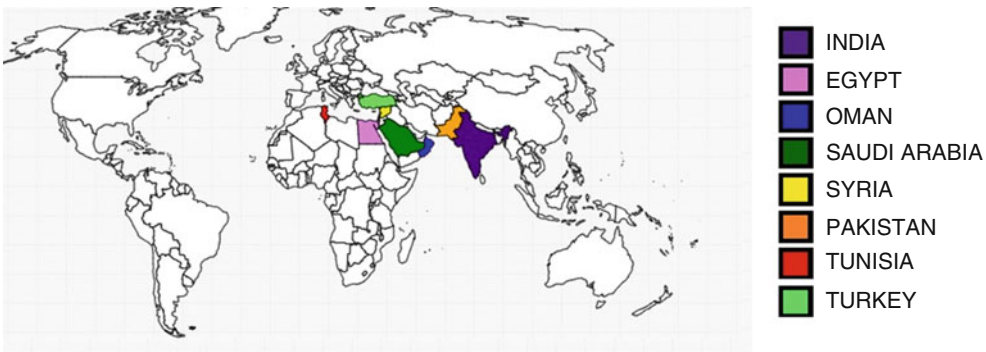


Fig. 1 Representation of geographical distribution of NS across the world

Fig. 2 Seeds of *N. sativa*

such compounds were subjected into various functional groups, the following results were obtained: carbonyl compounds (25%), phenols (1.7%), alcohols (0.9%), monoterpenes (46%), and esters (16%) (Aboutable et al. 1986). Approximately 18–24% thymoquinone and a total of 46% of various monoterpenes such as pinene and *p*-cymene were reported in volatile oil (El-Tahir et al. 1993).

Omar et al. (1999) provided a brief account about the presence of dithymoquinone, thymohydroquinone, thymoquinone, carvacrol, thymol, 6-methoxy-coumarin and 7-hydroxy-coumarin, oxy-coumarin, steryl-glucoside, and α-hedrin as well as enough amounts of tannins, essential fatty acids, flavonoids, ascorbic acid, amino acids, and few inorganic substances such as iron and calcium. As per the article of Weiss (2002), the seeds contain around 0.5% volatile oil that have seven main phyto-constituents, and their estimated proportions are as follows: thymoquinone (25%), *p*-cymene (31%), ethyl hexadecanoate (3%), α-pinene (9%), ethyl linoleate (9%), ethyl oleate (3%), and β-pinene (2%). Similarly Sharma et al. (2009) reported that thymoquinone was identified as the main component (up to 50%) alongside *p*-cymene (40%), pinene (up to 15%), fatty acid ethyl ester (10%), dithymoquinone, and thymohydroquinone. Other terpene derivatives were found only in trace amounts: carvacrol, carvone, limonene, 4-terpineol, and citronellol in the essential oil of NS (mean 0.5%, max. 1.5%). The conversion of thymoquinone to dithymoquinonene and higher oligo condensation products on storage results into the aromatic flavor of NS. Nickavara et al. (2003) from Iran used GC and GC-MS methods to investigate the chemical composition of the volatile oil of NS seeds and reported 32 compounds (86.7%). Among them, the major compounds were *trans*-anethole (38.3%), *p*-cymene (14.8%), limonene (4.3%), and carvone (4.0%). The GC and GC-MS results are given in Table 1 (Nickavara et al. 2003).

The main components of NS were *p*-cymene (33.8%) and thymol (26.8%), with only a small amount of thymoquinone (3.8%) reported by Moretti et al. (2004). Rajkapoor et al. (2002) reported the alkaloids present in the seeds to be nigellidine, nigellicin, tannin, steroid α-spinasterol, quinazoline, cholesterol, campesterol, stigmas 7-en-3-β-ol, stigmasterol, and flavonoids of trigillin quercetin-3-glucoside. Morikawa et al. (2004a, b) reported isolation of four dolabellane-type diterpene alkaloids from the seeds of NS. The active constituents, nigellidine and nigellone, were reported to contain an indazol nucleus (Rahman et al. 1995). Three flavonoid glycosides and triterpene saponins were also isolated and identified from NS together with four phospholipid classes: phosphatidylethanolamine, phosphatidylcholine, phosphatidylserine, and phosphatidylinositol (Merfort et al. 1997; Ramadan and Morsel 2002).

5 Pharmacological Studies

The extractives of NS showed many important pharmacological activities. The notability of the plant is due to its properties to cure many disorders. The detailed pharmacological studies of NS are given below:

5.1 Antioxidant Activity

NS essential oil has been found to possess great antioxidant activity. It has been observed that essential oil of NS affects the antioxidant enzyme status and myocardium of cyclosporine A-treated rats. It has also been reported that when pre-treatment with essential oil of NS was performed, it decreased the succeeding cyclosporine. Essential oil of NS showed antioxidant activity as it reduces the lipid peroxidation, development in antioxidant enzyme status, and cellular protein oxidation (Ebru et al. 2008). Essential oil of NS displayed many antioxidant activities verified by diphenylpicrylhydracyl. Thymoquinone (TQ) and other chemical components of NS like carvacrol, anethole, and 4-terpineol have radical scavenger property which is proved by using two TLC screening methods. These components have also been found to be effective OH radical scavenging agents,

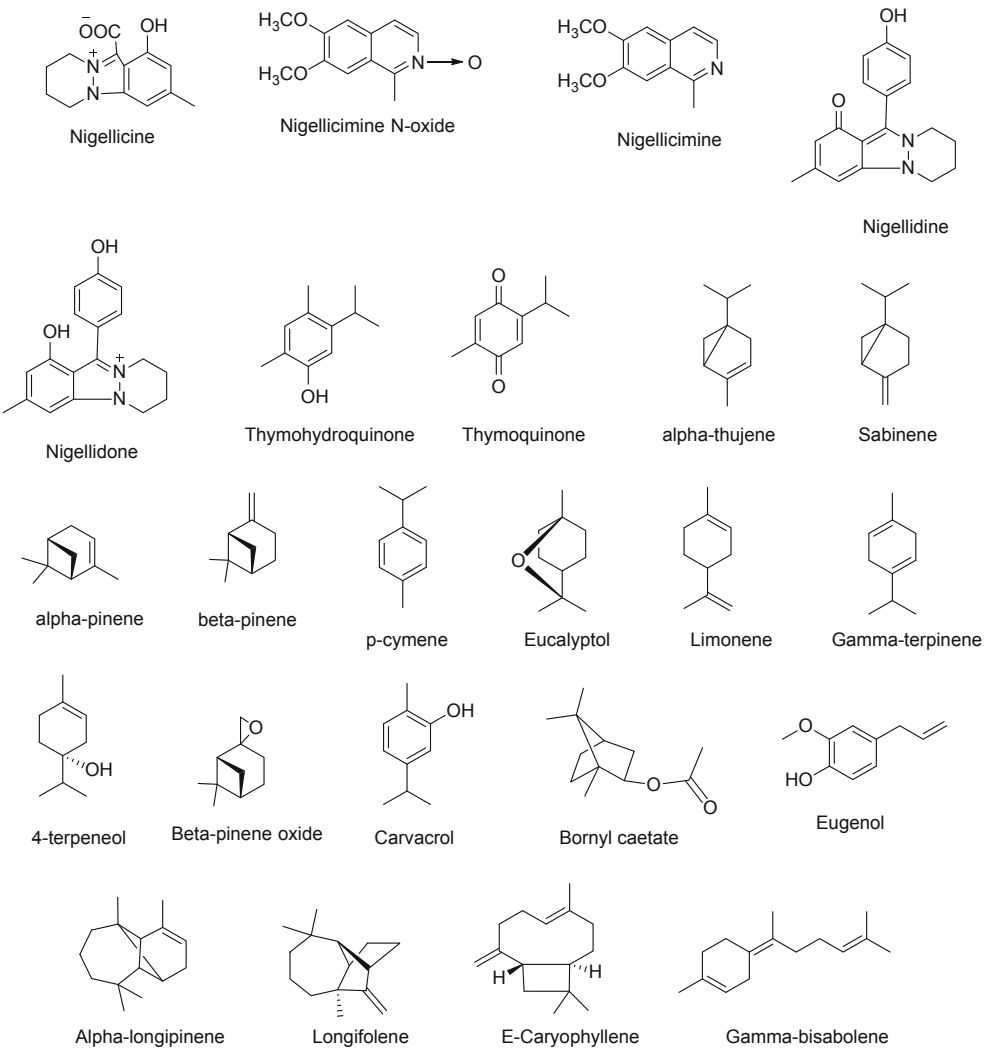


Fig. 3 Structures of chemical constituents of NS seeds

verified for nonenzymatic lipid peroxidation in liposomes and also deoxyribose degradation (Burits and Bucar 2000). The chemical constituents of NS like thymol, TQ, and dithymoquinone showed free-radical scavenging effect by attenuating reactive oxygen species (ROS) like hydroxyl radical, superoxide radical, and nascent oxygen, determined by chemiluminescence and spectrophotometric methods (Kruk et al. 2000). Due to the antioxidant activity of TQ, it showed protective effect against doxorubicin-induced nephrotoxicity (Badary et al. 2000) and doxorubicin-induced cardiotoxicity (Al-Shabanah et al. 1998; Nagi and Mansour 2000). It shows modulating effect and antitumor effect on bezo(a)pyrene-induced cancer in mice (Badary et al. 1999) and on 20-methylcholanthrene-induced fibrosarcoma tumor genesis (Badary and Gamal-el-Din 2001), respectively, due to antioxidant activity. NS seeds and their extracts have been reported to display antioxidant property, as they provide protection against damage caused by oxidation. Lado et al. (2004), Nagwa et al. (2006), and Adamu et al. (2010) have all observed that *Nigella* oils may be used as an antioxidant, while Musa et al. (2004) reported that the ethyl alcohol extract can also produce antioxidants and was able to extend the life span of mice. Recently, Ibraheem et al. (2010) reported that NS has antioxidant and calcium antagonist properties.

5.2 Antibacterial Activity

Essential oil extracted from NS seed by following various techniques, such as steam distillation (SD), dry steam distillation (DSD), hydrodistillation (HD), solvent extraction (SE), and supercritical fluid extraction (SFE-SD), has been reported to exert antibacterial activities (Islam et al. 2013). It has been found that the MIC (minimum inhibitory concentration) values of HD and SD extractives are 256 and 32 μg/mL, respectively, while for both SE-SD and SFE-SD, the value was 4 μg/mL. All NS essential oil samples have been observed to possess greater activity against gram-positive than gram-negative bacteria (Kokoska et al. 2008). The

Table 1 Chemical composition of the volatile constituents

Compound	%
1,3,5-Trimethyl benzene	0.5
1-Ethyl-2,3-dimethyl benzene	0.2
1-Methyl-3-propyl benzene	0.5
3-Methyl nonane	0.3
Anisaldehyde	1.7
Apiole	1.0
Carvacrol	1.6
Carvone	4.0
Dihydrocarvone	0.3
Dill apiole	1.8
Estragole	1.9
Fenchone	1.1
Limonene	4.3
Longifolene	0.7
Myrcene	0.4
Myristicin	1.4
n-Decane	0.4
n-Hexadecane	0.2
n-Nonane	1.7
n-Tetradecane	0.2
p-Cymene	14.8
p-Cymene-8-ol	0.4
Sabinene	1.4
Terpinen-4-ol	0.7
Thymoquinone	0.6
Total monoterpenoid alcohols	2.7
Total monoterpenoid hydrocarbons	26.9
Total monoterpenoid ketones	6.0
Total non-terpenoid hydrocarbons	4.0
Total phenyl propanoid compounds	46.1
Total sesquiterpene hydrocarbons	1.0
trans-Anethole	38.3
α-Longipinene	0.3
α-Phellandrene	0.6
α-Pinene	1.2
α-Thujene	2.4
β-Pinene	1.3
γ-Terpinene	0.5
Total compounds	86.7

Source: Malhotra (2012)

antibacterial activities of NS against clinical isolates of methicillin-resistant *Staphylococcus aureus* have been studied by Hannan et al. (2008). It has been found that different NS extracts show antibacterial activity against different bacteria. Different extracts of NS have shown antibacterial activity against 16 gram-negative and 6 gram-positive bacteria. It has been found that the most effective extracts for antibacterial activity are alkaloids and water extracts. Also, the gram-negative bacteria are more affected than gram-positive bacteria (Hanafy and Hatem 1991; Sokmen et al. 1997). The methanol extracts of seeds exhibit anti-plaque action by inhibiting *Streptococcus mutans*. Alcoholic extracts exhibit antibacterial activity against bacteria *Micrococcus pyogenes* var. *aureus*, *Shigella dysenteriae*, *S. sonnei*, *S. boydii*, *Vibrio cholera*, and *E. coli* (Ferdous et al. 1992). Alcohol extracts of NS seed showed inhibitory activity against the growth of *Staphylococcus aureus* at a conc. of 4 mg/disc with MIC ranges from 0.2 to 0.5 mg/mL (Hannan et al. 2008).

5.3 Antifungal Activity

The essential oil of NS seeds is reported to have appreciable activity against a large number of fungi (Agarwal et al. 1979; Bourrel et al. 1995; Aboul Ela et al. 1996). The inhibition of toxic substance named as "aflatoxin" formation by a number of medicinal plants including NS at different concentrations has been studied in the past. In one of these studies, the powdered seed and essential oil of NS efficiently inhibited the growth and production of aflatoxin from the toxigenic strain of *Aspergillus flavus* (El-Shayeb and Mabrouk 1984; El-Sayed et al. 1997; Ozcan 1998).

The aqueous extractives of NS seeds have inhibitory effect against candidiasis in mice (Bita et al. 2012). The ether extract of NS and TQ has been tested for the antidermatophyte activity against eight species of dermatophytes, in which four species are of *Trichophyton rubrum* and one each of *Trichophyton interdigitale*, *Trichophyton mentagrophytes*, *Epidermophyton floccosum*, and *Microsporum canis* using agar diffusion method (Ahmad et al. 2013). TQ and ether extracts of NS also show inhibitory activity against fungal strains (Aljabre et al. 2005). TQ exhibited high antifungal activity against *Aspergillus niger*, *Fusarium solani*, and *Scopulariopsis brevicaulis*, and the activity is found to be similar with that of drug amphotericin B (Aljabre et al. 2015a, b).

It is found that the MICs of the ether extracts of NS and TQ range between 10 and 40 mg/mL and 0.125 and 0.25 mg/mL, respectively (Aljabre et al. 2005). The NS seed oil has been found to possess antifungal activities against 20 fungi, including pathogenic and industrial strains. Many researchers have found that all the oil extracts of NS have antifungal activities, but a stronger and wider range of antifungal activities have been shown only by volatile oil. The MIC values of volatile oil of the NS seeds have been determined against three pathogenic fungi, and the lowest MIC has been found against *Aspergillus fumigatus* (Islam et al. 1989).

5.4 Anticancer Activity

There are a number of active chemical components that have been extracted from NS such as thymoquinone (TQ) and

alpha-hederin which possess anticancer activities (Aljabre et al. 2015a, b). Several experiments have been performed in mice and rats to explore the anticancer activity of NS. The anti-cancer activity of black seed was observed with activity of natural killer cells to 200–300% in patients with advanced cancer (Salim 2010). The essential oil ($IC_{50} = 0.6\%$ v/v) and ethyl acetate ($IC_{50} = 0.75\%$) extracts of NS have been found to be more toxic against the P815 cell line than their butanol extractives ($IC_{50} = 2\%$). It was found that tests on the BSR cell line manifest a higher cytotoxic effect of ethyl acetate extractive ($IC_{50} = 0.2\%$) than essential oil ($IC_{50} = 1.2\%$) (Ait et al. 2007). The ethyl alchol extracts of NS seeds have been reported for in vitro inhibition of cancer cells and endothelial cells progression (Medenica et al. 1997; Swamy and Tan 2000). The defensive effect of NS seeds against the oxidative stress and carcinogenesis induced by using methylnitrosourea in rats has been studied. Its protective ability is high (80%) against methylnitrosourea-induced oxidative stress and carcinogenesis (Mabrouk et al. 2002). The aqueous and alcoholic extracts, which are either alone or in combination with H_2O_2, were found to be effective in vitro in deactivating MCF-7 breast cancer (Farah and Begum 2003). So the above data elucidates the toxic effect of each extract against various types of tumor cell.

5.5 Antidiabetic Properties

The seeds and oil extracts of NS have been found to possess antidiabetic properties. The effects on oral glucose tolerance of the aqueous crude extract of NS have been investigated by using the electroshock technique. It has been found that the aqueous crude extract of NS directly inhibits the intestinal absorption. Therefore, the improvement of carbohydrate tolerance and body weight in experimental rats after oral intake of seeds authenticates the use of NS as antidiabetic agents (Meddah et al. 2009). Various HIV protease inhibitors such as nelfinavir and atazanavir (Fig. 4) have been exposed along with the NS seed extracts. The combination has been reported to decline the insulin secretion in rats. Due to reduced insulin secretion, there has been rapid decline in the mortality rate in the HIV-I-positive patients (Chandra et al. 2009).

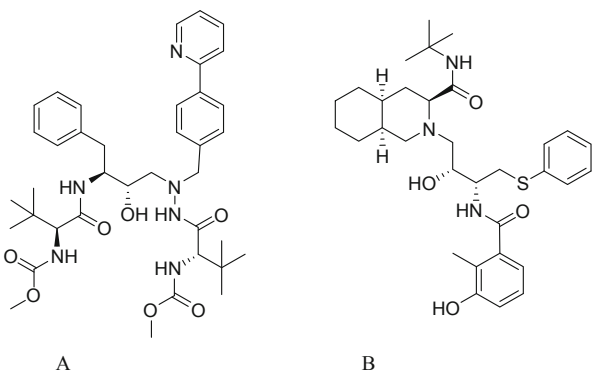


Fig. 4 Structure of atazanavir (**a**) and nelfinavir (**b**) as antidiabetic drugs, respectively

The defatted whole extracts of seeds have been evaluated for the insulin secretary effects in rats. The defatted extract has been divided into two subcategories as one with acidic and neutral molecules and the other with basic molecules. These subcategories have been tested in vitro in pancreatic islets of rats. The study revealed that the subcategories with basic molecules significantly enhanced the glucose-induced insulin release in rats indicating antidiabetic activity of the extract of NS (Rchid et al. 2004). Therefore, NS exhibits a protective effect in the diabetic rat by preserving insulin-producing pancreatic cells and inhibiting the oxidative stress in the cells (Kanter et al. 2004).

5.6 Antifertility Activity

A variety of reproductive responses of NS have been reported which include semen quality, follicle development, and effects related to pregnancy in both male and female individuals (Babazadeh et al. 2012). The seeds and extracts of NS have been used in albino rats to observe the effects on postmenopausal parameters. It has been found that the NS can be used for substituting the hormone therapy due to the presence of various beneficial effects (Parhizkar et al. 2016). The protein diet of Rahmani ewe lamb has been replaced with the protein diet rich in NS. It has been observed that the NS protein mixture has a wide range of beneficial role in altering the length of estrous cycle and conception rate (El-Harairy et al. 2006). Male lamb's performance has been found to be boosted when supplemented with NS (100–200 mg/kg body weight). The serum testosterone concentration was increased by NS. A group of broiler supplemented with the seeds and oil extracts of NS showed better semen characteristics which include increase in sperm mass motility, ejaculation volume, count, total sperm output, and viability percentage (Zanouny et al. 2013).

5.7 Anti-inflammatory Activity

TQ and other extracts of NS have significant anti-inflammatory activity. It has been studied that the ethanol extracts of NS seeds are used in the treatment of psoriasis, which is a general skin condition due to hyperproliferative, autoimmune skin disorder, and sometimes it is itchy and dolorous (Dwarampudi et al. 2012). Ahmed et al. (2014) reported that the ethanol extracts of NS showed anti-psoriatic effect. Also, NS oil is used generally in two forms which may be in balm and oral dosage form. The IC_{50} value of NS oil is

reported to be 23.9 μg/mL, which approximately equals to IC_{50} value of asiaticoside ($IC_{50} = 20.13$ μg/mL) (Jawad et al. 2014). NS oil is also used to cure acne vulgaris, which is the most common skin disease. Hadi and Ashor (2010) observed that using 20% NS oil extractives is better and less injurious in lotion formulation than using benzoyl peroxide. This is utilized in the treatment of mild to middle stage of acne vulgaris (Hadi and Ashor 2010).

5.8 Gastroprotective Properties

Wide-ranging gastroprotective activity of NS has been observed. The rats have been induced with gastric ulcer by using noxious chemical. The effects of aqueous extracts have been found to be significantly beneficial in the prevention of gastric ulcer and improving basal gastric secretion. The aqueous extract replenishes the depleted mucus content in the gastric mucosa indicating the gastroprotective activity of aqueous extract (Mofleh et al. 2008). Thymoquinone is examined against the noxious chemical-induced ulcer in rats and found to protect the gastric mucosa against the harmful effects of noxious chemical and support ulcer healing (Kanter et al. 2005; El-Dakhakhny et al. 2000). The aqueous extract of NS decreased the acidity in the gastric juice exhibiting gastroprotective activity in the acetyl salicylic acid-induced ulcer in rats (Akhtar et al. 1996).

5.9 Cardiovascular Activity

The seeds and extracts of NS have been employed to study the responses in the stimulation of cardiac activity. The seeds of NS have been administered orally to the normal rats to study their effect. The seeds enhanced the inherent contractile properties of the heart without increasing the cardiac workload (Al-Hariri et al. 2009; El-Bahai et al. 2009). The aqueous and macerated extracts from NS have been employed to examine their effects on heart rate and contractility of the heart. The powerful inhibitory effect on the heart rate and contractility has been noticed and found to be more effective than diltiazem (Fig. 5). The effect is attributed to calcium channel inhibition, and in plants it is due to the opening of potassium channels (Boskabady et al. 2005; Shafei et al. 2005).

Through the blockage of calcium ion channel, the active ingredient thymol is found to reduce the blood pressure (Gialni et al. 2001). Different doses of powdered seeds of NS have been administrated to albino rats to analyze the effect on the density of lipoprotein-cholesterol level. These powdered seeds lowered the level of low-density lipoprotein and enhanced the level of high-density lipoproteins which are essential for regular cardiovascular activities (Kocyigit et al. 2009).

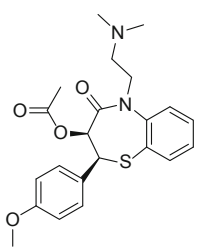


Fig. 5 Structure of diltiazem, anti-cardiovascular drug

5.10 Immunomodulatory Activities

Various synthetic antibiotics are used against various pathogenic organisms. Pathogenic organisms have been found to develop drug resistance against the administrated synthetic antibiotics. Therefore, compared to synthetic antibiotics, natural alternatives such as oil extracts and seeds of NS might be helpful. The seeds and purified constituents of NS have been used in the treatment of various diseases (Zeweil et al. 2008). Increased total plasma protein, albumin, and globulin were reported in rabbits upon supplementation with oil/extracts of NS (Tousson et al. 2011). The radioprotective activity of oil extracts of NS has been observed against hemopoietic harmful effect of gamma radiation. Before irradiation the oil extracts of NS were orally administrated which normalized the amplified concentration of malondialdehyde by decreasing the concentrations of catalase, plasma glutathione, and erythrocyte superoxide dismutase activities. Therefore, the oil extracts have been found to possess natural radioprotective activity and immunosuppressive effects on ionizing radiation (Assayed 2010). Significant declines in spontaneous motility, modification in general behavior, and normal body temperature were observed in methanol and aqueous extracts of oil suggesting depressant activity in the central nervous system (Khanna et al. 1993).

5.11 Memory and Learning Activities

In comparison with the other plant extracts, NS seed oil and its extracts are effective on the CNS and other actions related to spatial memory which involves special information, recognition, codifying, storing, and recovering by the brain (Kalat 2007). NS extracts are applied for acetylcholinesterase (AChE) activity inhibition.

The hydroalcoholic extracts of NS are involved in the central cholinergic enhancement against scopolamine-induced amnesia (Hosseini et al. 2015). The NS extractive oil shows mnemonic effect, cholinergic modulation, and oxidative stress mitigation (Raza et al. 2006). Khan et al.

(2008) reported that TQ of NS extract has neuroprotective properties on perceptional harm and other related dementias. It was also found that NS has antianxiety effect (Perveen et al. 2009). The hydroalcoholic extracts of NS have been reported to be useful in preventing the decadence of learning and memory activities shown in the pentylenetetrazole (PTZ)-induced epileptic model (Vafaee et al. 2015). The hydroalcoholic extracts of NS also reversed the harm of hypothyroidism linked with learning and memory in neonatal animals (Beheshti et al. 2016a, b). NS is also reported to affect the human mood, anxiety, and cognition (Sayeed Bin et al. 2014).

5.12 Anticonvulsant Activity

Since ancient times, epilepsy is one of the lethal disorders which is characterized by frequent seizures. In the treatment of epilepsy, the seeds and oil extracts of NS have been used since ancient times. In the tomb of Tutankhamun, it was selected as one of the entombed products as it was believed that it will cure cerebral malaria and epilepsy (Tahan and Bayram 2011; Mathur et al. 2011). The techniques such as maximal electroshock (MES) along with pentylenetetrazole (PTZ) have been used to determine the anticonvulsant activity of the seeds and oil extract of NS. Intraperitoneal insertion of thymoquinone has been found to diminish the duration of seizures (Yaman et al. 2010). Valproate (one of the major constituents among antiepileptic drugs) and oil extracts of NS were simultaneously tested for their ability to suppress the lethal and convulsive effects of PTZ in mice. The suppression activity has been found to be more pronounced in case of oil extract of NS than valproate in inhibiting PTZ-induced seizures (Abu-Zinadah 2009). Various constituents of NS like fixed oil, aqueous oil, and oil extracts have been investigated against MES- and PTZ-induced convulsion. It is observed that except the fixed oils, other constituents were found to be effective against PTZ convulsion (Ali and Meitei 2011).

6 Toxicological Properties

The seeds, extracts, and constituents of NS have been observed to have low toxicity. Intraperitoneal administrations of NS extract (50 mg/kg) to rats have been found to possess less significant effect on the activities of various enzymes (el-Daly 1998). The major constituent thymoquinone is characterized with very high lethal dosage value ranging between 1.52 and 3.77 g/kg (Badary et al. 1998). Supplementation of high dosage of NS extract causes hypoactivity and obstructed breathing. These high dosages declined the concentration of growth-stimulating hormone (GSH) in the liver, kidney, and heart. Due to the declined concentration of GSH in the kidney and liver, plasma metabolites and enzymes increased and caused damages in the organs (Badary et al. 1998). The rats have been treated with the NS fixed oil extracts to determine the toxicity level, and no significant effect was found in the levels of hepatic enzymes. However, the levels of thyroglobulin (TG), cholesterol, and glucose and count of leukocytes and platelets have been decreased. Therefore, the seeds and oil extracts of NS are of immense importance as they possess low level of toxicity (Zaghlol et al. 2012).

7 Concluding Remarks and Future Directions

The ethnobotanical and wide application of natural compounds especially plant derivatives have received great attention in recent years. The use of NS seeds and their constituents has been shown to exert multiple useful effects in the treatment of various ailments. It contains a wide range of pharmaceutical properties including antidiabetic, anticancer, immunomodulatory, gastroprotective, and many more. The seed oil/extracts of NS have been utilized as therapeutic agents since ancient times due to their least toxic effect. *N. sativa* has a great potential for nutraceutical and subject to further investigations.

References

Abdulelah HAA, Zainal-Abidin BAH (2007) *In vivo* antimalarial tests of *Nigella sativa* (Black Seed) different extracts. Am J Pharmacol Toxicol 2:46–50

Aboul Ela MA, El-Shaer NS, Ghanem NB (1996) Antimicrobial evaluation and chromatographic analysis of some essential and fixed oils. Pharmazie 51:993–994

Aboutable EA, El-Azzouny AA, Hammerschmidt FJ (1986) Aroma volatiles of *Nigella sativa* L. seeds. In: Brunke EJ (ed) Progress in essential oil research. Proceedings of the international symposium on essential oils. de Gruyter, Berlin, pp 44–55

Abu-Zinadah O (2009) Using nigella sativa oil to treat and heal chemical induced wound of rabbit skin. J King Abdulaziz Univ Sci 21:335–346

Adamu HM, Ekanem EO, Bulama S (2010) Identification of essential oil components from *Nigella sativa* seed by gas chromatography mass spectroscopy. Pak J Nutr 9:966–967

Agarwal R, Md K, Shrivastava R (1979) Antimicrobial and anthelmintic activities of the essential oil of *Nigella sativa* Linn. Indian J Exp Biol 17:1264–1265

Ahmad A, Husain A, Mujeeb M et al (2013) A review on therapeutic potential of *Nigella sativa*: a miracle herb. Asian Pac J Trop Biomed 3:337–352

Ait ML, Ait MH, Elabbadi N et al (2007) Anti-tumor properties of blackseed (*Nigella sativa* L.) extracts. Braz J Med Biol Res 40:893–847

Akhtar Ah, Ahmad KD, Gilani SN et al (1996) Antiulcer effect of aqueous extracts of *Nigella sativa* and *Pongamia pinnata* in rats. Fitoterapia 67:195–199

Akloul R, Benkaci-Ali F, Zerrouki M et al (2014) Composition and biological activities of the essential oil of *Nigella sativa seeds* isolated by accelerated microwave steam distillation with cryogenic grinding. Am J Essent Oil Nat Prod 1:23–33

Al-Hariri MT, Yar T, Bamosa AO et al (2009) Effects of two-months *Nigella sativa* supplementation on cardiac hemodynamics and adrenergic responsiveness. J Pak Med Assoc 59:363–368

Ali SA, Meitei KV (2011) *Nigella sativa* seed extract and its bioactive compound thymoquinone: the new melanogens causing hyperpigmentation in the wall lizard melanophores. J Pharm Pharmacol 63:741–746

Aljabre SH, Randhawa MA, Akhtar N et al (2005) Antidermatophyte activity of ether extract of *Nigella sativa* and its active principle, thymoquinone. J Ethnopharmacol 101:116–119

Aljabre SHM, Alakloby OM, Randhawa MA (2015a) Dermatological effects of *Nigella sativa*. J Dermatol Dermatol Surg 10:92–98

Aljabre SHM, Alakloby OM, Randhawa MA (2015b) Dermatological effects of *Nigella sativa*. J Dermatol Dermatol Surg 19:92–98

Al-Shabanah OA, Badary OA, Nagi MN et al (1998) Thymoquinone protects against doxorubicin induced cardiotoxicity without compromising its antitumor activity. J Exp Clin Cancer Res 17:193–198

Al-Yahya MA (1986) Phytochemical studies of the plants used in traditional medicine of Saudi Arabia. Fitoterapia 57:179–182

Assayed ME (2010) Radioprotective effects of black seed (*Nigella sativa*) oil against hemopoietic damage and immunosuppression in gamma-irradiated rats. Immunopharmacol Immunotoxicol 32:284–296

Babazadeh B, Sadeghnia HR, Safarpour Kapurchal E et al (2012) Protective effect of *Nigella sativa* and thymoquinone on serum/glucose deprivation induced DNA damage in PC12 cells. Avicenna J Phytomed 2:125–132

Babyan VK, Kottunga D, Halaby GA (1978) Proximate analysis, fatty acid and amino acid composition of *Nigella sativa* seeds. J Food Sci 43:1314–1315

Badary OA, Gamal-el-Din AM (2001) Inhibitory effects of thymoquinone against 20-methylcholanthrene induce fibrosarcoma tumoriogenesis. Cancer Detect Prev 25:362–368

Badary OA, Al-Shabanah OA, Nagi MN et al (1998) Acute and subchronic toxicity of thymoquinone in mice. Drug Dev Res 44:56–61

Badary OA, Al-Shabanah OA, Nagi MN et al (1999) Inhibition of benzopyrene induced forestomach carcinogenesis in mice by thymoquinone. Eur J Cancer Prev 8:435–440

Badary OA, Abdel-Naim AB, Abdel-Wahab MH et al (2000) The influence of thymoquinone on doxorubicin-induced hyperlipidemic nephropathy in rats. Toxicology 143:219–226

Beheshti F, Hosseini M, Vafaee F et al (2016a) Feeding of *Nigella sativa* during neonatal and juvenile growth improves learning and memory of rats. J Tradit Complement Med 6:146–152

Beheshti F, Hosseini M, Shafei MN et al (2016b) The effects of *Nigella sativa* extract on hypothyroidism-associated learning and memory impairment during neonatal and juvenile growth in rats. Nutr Neurosci 20:49–59

Benhaddou-Andaloussi A, Martineau L, Vuong T et al (2011) The *in vivo* antidiabetic activity of *Nigella sativa* is mediated through activation of the AMPK pathway and increased muscle Glut4 content. Evid-Based Complement Alternat Med 2011:1–9

Bita A, Rosu AF, Calina D et al (2012) An alternative treatment for Candida infections with *Nigella sativa* extracts. Eur J Hosp Pharm 19:162

Boskabady MH, Shafei MN, Parsaee H (2005) Effects of aqueous and macerated extracts from *Nigella sativa* on guinea pig isolated heart activity. Pharmazie 60:943–948

Bourrel C, Dargent R, Vilrem G et al (1995) Chemical analysis and fungistatic properties of some essential oils in a liquid medium. Effects on hyphal morphogenesis. Riv Ital EPPOS 6:31–42

Burits M, Bucar F (2000) Antioxidant activity of *Nigella sativa* essential oil. Phytother Res 14(5):323–328

Chandra S, Mondal D, Agrawal KC (2009) HIV-1 protease inhibitor induced oxidative stress suppresses glucose stimulated insulin release: protection with thymoquinone. Exp Biol Med 234:442–453

Dwarampudi LP, Palaniswamy D, Nithyanantham M et al (2012) Antipsoriatic activity and cytotoxicity of ethanolic extract of *Nigella sativa* seeds. Pharmacogn Mag 8:268–272

Ebru U, Burak U, Yusuf S et al (2008) Cardioprotective effects of *Nigella sativa* oil on cyclosporine A-induced cardiotoxicity in rats. Basic Clin Pharmacol Toxicol 103:574–580

El-Bahai MN, Al-Hariri MT, Yar T et al (2009) Cardiac inotropic and hypertrophic effects of *Nigella sativa* supplementation in rats. Int J Cardiol 31:115–117

El-Dakhakhny M, Barakat M, El-Halim MA et al (2000) Effects of *Nigella sativa* oil on gastric secretion and ethanol induced ulcer in rats. J Ethnopharmacol 72:299–304

el-Daly ES (1998) Protective effect of cysteine and vitamin E, *Crocus sativus* and *Nigella sativa* extracts on cisplatin induced toxicity in rats. J Pharm Belg 53:87–95

El-Harairy MA, Gabr MG, El-Ayouty SA et al (2006) Effect of feeding level and replacement of *Nigella sativa* meal in diet of Rahmani ewe lambs on: 2. Onset of puberty, oestrous activity and conception rate. Egypt J Sheep Goat Desert Anim Sci 1:171–186

El-Sayed AAAM, Hussiney HA, Yassa AI (1997) Constituents of *Nigella sativa* oil and evaluation of its inhibitory effect on growth and aflatoxin production by *Aspergillus parasiticus*. *Dtsch Lebensm Rundsch* 93:149–152

El-Shayeb NMA, Mabrouk SS (1984) Utilization of some edible and medicinal plants to inhibit aflatoxin formation. Nutr Rep Int 29:273–282

El-Tahir KEH, Ashour MMS, Al-Harbi MM (1993) The respiratory effects of the volatile oil of the black seed in guinea pigs – elucidation of the mechanism of action. Gen Pharmacol 24:1115–1122

Farah IO, Begum RA (2003) Effect of *Nigella sativa* and oxidative stress on the survival pattern of MCF-7 breast cancer cells. Biomed Sci Instrum 39:359–364

Ferdous AJ, Islam SN, Ahsan M et al (1992) In vitro antibacterial activity of the volatile oil of *Nigella sativa* seeds against multiple drug resistant isolates of Shigella species and isolates of *Vibrio cholera* and *Escherichia coli*. Phytother Res 6:137–140

Forouzanfar F, Bazzaz BSF, Hosseinzadeh H (2014) Black cumin (*Nigella sativa*) and its constituent (thymoquinone): a review on antimicrobial effects. Iran J Basic Med Sci 17:929–938

Gialni AH, Shaheen F, Shakir T (2001) Thymol lowers blood pressure through blockade of calcium channels. Fund Clin Pharmacol 15:163

Gilani AH, Aziz N, Khurram IM, Chaudhary KS et al (2001) Bronchodilator, spasmolytic and calcium antagonist activities of *Nigella sativa* seeds (Kalonji): a traditional herbal product with multiple medicinal uses. J Pak Med Assoc 51:115–120

Gilani A, Jabeen Q, Ullahkhan M (2004) A review of medicinal uses and pharmacological activities of *Nigella sativa*. Pak J Biol Sci 7:441–451

Goreja WG (2003) Black seed: nature's miracle remedy. Amazing Herbs Press, New York

Hadi NA, Ashor AW (2010) *Nigella sativa* oil lotion 20% vs. benzoyl peroxide lotion 5% in the treatment of mild to moderate acne vulgaris. Iraq Postgrad Med J 9:371–376

Hanafy MS, Hatem ME (1991) Studies on the antimicrobial activity of *Nigella sativa* seed (black cumin). J Ethnopharmacol 34:275–327

Hannan A, Saleem S, Chaudhary S et al (2008) Antibacterial activity of *Nigella sativa* against clinical isolates of methicillin resistant *Staphylococcus aureus*. J Ayub Med Coll Abbottabad 20:72–74

Hosseini M, Mohammadpour T, Karami R et al (2015) Effects of the hydroalcoholic extract of *Nigella sativa* on scopolamine-induced spatial memory impairment in rats and its possible mechanism. Chin J Integr Med 21:438–444

Ibraheem NK, Ahmed JH, Hassan MK (2010) The effect of fixed oil and water extracts of *Nigella sativa* on sickle cells: an in vitro study. Singapore Med J 51:230–234

Islam SK, Ahsan M, Hassan CM et al (1989) Antifungal activities of the oils of *Nigella sativa* seeds. Pak J Pharm Sci 2:25–28

Islam MH, Ahmad IZ, Salman MT (2013) Antibacterial activity of *Nigella sativa* seed in various germination phases on clinical bacterial strains isolated from human patients. E3 J Biotechnol Pharm Res 4:8–13

Jawad HA, Azhar YI, Khalil IAH (2014) Evaluation of efficacy, safety and antioxidant effect of *Nigella sativa* in patients with psoriasis: a randomized clinical trial. J Clin Exper Invest 5:186–193

Kalat JW (2007) Biological psychology, 9th edn. Thomson Wadsworth, Toronto

Kanter M, Coskun O, Korkmaz A et al (2004) Effects of *Nigella sativa* on oxidative stress and beta-cell damage in streptozotocin-induced diabetic rats. Anat Rec A Discov Mol Cell Evol Biol 279:685–691

Kanter M, Demir H, Karakaya C et al (2005) Gastroprotective activity of *Nigella sativa* L oil and its constituent, thymoquinone against acute alcohol-induced gastric mucosal injury in rats. World J Gastroenterol 11:6662–6666

Khan A, Khuwaja G, Khan MB et al (2008) Effect of thymoquinone on streptozotocin model of cognitive impairment in rats. Ann Neurosci 15:94

Khanna Y, Zaidi FA, Dandiya PC (1993) CNS and analgesic studies on *Nigella sativa*. Fitoterapia 64:407–410

Khare CP (2004) Encyclopedia of Indian medicinal plants. Springer, New York

Kocyigit Y, Atamer Y, Uysal E (2009) The effect of dietary supplementation of *Nigella sativa* L. on serum lipid profile in rats. Saudi Med J 30:893–896

Kokoska L, Havlik J, Valterova I et al (2008) Comparison of chemical composition and antibacterial activity of *Nigella sativa* seed essential oils obtained by different extraction methods. J Food Prot 71:2475–2480

Kruk I, Michalska T, Lichszteld K et al (2000) The effect of thymol and its derivatives on reactions generating reactive oxygen species. Chemosphere 41:1059–1064

Lado CM, Then I, Varga ES et al (2004) Antioxidant property of volatile oils determined by ferrous reducing agent. Z Naturforsch 59c:354–358

Mabrouk GM, Moselhy SS, Zohny EM et al (2002) Inhibition of methylnitrosourea induced oxidative stress and carcinogenesis by orally administered bee honey and Nigella grains in Sprague Dawley rats. J Exp Clin Cancer Res 21:341–346

Malhotra SK (2012) Nigella. In: Handbook of herbs and spices, 2nd edn. Woodhead Publishing, Cambridge, pp 391–416

Mathur ML, Gaur J, Sharma R et al (2011) Antidiabetic properties of a spice plant *Nigella sativa*. J Endocrinol Metab 1(1):1–8

Meddah B, Ducroc R, El-Abbes-Faouzi M et al (2009) *Nigella sativa* inhibits intestinal glucose absorption and improves glucose tolerance in rats. J Ethnopharmacol 21:419–424

Medenica R, Janssens J, Tarsenko A et al (1997) Anti-angiogenic activity of *Nigella sativa* plant extract in cancer therapy. Proc Annu Meet Am Assoc Cancer Res 38:A1377

Merfort I, Wray V, Barakat HH et al (1997) Flavonol triglycosides from seeds of *Nigella sativa*. Phytochemistry 46:359–363

Mofleh IAA, Alhaider AA, Mossa JS et al (2008) Gastroprotective effect of an aqueous suspension of black cumin *Nigella sativa* on necrotizing agents-induced gastric injury in experimental animals. Saudi J Gastroenterol 14:128–134

Moretti A, Antuono D, Fi Lippo L et al (2004) Essential oil of *Nigella sativa* L. and *Nigella damascena* L. seed. J Essent Oil Res 16:182–183

Morikawa T, Xu F, Kashima Y et al (2004a) Novel dolabellane type diterpene alkaloid with lipid metabolism promoting activities from the seeds of *Nigella sativa*. Org Lett 6:869–872

Morikawa T, Xu F, Ninomiya K et al (2004b) Nigellamines A3, A4, A5 and C ne dolabellane type diterpene alkaloid with lipid metabolism promoting activities from the Egyptian medicinal food black cumin. Chem Pharm Bull 52:494–497

Musa D, Dilsiz N, Gumushan H, Ulakoglu G et al (2004) Antitumor activity of an ethanol extract of *Nigella sativa* seeds. Biol Brat 59:735–740

Nagi MN, Mansour MA (2000) Protective effect of thymoquinone against doxorubicin induced cardiotoxicity in rats: a possible mechanism of protection. Pharmacol Res 41:283–289

Nagwa M, El-Sawi, Hana GM (2006) Effect of *Nigella sativa* and activated charcoal as antioxidant on verrucarin induced hepatotoxicity in male rats. Adv Phytomed 2:133–153

Nickavara B, Mojaba F, Javidniab K et al (2003) Chemical composition of the fixed and volatile oils of *Nigella sativa* L. from Iran. Z Naturforsch 58c:629–631

Omar A, Ghosheh S, Abdulghani A et al (1999) High performance liquid chromatographic analysis of the pharmacologically active quinones and related compounds in the oil of the black seed. J Pharm Biomed Anal 19:757–762

Omojate GC, Enwa FO, Jewo AO et al (2014) Mechanisms of antimicrobial actions of phytochemicals against enteric pathogens – a review. J Pharm Chem Biol Sci 2:77–85

Ozcan M (1998) Inhibitory effects of spice extracts on the growth of *Aspergillus parasiticus*. NRRL2999 strain. Z Lebensm Unters Forsch 207:253–255

Parhizkar S, Latiff LA, Parsa A (2016) Effect of *Nigella sativa* on reproductive system in experimental menopause rat model. Avicenna J Phytomed 6:95–103

Perveen T, Haider S, Kanwal S (2009) Repeated administration of *Nigella sativa* decreases 5-HT turnover and produces anxiolytic effects in rats. Pak J Pharm Sci 22:139–144

Rahman A-U, Malik S, Sadiq HS et al (1995) Nigellidine – a new indazole alkaloid from the seeds of *Nigella sativa*. Tetrahedron Lett 36:1993–1996

Rajkapoor B, Anandan, Jayakar B (2002) Anti-ulcer effect of *Nigella sativa* L. against gastric ulcers in rats. Curr Sci 82:177–179

Ramadan RF, Morsel JT (2002) Characterization of phospholipid composition of black cumin (*Nigella sativa* L.) seed oil. Nahrung 46:240–244

Raza M, El-Hadiyah TM, Al-Shabanah OA (2006) *Nigella sativa* seed constituents and anxiety relief in experimental models. J Herbs Spices Med Plants 12:153–164

Rchid H, Chevassus H, Nmila R et al (2004) Nigella sativa seed extracts enhance glucose-induced insulin release from rat-isolated Langerhans islets. Fundam Clin Pharmacol 18:525–529

Salim EI (2010) Cancer chemopreventive potential of volatile oil from black cumin seeds, *Nigella sativa* L., in a rat multiorgan carcinogenesis bioassay. Oncol Lett 1:913–924

Sayeed Bin MS, Shams T, Fahim Hossain S et al (2014) *Nigella sativa* L. seeds modulate mood, anxiety and cognition in healthy adolescent males. J Ethnopharmacol 152:156–162

Shafei MN, Boskabady MH, Parsaee H (2005) Effect of aqueous extract from *Nigella sativa* L. on guinea pig isolated heart. Indian J Exp Biol 43:635–639

Sharma PC, Yelne MB, Dennis TJ (2005) Database on medicinal plants used in Ayurveda, vol 6. CCRAS, New Delhi, pp 420–440

Sharma NK, Ahirwar D, Jhade D et al (2009) Medicinal and pharmacological potential of *Nigella sativa*: a review. Ethnobot Rev 13:946–955

Sokmen A, Jones BM, Erturk M (1997) The *in vitro* antibacterial activity of Turkish medicinal plants. J Ethnopharmacol 67:79–86

Swamy SMK, Tan BHH (2000) Cytotoxic and immunopotentiating effects of ethanolic extract of *Nigella sativa*. J Ethnopharmacol 70:1–7

Tahan M, Bayram I (2011) Effect of using black cumin (*Nigella sativa*) and parsley (*Petroselinum crispum*) in laying quail diets on egg yield, egg quality and hatchability. Archiva Zootechnica 14(4):39–44

Tasawar Z, Siraj Z, Ahmad N et al (2011) The effects of *Nigella sativa* (Kalonji) on lipid profile in patients with stable coronary artery disease in Multan, Pakistan. Pak J Nutr 10:162–167

Tousson E, El-Moghazy M, El-Atrsh E (2011) The possible effect of diets containing *Nigella sativa* and *Thymus vulgaris* on blood parameters and some organs structure in rabbit. Toxicol Ind Health 27:107–116

Vafaee F, Hosseini M, Hassanzadeh Z et al (2015) The effects of *Nigella sativa* hydro-alcoholic extract on memory and brain tissues oxidative damage after repeated seizures in rats. Iran J Pharm Res 14:547–557

Vuorelaa P, Leinonenb M, Saikkuc P et al (2004) Natural products in the process of finding new drug candidates. Curr Med Chem 11:1375–1389

Weiss EA (2002) Spices crops. CABI, Wallingford, pp 356–360

Yaman I, Durmus AS, Ceribasi S et al (2010) Effects of *Nigella sativa* and silver sulfadiazine on burn wound healing in rats. Vet Med 55:619–624

Zaghlol DAA, Kamel ES, Mohammed DS et al (2012) The possible toxic effect of different doses of *Nigella sativa* oil on the histological structure of the liver and renal cortex of adult male albino rats. Egypt J Histol 35:127–136

Zanouny AI, Abd-El moty AKI, El-Barody MAA et al (2013) Effect of supplementation with *Nigella sativa* seeds on some blood metabolites and reproductive performance of Ossimi male lambs. Egypt J Sheep Goats 8:47–56

Zeweil HS, Ahmed MH, El-Adawy MM (2008) Evaluation of substituting nigella seed meal as a source of protein for soybean meal in diets of New Zealand white rabbits. In: 9th World Rabbit Congress, 10–13 June, Verona, pp 863–868

Babool (*Acacia nilotica*)

Ramesh C. Gupta, Robin B. Doss, Rajiv Lall, Anita Sinha, Ajay Srivastava, and Jitendra K. Malik

Abstract

The importance of *Acacia* plants in animal nutrition and in the prevention and treatment of human and animal diseases has been recognized for centuries. Babool extract, obtained from *Acacia nilotica* (also known as gum Arabica tree), is very rich in secondary metabolites such as tannins, flavonoids, alkaloids, terpenes, fatty acids, etc. These compounds exert antioxidative, anti-inflammatory, anthelmintic, antidiarrheal, antispasmodic, antihypertensive, antibacterial, antifungal, antidiabetic, antiplatelet aggregatory, antiplasmodial, antimutagenic, anticancer, acetylcholinesterase-inhibiting, diuretic, antipyretic, analgesic, and many other effects. This chapter describes various aspects of babool with special emphasis on its nutritional value and applications in prevention and treatment of diseases in animals.

Keywords

Nutraceuticals · Veterinary nutraceuticals · Babool · Animal health

1 Introduction

Babool (*Acacia nilotica*) is a tropical tree, which can be 15–18 m high and 2–3 m in diameter. The tree is native to the Indian and African subcontinents. Other names for babool are babul, booni, babbula, Egyptian thorn, Egyptian acacia, Indian gum arabic, thorn mimosa, thorny acacia, prickly acacia, black piquant, kikar, sant tree, goma arabica, acacia de cayenne, gommier rouge, and many others. *Acacia nilotica* has several synonyms, such as *Acacia arabica* (Lam.) Wild, *Acacia arabica* var. *cupressiformis* J. Stewart, *Acacia arabica* var. Indica Benth., *Acacia arabica* var. *tomentosa* Benth., Acacia *benthamii* Rochebr., *Acacia nilotica* subsp. *adansonii* (Guill. and Perr.) Brenan, *Acacia scorpioides* (L.) W. Wight, *Acacia subalata* Vatke, *Acacia vera* Wild., and many others.

Babool has many chemical compounds, including tannins, flavonoids, alkaloids, terpenes, fatty acids, etc. These compounds exert antioxidative, anti-inflammatory, anthelmintic, antidiarrheal, antispasmodic, antihypertensive, antibacterial, antiviral, antifungal, antidiabetic, antiplatelet aggregatory, antiplasmodial, antimutagenic, anticancer, acetylcholinesterase inhibitory, diuretic, antipyretic, analgesic, and many other biological and pharmacological effects (Rather et al. 2015). Currently, many phytoconstituents of this plant are used as therapeutic drugs, while others are under investigation for novel uses. This chapter describes various aspects of babool, especially its nutritional value and biological and pharmacological effects in the health and diseases of animals.

2 Chemical Constituents in Babool

Babool (*Acacia nilotica*) is of significant nutritional, nutraceutical, and pharmaceutical importance. Abbasian et al. (2015) reported that mature and dry seeds of babool contain potassium, iron, zinc, copper, and manganese (2.1, 203.1, 108.7, 322.7, and 1.09 g/100 g, respectively). The oil, crude protein, and crude fiber contents in the seeds were found to be 4.1, 25.3, and 28.4% (fresh weight basis), respectively.

At least 66 chemical compounds have been identified in various parts of babool (Rather et al. 2015). The main

R. C. Gupta (✉) · R. B. Doss
Toxicology Department, Breathitt Veterinary Center, Murray State University, Hopkinsville, KY, USA
e-mail: rgupta@murraystate.edu

R. Lall · A. Sinha · A. Srivastava
Vets Plus Inc., Menomonie, WI, USA

J. K. Malik
Division of Pharmacology and Toxicology, Indian Veterinary Research Institute, Dehradun, Uttarakhand, India

R. C. Gupta et al. (eds.), *Nutraceuticals in Veterinary Medicine*, https://doi.org/10.1007/978-3-030-04624-8_8

alkaloids and amines present include dimethyltryptamine, 5-methoxydimethyltryptamine, and N-methyltryptamine. The extract has *D*-pinitol, kaempferol, gallic acid, ellagic acid, (+/−) catechin, (−) epigallocatechin, and rutin. Babool extract has an anti-inflammatory compound, androstene steroid. In addition, the extract has cyclitols, fatty acids (palmitic acid, stearic acid, arachidic acid, oleic acid, linoleic acid, and coronaric acid), seed oils, nonprotein amino acids, terpenes (niloticane, lupenone, and lupeol), saponins, hydrolyzable tannins, flavonoids, and niloticane (Malviya et al. 2011). The extract also contains a total phenolic content ranging from 9.2 to 16.5% (Bushra et al. 2007) and tannins and gallic acid from 24 to 42% (Rahaman 2010). In some studies, tannin content in *A. nilotica* is reported at 18–27%, but in *A. nilotica* subsp. indica, the level could be as high as 50% (Kumari et al. 2014). Babool pods have been found to contain gallic acid, m-digallic acid, (+)-catechin, chlorogenic acid, gallolylated flaven-3, 4-diol robidandiol (7, 3, 4, 5-tetrahydroxyflavan-3,4-diol), kaempferol, umbelliferone, androstene steroid, D-pinitol, carbohydrate, and catechin-5-galloyl ester (Singh et al. 2009a, b; Prathapa Reddy et al. 2018).

The secondary metabolites described in *Acacia nilotica* Delile included naringenin, niloticane, and several galloyl and catechin derivatives isolated from the bark (Khalid et al. 1989; Malan 1991; Eldeen et al. 2010), an androstene steroid from the aerial parts (Chaubal et al. 2003), flavonol glycosides from the seeds (Chauhan et al. 2000), triterpenes botulin and β-amyrin from the roots (Prakash and Garg 1981), arabinobioses from the gum (Chalk et al. 1968), and acanilol A and acanilol B (Ahmadu et al. 2009), together with the known triterpene lupenone, from the stem bark. For further details on chemical constituents in different parts of babool, readers are referred to recent publications (Rana 2018; Prathapa Reddy et al. 2018).

3 Nutritional Value of Babool

In the subcontinents of India and Africa, and other tropical regions, babool (*Acacia nilotica*) is used as an inexpensive source of protein for livestock (Mlambo 2003; Mousa 2011; Paswan et al. 2016). Babool contains about 13% crude protein and about 87% or more organic matter. Bargali and Bargali (2009) found that babool fruit (pods and seeds) contained 12% protein, 2% fat, 15.36% crude fiber, 5.26% ash, 5.45% tannins, 0.26% phosphorus, 0.64% calcium, 0.13% magnesium, 1.28% potassium, 6.43% copper, 28.50 mg/kg zinc, 2650 mg/kg manganese, and 100 mg/kg iron. Recently, Abdullah et al. (2018) evaluated the effect of babool pods on nutrient digestibility, nitrogen balance, and rumen liquor parameters (pH, total protozoa count, protein concentration, and enzyme activity) in rams. The findings revealed that inclusion of babool pods at the rate of 1.5 or 3.0% of the concentrate (equivalent to a tannin concentration of 2.9 and 4.6 g/kg) for 3 weeks significantly improved the total feed intake and the digestibility of crude protein, while the digestibility of dry matter and crude fiber was significantly decreased. Values of nitrogen intake and nitrogen retained were significantly increased by babool supplement. Rams receiving babool showed low protozoa count, protein concentration, and enzymes (α-amylase, cellulase, and protease) in the rumen content, without any change in pH. It was concluded that babool supplement can be used as a natural protein protectant in ruminants by forming tannin-protein complexes in the rumen to maximize the availability of amino acids in the lower digestive tract. The significance of tannins from *A. nilotica* and other acacia plants in the ruminants ration is well documented (Mangan 1988; Scalbert 1991; Mlambo 2003; Mueller 2006). Also, babool pods at the rate of 1.5 or 3.0% can increase the protein digestibility as well as the nitrogen retained in the body. Abbasian et al. (2015) found significant levels of minerals in the seeds of babool. Therefore, babool pods/seeds can be recommended as a dietary supplement of high-protein content and trace and essential minerals to livestock.

4 Pharmacotherapeutic Effects

The leaves, roots, bark, flowers, pods/seeds, branches, and gum extracts of babool have been used in various Ayurvedic, Unani, Chinese, Egyptian, and other traditional medicines for centuries. In general, acacia plants are very rich in bioactive secondary compounds which can be indicated in the promotion of health and prevention and treatment of human and animal ailments. This fact can be substantiated with a few examples, such as triterpenoid and saponins in cancer; glucosides as diuretic and natriuretic; saponins, tannins, and flavonoids in digestive disorders; polyphenols as antioxidants; and tryptamine, tannins, saponins, and organic acids as antiplasmodial (Saini et al. 2008).

Although *A. nilotica* has many medicinal properties, some of them are described here in brief, while others are listed in Table 1.

4.1 Antioxidative and Anti-inflammatory

The extracts from various parts of babool contain many chemical constituents that possess metal chelation, free radical scavenging, and antioxidative properties. Antioxidative activity can be attributed to kaempferol, umbelliferone, and many phenolic compounds present in the babool extracts. In in vitro studies, Singh et al. (2008, 2010) demonstrated that kaempferol and umbelliferone exhibited antioxidative

Table 1 Phytoconstituents in babool (*Acacia nilotica*) and their biological and pharmacological properties

Biological/ pharmacological activity	Bioactive phytoconstituents	References
Antioxidative and free radical scavenging	Kaempferol, umbelliferone, gallic acid, ellagic acid, epicatechin, rutin, tannins	Singh et al. (2008, 2009a), Kalaivani and Mathew (2010), Rajbir et al. (2010), El-Toumy et al. (2011), Abuelgassim (2013a), Rasool et al. (2013), Sokeng et al. (2013), Mohan et al. (2014)
Anti-inflammatory	Androstene, peltogynoids (acanilol A and acanilol B), cassane diterpene (niloticane), triterpene (lupenone)	Dafallah and Al-Mustafa (1996), Chaubal et al. (2003), Ahmadu et al. (2009), Eldeen et al. (2010), Jigam et al. (2010), Sokeng et al. (2013)
Immunostimulatory	Flavonoids, alkaloids, phenolics, steroids, terpenoids, saponins, and tannins	Umaru et al. (2016)
Antibacterial, antiviral, and antifungal	Terpenoids, polyphenols, tannins, alkaloids, saponins, glycosides, flavone, quercetin 3-gallate, nilobamate	Bhargava et al. (1998), Mustafa et al. (1999), Hussein et al. (2000), Elizabeth et al. (2005), Banso (2009), Mohamed et al. (2010), Pai et al. (2010), Vijayasanthi et al. (2011), Fatima et al. (2012), Mbatchou and Oumar (2012), Oladosu et al. (2013), Raheel et al. (2013), Bashir et al. (2014), Dev et al. (2014), Rai et al. (2014), Shanker et al. (2014), Sharma et al. (2014a, b), Srivastava et al. (2014), Abbas and Elhag (2015)
Periodontitis and otitis	Tannins	Pai et al. (2010), Sharma et al (2014)
Antidiarrheal and anthelmintic	Tannins	Agunu et al. (2005), Misar et al. (2008), Bachaya et al. (2009)
Antiplasmodial	Alkaloids, tannins, terpenoids	El-Tahir et al. (1999), Jigam et al. (2010), Alli et al. (2011, 2016), Bapna et al. (2014)
Antidiabetic, hypoglycemic, and antiplatelet aggregatory	Tannins, tannic acid, kaempferol, umbelliferone	Shah et al. (1997), Liu et al. (2005), Ahmad et al. (2008), Asad et al. (2011), Omara et al. (2012), Abuelgassim (2013b), Kumari et al. (2014), Roozbeh et al. (2017)
Antihypertensive and antispasmodic	Triterpenoids	Gilani et al. (1999), Jangade et al. (2014)
Antihypercholesterolemic/ hypolipidemic	Saponins, glycosides, tannin	Ahmad et al. (2008), Tanko et al. (2014)
Antipyretic and analgesic	Polysaccharides, organic acids, flavonoids	Dafallah and Al-Mustafa (1996), Jigam et al. (2010), Alli et al. (2014), Safari et al. (2016)
Gastroprotective	Polyphenols	Bansal and Goel (2012)
Hepatoprotective	Flavonoids, alkaloids, phenolics, steroids, terpenoids, saponins, tannins	Kannan et al. (2013)
Diuretic	Saponins, alkaloids, glycosides	Krishna et al. (2011)
Anti-asthmatic		Sonibare and Gbile (2008)
Anti-acetylcholinesterase	Diterpene niloticane	Eldeen et al. (2005), Krowch and Okello (2009)
Antimutagenic and anticancer	Polyphenols, γ-sitosterol, gallocatechin-5-0-gallate	Meena et al. (2006), Singh et al. (2009b), Sakthivel et al. (2012), Sundarraj et al. (2012)
Prolactin release and milk production	–	Sawadogo et al. (1989), Lompo-Ouedraogo et al. (2004)
Molluscicidal	Phenolic tannins	Hussein Ayoub (1982), Hussein (1982)
Larvicidal	p-Pinitol	Chaubal et al. (2005)
Metal chelation	Phenolic compounds	Singh et al. (2009a)

activity in a dose-dependent manner. Singh et al. (2009a) also reported free radical scavenging and metal chelation effects of babool's green pod extracts.

Babool pods and seeds are an easily accessible source of natural antioxidants, which can be used as supplement to aid the therapy of free radical-mediated diseases such as cancer, diabetes, inflammation, etc. (Amos et al. 1999; Pareek and Choudhry 2013). In several other studies, it was reported that the extracts of babool have strong free radical scavenging and antioxidative activities, which may be due to hydroxyl groups existing in the phenolic compounds (Kalaivani and Mathew 2010; Sultana et al. 2007; Singh and Arora 2007). Vadivel and Biesalski (2012) also found that the methanolic extract of *A. nilotica* seed materials contain a total free phenolic content of 14.57 ± 1.69 g catechin equivalent/ 100 g extract. The levels of ferric reducing antioxidant power (FRAP, 1840 mmol Fe^{2+}/mg extract), inhibition of β-carotene degradation (53.26%), and radical scavenging activity against DPPH (64.91%) and superoxide (53.23%) radicals were reported (reviewed in Pareek and Choudhry 2013). Some studies also provided evidence that among all extracts, the acetone extract exhibited the highest antioxidative activity, and this was related to total phenolic content (Sundaram and Mitra 2007; Rather et al. 2015).

Phytoconstituents, such as androstene, peltogynoids (acanilol A and acanilol B), and triterpene (lupenone), present in the stem bark of *Acacia nilotica* (L.) Delile are reported to exert anti-inflammatory activity (Ahmadu et al. 2009). Ahmadu et al. (2009) tested acanilol A and acanilol B as kinase inhibitors against CDK1, GSK3, CK1, and DYRK1A and found acanilol B as a DYRK1A inhibitor with an IC_{50} value of 19 μM. Eldeen et al. (2010) demonstrated that cassane diterpene niloticane from the bark extract exhibited COX-1 and COX-2 inhibitory effect with IC_{50} values of 3.6 μM and 189 μM, respectively. Chaubal et al. (2003) attributed anti-inflammatory activity to 3-β-acetoxy-17-β-hydroxyandrost-5-ene present in the aerial parts of babool. In in vivo studies, carrageenan- or formalin-induced paw edema model and cotton pellet-induced granuloma model in rats, *A. nilotica* extract significantly reduced the inflammatory reaction (Dafallah and Al-Mustafa 1996; Sokeng et al. 2013; Safari et al. 2016).

4.2 Antimicrobial

The leaves, flowers, pods/seeds, bark, and root of *A. nilotica* have been extensively studied for their antimicrobial (antibacterial, antiviral, and antifungal) activity. Banso (2009) reported that the stem bark extract of the plant possessed certain bioactive constituents including terpenoids, tannins, saponins, and glycosides. The antimicrobial activity of the extracts was assayed against *Streptococcus viridans*, *Staphylococcus aureus*, *E. coli*, *Bacillus subtilis*, and *Shigella sonnei*. The plant extract exhibited antimicrobial activity against all the test microorganisms. *B. subtilis* was found to be the most susceptible, and *Candida albicans* was the most resistant to the plant extract. The minimum inhibitory concentration of the extract ranged between 35 and 50 mg/ml, while the minimum bactericidal concentration ranged between 35 and 60 mg/ml. Fatima et al. (2012) assessed antibacterial activity of leaf bark and root extracts (aqueous and ethyl acetate) of *A. nilotica* (L.) Del. against *Xanthomonas malvacearum* bacteria and found that ethyl acetate extracts of the root seem to contain greater antibacterial components than the pure antibiotic (streptomycin or tetracycline), with a concentration of 500 μg/ml. Saini et al. (2008) reported that the methanolic extract of *A. nilotica* pods shows antimicrobial activity against *E. coli*, *S. aureus*, and *A. niger*.

Dev et al. (2014) examined antimicrobial activity of aqueous, chloroform, ethanol, and methanol extracts of different parts (stem, leaf, seed) of *A. nilotica* (L.) Del. against *E. coli*, *Agrobacterium tumefaciens*, *Bacillus aureus*, *Candida glabrata*, and *Aspergillus niger*. Only the methanolic extract showed good activity against all bacteria and fungi (except *A. niger*) due to the presence of alkaloids, saponins, flavonoids, tannins, and glycosides in the leaf extract. Rani and Khullar (2004) observed moderate antimicrobial activity of methanol and aqueous extracts of *A. nilotica* toward multidrug-resistant *Salmonella typhi*.

In some studies, antimicrobial activity of *A. nilotica* has been reported against pathogens involved in periodontitis (including *Streptococcus mutans*; Sharma et al. 2014a, b) and otitis (Pai et al. 2010).

Like some other plant products (Vanden Berghe et al. 1986; Vlietinck and Vanden Berghe 1991; Vlietinck et al. 1997), *A. nilotica* extract has been reported to exert antiviral activity against fowl pox, Newcastle disease, and hepatitis C virus (Hussein et al. 2000; Mohamed et al. 2010).

Antimicrobial activity of babool extracts appears to be due to hydrophilic compounds such as polyphenols, polysaccharides, terpenoids, tannins, alkaloids, saponins, glycosides, flavone, and quercetin 3-gallate. The antifungal activity may be attributed to polyphenols and nilobamate (Bhargava et al. 1998; Mbatchou and Oumar 2012; Rai et al. 2014).

A dental chew formulation ACANIL (Vets Plus Inc, Menomonie, WI, USA), which contains babool extract and white curcumin, showed a great effect on reducing halitosis in dogs. In in vitro studies, ACANIL has shown a zone of inhibition on microbial colonies, and the effect was comparable to chlorhexidine (data presented at the 26th American Dental Congress 2017, Philadelphia, PA, USA). In proof of concept clinical studies, ACANIL has been found significantly effective (unpublished).

It is suggested that babool extract can be used as an antimicrobial nutraceutical in humans and animals.

4.3 Antiplasmodials

A good number of plant extracts have been found to possess antiplasmodial activity (Ibrahim et al. 1991; El-Tahir et al. 1999; Alli et al. 2016). El-Tahir et al. (1999) reported that ethyl acetate extract of *A. nilotica*, by having tannins and terpenoids, exerted a very strong inhibitory potential (IC_{50} = 1.5 μg/ml) against *Plasmodium falciparum*. The methanol extract of *A. nilotica* seed exerted high activity with an IC_{50} value of 0.9 μg/ml. The husk also revealed highly potent antiplasmodial activity where the methanol extract and the water extract showed IC_{50} values of 4.9 and 7.5 μg/ml, respectively. Recently, Alli et al. (2016) demonstrated that a particular fraction (F-1 rich in alkaloids and phenolics) of *A. nilotica* root (50 and 100 mg/kg body wt) produced a significant and dose-dependent reduction in *Plasmodium berghei*-infected mice compared to the control and also significantly increased the survival time of the mice compared to the control group. The same fraction also ameliorated malaria-induced anemia by improving PCV in

treated mice. However, this fraction of *A. nilotica* could not reverse the reduced body temperature and weight loss associated with rodent malaria. In several other studies, roots, twigs, and other parts of *A. nilotica* extracts have shown strong antiplasmodial potential (Alli et al. 2011; Bapna et al. 2014). Taking all findings into consideration, *A. nilotica* extract (particularly Fraction-1) appears to be an alternative therapy to conventional drugs which have an issue of drug resistance.

4.4 Anticholinesterase

Eldeen et al. (2005) reported that *A. nilotica* possesses anticholinesterase properties. Krowch and Okello (2009) further demonstrated the activity of *A. nilotica* root in an aqueous extraction (IC_{50} 0.079 mg/ml) to be about tenfold more potent than with leaf (IC_{50} 0.7 and 0.5 mg/ml for ethyl acetate and ethanol extracts, respectively) and bark (IC_{50} 1.3 mg/ml ethyl acetate extraction). Acetylcholinesterase inhibition kinetics revealed a concentration-dependent mixed type inhibition (noncompetitive uncompetitive), similar to that found with galantamine. *A. nilotica* extract was found not to be as strong AChE inhibitor as galantamine. However, by having antioxidative, anti-inflammatory, and acetylcholinesterase (AChE) properties, *A. nilotica* could provide the basis as a novel poly-pharmacological treatment of chronic cognition syndrome in senior dogs and cats.

4.5 Anti-diabetic, Hypoglycemic, and Hypolipidemic

Currently, a variety of herbal treatments are recommended for the management of type 2 diabetes. Karau (2013) reported that the aqueous extract of *A. nilotica* exerts an antidiabetic effect which may be due to the release of insulin from pancreatic β-cells. Babool is known to have a very high content of tannins, and tannic acid stimulates the transport of glucose and inhibits adipocyte differentiation, thereby producing an antidiabetic effect (Liu et al. 2005; Kumari et al. 2014). In alloxan-induced diabetic rabbits, methanol extract of *A. nilotica* pods (400 mg/kg body wt) showed significant reductions in blood glucose, plasma total cholesterol, triglyceride, and low-density lipids. In a similar study conducted on alloxan-induced diabetic rats, methanolic extract of fruits of this plant did not significantly reduce serum glucose but did reduce serum levels of triglycerides and low-density lipoprotein cholesterol (Abuelgassim 2013a, b). However, in the streptozotocin-induced diabetic rat model, methanolic extract of *A. nilotica* pod extract (150 and 300 mg/kg body wt/day for 60 days) lowered blood glucose levels, restored serum urea, and creatinine levels as well as the normal histopathological architecture of the kidney (Omara et al. 2012). Asad et al. (2011) found that *A. nilotica* leaf extract (300 mg/kg body wt) produced hypoglycemic and antiplatelet aggregation effects in streptozotocin-induced diabetic rats.

Pareek and Choudhry (2013) assessed the effect of babool pods powder (2, 3, or 4 g/day) on blood glucose and lipid levels in type 2 diabetic subjects. After 4 weeks of treatment, the patients showed reduced fasting blood glucose (10–19%), postprandial (7–35%), triglyceride (6–18%), LDL (7–10%), total cholesterol (5–11%), VLDL (7–15%), HDL cholesterol (5–10%), and blood pressure (8–13%). Significant changes occurred in the postprandial glucose, triglyceride, VLDL cholesterol, and blood pressure of the subjects receiving 4 g/day dose. In several other studies, babool pod products have been found to exert antidiabetic, hypoglycemic, and hypolipidemic effects (Ahmad et al. 2008; Rahaman 2010; Roozbeh et al. 2017). The observed antihyperglycemic effect of *A. nilotica* extracts in diabetes may be due to multiple mechanisms: (1) increased insulin release from pancreatic β-cells, (2) antioxidative effect, (3) anti-inflammatory effect, and (4) increased glucose transport to tissues from circulation. In conclusion, it can be suggested that a diet supplemented with babool products will produce antidiabetic effects and reduce risk factors associated with cardiovascular and renal diseases.

4.6 Antipyretic and Analgesic

Alli et al. (2014) investigated the effect of an aqueous extract of *A. nilotica* root on pain and fever in rats. These investigators used Brewer's yeast suspension to induce pyrexia and the hot plate, tail immersion, and acetic acid-induced writhing tests as nociceptive models for the analgesic study. In a dose-dependent manner, the extract produced significant reduction in rectal temperature at 200 and 400 mg/kg body wt. At these dose levels, significant analgesic activities were observed in the hot plate, tail immersion, and acetic acid-induced writhing, and the effects were comparable to acetaminophen (150 mg/kg body wt). In a recent study, Safari et al. (2016) demonstrated antinociceptive (not dose-dependent), anti-inflammatory (80.07%), and antipyretic effects (98.89%) of aqueous extract of *A. nilotica* bark (150 mg/kg) in mice. These studies provided scientific support for the use of *A. nilotica* root and bark extracts for fever, inflammation, and pain.

4.7 Antihypertensive and Antispasmodic

Gilani et al. (1999) reported that a methanol extract of babool pods caused a dose-dependent (3–30 mg/kg) fall in arterial

blood pressure, and the observed effect was independent of muscarinic receptor stimulation or adrenoceptor blockage. In the in vitro studies, these investigators found that the plant extract produced a dose-dependent (0.3–3 mg/ml) inhibitory effect on force and rate of spontaneous contractions in guinea pig atria. Similarly, it inhibited the spontaneous contraction of rabbit jejunum in a concentration-dependent (0.1–3 mg/ml) manner. The extract inhibited K^+-induced contractions in rabbit jejunum at a similar concentration range, which suggests that the antispasmodic action of babool is mediated through calcium channel blockage, and this may also be responsible for the antihypertensive effect.

4.8 Antimutagenic and Anticancer

This antihypertensive effect appears to be independent of muscarinic acetylcholine receptor stimulation or adrenoceptor blockade. The same extract is also known to exert antispasmodic activity (Gilani et al. 1999). Lompo-Ouedraogo et al. (2004) demonstrated that an aqueous extract of babool stimulated milk production (59% greater) and prolactin release in female rats. This could consequently be helpful in lactating animals and women. In Africa, babool extract has been used for cough, asthma, diarrhea, dysentery, conjunctivitis, skin diseases, tumors, cancers, and leprosy treatment, and in Egypt for diabetes mellitus treatment.

In a recent study, Umaru et al. (2016) treated rats with babool pod aqueous extract (50, 100, 200, and 400 mg/kg) daily for 21 days and found that the extract had immunostimulatory and anti-hemostatic properties.

Other ethnopharmacological claims include antimicrobial (antibacterial, antifungal, antimalarial), antidiarrheal, antioxidant, antispasmodial, antihypertensive, antidiabetic, antimutagenic, anti-inflammatory, analgesic, antiplatelet, anticancer, and molluscicidal activities (Amos et al. 1999; Rajvaidhya et al. 2015). It has been used as an anthelmintic in ethnoveterinary medicine (Bachaya et al. 2009). A 50% ethanolic extract of the stem bark in a preliminary biological screening exhibited antiprotozoal activity against *Entamoeba histolytica* in dogs and cats.

5 Toxicity and Safety

Al-Mustafa (2000) found a low toxicity potential of babool extract in rats receiving 2% and 8% acacia diet for 2 and 4 weeks. There was no change in serum biomarkers for hepatic and renal functions, fasting glucose, and triglycerides. No histopathological changes in liver sections and no deaths in animals were noted. Alli et al. (2015) reported that the aqueous extract of *Acacia nilotica* root was found to be safe in a single acute dose (50, 300, and 2000 mg/kg body wt) in mice. The estimated oral LD_{50} in mice is 5000 mg/kg. The IP LD_{50} in mice was reported to be 500 mg/kg (Bhakuni et al. 1969). In a 28-day subacute study (125, 250, and 500 mg/kg babool extract) in rats, doses higher than 250 mg/kg body wt appeared to cause hepatotoxicity (Alli et al. 2015). There was no evidence of nephrotoxicity in the subacute toxicity study. These authors suggested NOAEL <250 mg/kg body wt.

6 Concluding Remarks and Future Directions

Babool has been widely used for multipurpose (nutritional, nutraceutical, and pharmacological) in human and animal medicine for centuries. Different parts of babool (bark, root, fruits/pods, leaves, and gum) have different chemical constituents, and accordingly their applications differ in disease conditions. The root portion of the tree is widely used in traditional treatment of diseases because of its wide margin of safety. Antioxidative and anti-inflammatory properties of *A. nilotica* play significant roles in ameliorating various diseases. Based on acute toxicity in mice, aqueous extract of babool root extract is safe up to a dose of 2000 mg/kg; and its NOEL in rats is reported to be <250 mg/kg/day. Repeat exposure at higher doses may cause hepatotoxicity.

References

Abbas ZTEM, Elhag WI (2015) *In vitro* antibacterial activity of *Acacia nilotica* methanolic extract against wound infection pathogen. Am J Res Commun 3:111–121

Abbasian K, Asgarpanah J, Ziarati P (2015) Chemical composition profile of *Acacia nilotica* seed growing wild in south of Iran. Orient J Chem 31(2):1027–1033

Abdullah MAM, Farghaly MM, Youssef IMI (2018) Effect of feeding *Acacia nilotica* pods to sheep on nutrient digestibility, nitrogen balance, ruminal protozoa and rumen enzymes activity. J Anim Physiol Anim Nutr 102:662–669

Abuelgassim AO (2013a) Antioxidant potential of date palm leaves and *Acacia nilotica* fruit in comparison with other four common Arabian medicinal plants. Life Sci J 10:3405–3410

Abuelgassim AO (2013b) Effect of *Acacia nilotica* fruit extract on serum glucose and lipid concentrations in alloxan-induced diabetic rats. Pak J Biol Sci 16(21):1398–1402

Agunu A, Yusuf S, Andrew GO et al (2005) Evaluation of five medicinal plants used in diarrhea treatment in Nigeria. J Ethnopharmacol 101:27–30

Ahmad M, Zaman F, Sharif T et al (2008) Antidiabetic and hypolipidemic effects of aqueous methanolic extract of *Acacia nilotica* pods in alloxan-induced diabetic rabbits. Scand J Lab Anim Sci 35(1):29–34

Ahmadu A, Abdulkarim A, Grougnet R et al (2009) Two new peltogynoids from *Acacia nilotica* Delile with kinase inhibitory activity. Planta Med 75:1–3

Alli LA, Adesokan AA, Salawu OA et al (2011) Antiplasmodial activity of aqueous root extract of *Acacia nilotica*. Afr J Biochem Res 5:214–219

Alli LA, Nafiu MO, Adesokan AA et al (2014) Antipyretic and analgesic activities of aqueous extract of *Acacia nilotica* root. Biokemistri 26:55–62

Alli LA, Adesokan AA, Salawu OA, Akanji MA (2015) Toxicological studies of aqueous extract of *Acacia nilotica* root. Interdisc Toxicol 8 (1):48–54

Alli A, Adesokan AA, Salawu AO (2016) Antimalarial activity of fractions of aqueous extract of *Acacia nilotica* root. J Intercult Ethnopharmacol 5(2):180–185

Al-Mustafa ZH (2000) A study on the toxicology of *Acacia nilotica*. Am J Chin Med 28:123–129

Amos S, Akah CJ, Odukwe KS, Wambede C (1999) The pharmacological effects of an aqueous extract from *Acacia nilotica* seeds. Phytother Res 13:683–685

Asad M, Munir TA, Afzal N (2011) *Acacia nilotica* leave extract and glyburide: comparison of fasting blood glucose, serum insulin, β-thromboglobulin levels and platelet aggregation in streptozotocin induced diabetic rats. J Pak Med Assoc 61:247–251

Bachaya HA, Iqbala Z, Khan MN et al (2009) Anthelmintic activity of *Ziziphus nummularia* (bark) and *Acacia nilotica* (fruit) against *Trichostrongylid* nematodes of sheep. J Ethnopharmacol 123:325–329

Bansal VK, Goel RK (2012) Gastroprotective effect of *Acacia nilotica* young seedless pod extract: role of polyphenolic constituents. Asian Pac J Trop Med 5:523–528

Banso A (2009) Phytochemical and antibacterial investigation of bark extracts of *Acacia nilotica*. J Med Pants Res 3:82–85

Bapna S, Ramaiya M, Chowdhary A (2014) Antimalarial potential of plants used as chewing sticks for oral hygiene in rural areas of Rajasthan, India. Am J Ethnomed 1:319–325

Bargali K, Bargali SS (2009) *Acacia nilotica*: a multipurpose leguminous plant. Nat Sci 7(4):11–19

Bashir HS, Mohammed AM, Magsoud AS et al (2014) Isolation and identification of two flavonoids from *Acacia nilotica* (*Leguminoseae*) leaves. J Forst Prod Indust 3(5):211–215

Bhakuni DS, Dhar ML, Dhar MM et al (1969) Screening of Indian plants for biological activity. Part II. Indian J Exp Biol 7:250–262

Bhargava A, Srivastava A, Kumbhare VC (1998) Antifungal activity of polyphenolic complex of *Acacia nilotica* bark. Indian Forest 124:292–298

Bushra S, Farooq A, Roman P (2007) Antioxidant activity of phenolic components present in barks of *Azarichta indica*, *Terminalia arjuna*, and *Acacia nilotica*, and *Eugenia jambolana* Lam trees. Food Chem 104(3):148–161

Chalk RC, Stoddart JF, Szarek WA et al (1968) Isolation of two arabinobioses from *Acacia nilotica* gum. Can J Chem 46:2311–2313

Chaubal R, Mujumdar AM, Puranik VG et al (2003) Isolation and X-ray study of an anti-inflammatory active androstene steroid from *Acacia nilotica*. Planta Med 69:287–288

Chaubal R, Pawar PV, Hebbalkar GD et al (2005) Larvicidal activity of *Acacia nilotica* extracts and isolation of p-pinitol-a bioactive carbohydrate. Chem Biodivers 2:684–688

Chauhan D, Singh J, Siddiqui IR (2000) Isolation of two flavonol glycosides from the seeds of *Acacia nilotica*. Indian J Chem 39 (B):719–722

Dafallah AA, Al-Mustafa Z (1996) Investigation of the anti-inflammatory activity of *Acacia nilotica* and *Hibiscus sabdariffa*. Am J Chin Med 24:263–269

Dev SNC, De K, Singh S (2014) Antimicrobial activity and phytochemical analysis of *Acacia nilotica* (L.) Del. Indian J Appl Pure Biol 29:331–332

Eldeen IMS, Elgorashi EE, Staden J (2005) Antibacterial, anti-inflammatory, and anti-cholinesterase and mutagenic effects of extracts from some trees used in South African traditional medicine. J Ethnopharmacol 102:457–464

Eldeen IM, Heerden V, Staden JV (2010) *In vitro* biological activities of niloticane, a new bioactive cassane diterpene from the bark of *Acacia nilotica* subsp. kraussiana. J Ethnopharmacol 128:555–560

Elizabeth KM, Sireesha D, Rao KN et al (2005) Antimicrobial activity of *Acacia nilotica*. Asian J Chem 18:191–195

El-Tahir A, Satti GMH, Khalid SA (1999) Antiplasmodial activity of selected Sudanese medicinal plants with emphasis on *Acacia nilotica*. Phytother Res 13:474–478

El-Toumy SA, Mohamed SM, Hassan EM et al (2011) Phenolic metabolites from *Acacia nilotica* flowers and evaluation of its free radical scavenging activity. J Am Sci 7:287–295

Fatima S, Baig MR, Baig M et al (2012) Antimicrobial activity of *Acacia nilotica* (L.) Del. Plant extract against *Xanthomonas malvacearum* bacteria. Int Multidiscipl Res J 2:48–49

Gilani AH, Shaheen F, Zaman M et al (1999) Studies on antihypertensive and antispasmodic activities of methanol extract of *Acacia nilotica* pods. Phytother Res 13:665–669

Hussein A (1982) Molluscicidal properties of *Acacia nilotica*. Planta Med 46:181–183

Hussein Ayoub SM (1982) Molluscicidal properties of *Acacia nilotica* subspecies tomentosa and astringens II. J Trop Med Hyg 88 (3):201–203

Hussein G, Miyashiro H, Nakamura N et al (2000) Inhibitory effects of Sudanese medicinal plant extracts on hepatitis C virus (HCV) protease. Phytother Res 14:510–516

Ibrahim AM, Phillipson JD, Warhurst DC et al (1991) The potential antimalarial activity of some Sudanese plants. Trans Roy Soc Trop Med Hyg 85:310–318

Jangade NM, Nagargoje PB, Shirote PJ (2014) Isolation, phytochemical and biological evaluation of *Acacia nilotica* (L.) Wild. leaf extract. Int J Pharmacog Phytochem Res 6:179–182

Jigam AA, Akanya HO, Ogbadoyi EO et al (2010) *In vivo* antiplasmodial, analgesic and anti-inflammatory effects of the root extracts of *Acacia nilotica* Del (*Leguminoseae*). Asian J Exp Biol Sci 1:315–320

Kalaivani T, Mathew L (2010) Free radical scavenging activity from leaves of *Acacia nilotica* (L.) Wild. ex Delile, an Indian medicinal tree. Food Chem Toxicol 48:298–305

Kannan LN, Sakthivel KM, Guruvayoorappan C (2013) Protective effect of *Acacia nilotica* (L.) against acetaminophen-induced hepatocellular damage in Wistar rats. Adv Pharmacol Sci 2013:987692

Karau GM (2013) Biosprospecting of antidiabetic compounds from selected medicinal plants for the management of diabetes mellitus in Mbeere and Meru, Kenya. PhD Thesis

Khalid SA, Yagi SM, Khritova P et al (1989) (+)-Catechin-5-galloyl ester as a novel natural polyphenol from the bark of *Acacia nilotica* of Sudanese origin. Planta Med 55:556–558

Krishna PSR, Lavanya B, Sireesha P et al (2011) Comparative study of *Acacia nilotica* and *Acacia sinuata* for diuretic activity. Der Pharmacia Sinc 2(6):17–22

Krowch CM, Okello EJ (2009) Kinetics of acetylcholinesterase inhibitory activities by aqueous extracts of *Acacia nilotica* (L.) and *Rhamnus prinoides* (L'Hér.). Afr J Pharm Pharmacol 3:469–475

Kumari M, Jain S, Dave R (2014) Babul (*Acacia nilotica*) a potential source of tannin and its suitability in management of type II diabetes. Nutr Food Sci 44(2):116–119

Liu X, Kim J-K, Li Y et al (2005) Tannic acid stimulates glucose transport and inhibits adipocyte differentiation in 3T3-L1 cells. Am Soc Nutr Sci 135:165–171

Lompo-Ouedraogo Z, van der Heide D, van der Beek EM et al (2004) Effect of aqueous extract of *Acacia nilotica* ssp *adansonii* on milk production and prolactin release in the rat. J Endocrinol 182:257–266

Malan E (1991) Derivatives of (+)-catechin-5-gallate from the bark of *Acacia nilotica*. Phytochemistry 30:2737–2739

Malviya S, Rawat S, Kharia A, Varma M (2011) Medicinal attributes of *Acacia nilotica* Linn. A comprehensive review on ethnopharmacological claims. Int J Pharm Life Sci 2(6):830–837

Mangan J (1988) Nutritional effects of tannins in animal feeds. Nutr Res Rev 1:209–231

Mbatchou VC, Oumar AA (2012) Antifungal activity of nilobamate isolated from *Acacia nilotica* Wild. Phytopharmacology 3:208–213

Meena PD, Kaushik P, Shukla S et al (2006) Anticancer and antimutagenic properties of *Acacia nilotica* (Linn.) on 7,12-dimethylbenz(a)anthracene-induced skin papillomagenesis in Swiss albino mice. Asian Pac J Cancer Prev 7(4):627–632

Misar A, Bhagat R, Mujumdar AM (2008) Antidiarrheal activity of *Acacia nilotica* Wild. bark methanol extract. Hindustan Antibiot Bull 50:14–20

Mlambo V (2003) Modifying the nutritional effects of tannins present in Acacia and other tree fruits offered as protein supplements to goats in Zimbabwe. PhD Thesis, University of Reading, Reading, UK, p 273

Mohamed LT, Bushra EIS, Abdelrahman MN (2010) The antibacterial, antiviral activities and phytochemical screening of some Sudanese medicinal plants. Eur Asian J Biosci 4:8–16

Mohan S, Thiagarajan K, Chandrasekaran R et al (2014) *In vitro* protection of biological macromolecules against oxidative stress and *in vivo* toxicity evaluation of *Acacia nilotica* (L.) and ethyl gallate in rats. BMC Complement Altern Med 14:257–270

Mousa M (2011) Effect of feeding acacia as supplements on the nutrient digestion, growth performance, carcass traits and some blood constituents of Awassi lambs under the conditions of North Sinai. Asian J Anim Sci 5:102–117

Mueller H (2006) Unravelling the conundrum of tannins in animal nutrition and health. J Sci Food Agr 86:2010–2037

Mustafa NK, Tanira MOM, Dar FK et al (1999) Antimicrobial activity of *Acacia nilotica* subsp. *nilotica* fruit extracts. Pharm Pharmacol Commun 5:583–586

Oladosu P, Isu NR, Ibrahim K et al (2013) Time kill-kinetics antibacterial study of *Acacia nilotica*. Afr J Microbiol 7:5248–5252

Omara EO, Nadab SA, Farraga ARH et al (2012) Therapeutic effect of *Acacia nilotica* pods extract on streptozotocin induced diabetic nephropathy in rat. Phytomedicine 19(12):1059–1067

Pai MBP, Prashant GM, Murlikrishna KS et al (2010) Antifungal efficacy of *Punica granatum*, *Acacia nilotica*, *Cuminum cyminum* and *Foeniculum vulgare* on *Candida albicans*: an *in vitro* study. Indian J Dent Res 21:334–336

Pareek P, Choudhry M (2013) Management of type 2 diabetics by Indian gum arabic (*Acacia nilotica*) pods powder. Int J Food Nutr Sci 2(2):77–83

Paswan JK, Kumar K, Kumar S et al (2016) Effect of feeding *Acacia nilotica* pod meal on hematobiochemical profile and fecal egg count in goats. Vet World 9:1400–1406

Prakash L, Garg G (1981) Chemical constituents of the roots of *Millingtonia hortensis* L. and *Acacia nilotica* (L.) Del. J Indian Chem Soc 58:96–97

Prathapa Reddy M, Shantha TR, Naveen Kumar SP et al (2018) Pharmacognostical studies on fruits of babbula-*Acacia nilotica* (L.) Delile. Int J Herbal Med 6(2):115–120

Rahaman O (2010) A review of uses *Acacia nilotica* (Booni) in alternative medicine. SearchWarp.com

Raheel R, Ashraf M, Ejaz S et al (2013) Assessment of the cytotoxic and antiviral potential of aqueous extracts from different parts of *Acacia nilotica* (Linn) Delile against Peste des petits ruminants virus. Environ Toxicol Pharmacol 35:72–81

Rai SP, Prasad MS, Singh K (2014) Evaluation of the antifungal activity of the potent fraction of hexane extract obtained from the bark of *Acacia nilotica*. Int J Sci Res 3:730–738

Rajbir S, Bikram S, Sukhpreet K et al (2010) Umbelliferone-an antioxidant isolated from *Acacia nilotica* (L.) Wild. ex Del. Food Chem 120 (3):825–830

Rajvaidhya S, Nagori BP, Singh GK et al (2015) A review of *Acacia arabica* – an Indian medicinal plant. Int J Pharmaceut Sci Res 1.11:90.2

Rana D (2018) A review of ethnomedicine, phytochemical and pharmacological properties of *Acacia nilotica* (babool/kikar). Int J Biol Pharmac Allied Sci 7(5):856–863

Rani P, Khullar N (2004) Antimicrobial evaluation of some medicinal plants for their anti-enteric potential against multi-drug resistant *Salmonella typhi*. Phytother Res 18:670–673

Rasool N, Tehseen H, Riaz M et al (2013) Cytotoxicity studies and antioxidant potential of *Acacia nilotica* roots. Int J Chem Biochem Sci 3:34–41

Rather LJ, Islam S-U, Mohammad F (2015) *Acacia nilotica* (L.): a review of its traditional uses, phytochemistry, and pharmacology. Sustain Chem Pharm 2:12–30

Roozbeh N, Darvish L, Abdi F (2017) Hypoglycemic effects of *Acacia nilotica* in type II diabetes: a research proposal. BMC Res Notes 10:331

Safari VZ, Kamau JK, Nthiga PM et al (2016) Antipyretic, anti-inflammatory and antinociceptive activities of aqueous bark extract of *Acacia nilotica* (L.) Delile in albino mice. J Pain Manage Med 2 (2):113

Saini ML, Saini R, Roy S et al (2008) Comparative pharmacognostical and antimicrobial studies of *acacia species* (*Mimosaceae*). J Medic Plant Res 2(12):378–386

Sakthivel KM, Kannan N, Angeline A et al (2012) Anticancer activity of *Acacia nilotica* (L.) Wild ex. Delile subsp. indica against Dalton's ascitic lymphoma induced solid and ascitic tumor model. Asian Pac J Cancer Prev 13(8):3989–3995

Sawadogo L, Sepehri H, Houdebine LM (1989) Mise en evidence d'un facteur stimulant la sécrétion de PRL et d'hormone de croissance dans la drè che de brasserie. Reprod Nutr Dev 29:139–146

Scalbert A (1991) Antimicrobial properties of tannins. Phytochemistry 30:3875–3883

Shah BH, Safdar B, Virani SS et al (1997) The antiplatelet aggregatory activity of *Acacia nilotica* is due to blockade of calcium influx through membrane calcium channels. Gen Pharmacol 29:251–255

Shanker K, Krishna MG, Bhagavan RM et al (2014) Efficacy of leaves extract of *Acacia nilotica* against *Pseudomonas aeruginosa* with reference to disc diffusion method. Res J Pharmacogn Phytochem 6:96–98

Sharma A, Sankhla B, Parker SM et al (2014a) Effect of traditionally used neem and babool chewing stick (datum) on *Streptococcus mutans*: an *in vitro* study. J Clin Diagn Res 8(7):ZC15–ZC17

Sharma C, Aneja KR, Surain P et al (2014b) *In vitro* evaluation of antimicrobial spectrum of *Acacia nilotica* leaves and bark extracts against pathogens causing otitis infection. J Innov Biol 1(1):051–056

Singh R, Arora S (2007) Attenuation of free radicals by acetone extract/fraction of *Acacia nilotica* Wild (L.) ex Del. J Chin Clin Med 2:196–203

Singh R, Singh B, Singh S et al (2008) Anti-free radical activities of kaempferol isolated from *Acacia nilotica* (L.) Wild. ex. Del. Toxicol In Vitro 22(8):1965–1970

Singh BN, Singh BR, Singh RL et al (2009a) Antioxidant and anti-quorum sensing activities of green pods of *Acacia nilotica* L. Food Chem Toxicol 47:778–786

Singh BN, Singh BR, Sarma BK et al (2009b) Potential chemoprevention of N-nitrosodiethylamine- induced hepato-carcinogenesis by polyphenolics from *Acacia nilotica* bark. Chem Biol Interact 181 (1):20–28

Singh R, Singh B, Singh S et al (2010) Umbelliferone-an antioxidant isolated from *Acacia nilotica* (L.) Wild. ex Del. Food Chem 120:825–830

Sokeng SD, Koubé J, Dongmo F et al (2013) Acute and chronic anti-inflammatory effects of the aqueous extract of *Acacia nilotica* (L.) Del. (*Fabaceae*) pods. Acad J Med Plants 1:001–005

Sonibare MA, Gbile ZO (2008) *Acacia nilotica* is good for the treatment of asthma. Afr J Tradit Complement Altern Med 5:345

Srivastava M, Kumar G, Mohan R et al (2014) Phytochemical studies and antimicrobial activity of babool seeds. J Sci Ind Res 73:724–728

Sultana B, Anwar F, Przybylski R (2007) Antioxidant activity of phenolic components present in bark of *Azadirachta indica*, *Terminalia arjuna*, *Acacia nilotica*, and *Eugenia jambolana* Lam. trees. Food Chem 104:1106–1114

Sundaram R, Mitra SK (2007) Antioxidant activity of ethyl acetate soluble fraction of *Acacia nilotica* bark in rats. Indian J Pharmacol 39:33–38

Sundarraj S, Thangam R, Sreevani V et al (2012) γ-Sitosterol from *Acacia nilotica* L. induces G2/M cell cycle arrest and apoptosis through c-Myc suppression in MCF-7 and A549 cells. J Ethnopharmacol 141(3):803–809

Tanko Y, Abdulazeez A, Muhammad A et al (2014) Effect of methanol crude leaves extract and aqueous fraction of *Acacia nilotica* on lipid profile and liver enzymes on alloxan-induced diabetic rats. Ann Exp Biol 2:36–40

Umaru B, Mahre S, Dogo HM et al (2016) Effects of aqueous pod extract of *Acacia nilotica* on white blood cells, platelets and clotting time in albino rats. Am J Pharmacol Pharmacother 3 (3):1–6

Vadivel V, Biesalski HK (2012) Total phenolic content, *in vitro* antioxidant activity and type II diabetes relevant enzyme inhibition properties of methanolic extract of traditionally processed underutilized food legume, *Acacia nilotica* (L.) Wild ex. Delile. Int Food Res J 19(2):593–601

Vanden Berghe DA, Vlietinck AJ, Van Hoof L (1986) Plant products as potential antiviral agents. Bull de l'institut Pasteur 84:101–147

Vijayasanthi M, Kannan V, Venkataswamy R, Doss A (2011) Evaluation of the antibacterial potential of various solvent extracts of *Acacia nilotica* Linn. leaves. J Drug Med 4:91–96

Vlietinck AJ, Vanden Berghe DA (1991) Can ethnopharmacology contribute to the development of antiviral drugs. J Ethnopharmacol 32:141–153

Vlietinck AJ, de Bruyne T, Vanden Berghe DA (1997) Plant substances as antiviral agents. Curr Organ Chem 1:307–344

Glucosinolates and Organosulfur Compounds

Karyn Bischoff

Abstract

Glucosinolates are compounds commonly found in plants of the Brassicaceae family. Many common edible plants, such as broccoli, cabbage, cauliflower, mustard, and horseradish, are members of the Brassicaceae family, and the strong flavors of these plants correlate with high glucosinolate concentrations. Recent studies have found beneficial effects of glucosinolates, including regulatory functions in inflammation, stress response, phase I metabolism, antioxidant activities, and direct antimicrobial properties. Future studies will no doubt find more benefits of these chemicals. However, feeding rations high in brassicae to livestock is likely to have adverse effects, some life-threatening, and may add unwanted flavors to animal products. The high sulfur content of many brassicas can cause trace mineral deficiencies and polioencephalomalacia. Therefore, while Brassicaceae-derived feeds can be a good source of nutrition, it's best to avoid overusing them.

Keywords

Glucosinolates · Brassica · Canola · Rapeseed · Erucic acid · Broccoli · Cabbage · Cauliflower · Mustard

1 Introduction

The first known glucosinolate was isolated in 1831 (Dinkova-Kostova and Kostov 2012). Glucosinolates and other organosulfur compounds are found in the Brassica family of plants, which includes the cruciferous vegetables: cabbage, broccoli, cauliflower, kale, mustards, etc. The Brassicaceae family also includes the important oil seed crop canola or rapeseed, *Brassica napus* (Burel et al. 2000). Many brassicas are late-season crops; thus they remain palatable after other plants have died (Burrows and Tyrl 2013). Glucosinolates are present in all species in the Brassicaceae family. Different species can typically produce up to at least 40 glucosinolates (Halkier and Gershenzon 2006). Concentrations of glucosinolates and related chemicals are dependent on portion of the plant (leaves, flowers, roots, stems, seeds, etc.), species and variety of plant, season, and environmental conditions. Hot, dry conditions are likely to increase plant glucosinolate concentrations (Tripathi and Mishra 2007). Glucosinolate concentrations in foliage can range from 1000 ppm to at least 3000 ppm, depending on the species. Concentrations in roots and seeds are often higher, up to 60,000 ppm in mustard seed (*B. nigra*). Glucosinolate metabolites, other sulfur-containing compounds, and erucic acid in *Brassica* are the defensive chemicals of plants (Cavaiuolo and Ferrante 2014; Vig et al. 2009). Consequently, these chemicals are bioactive and can have beneficial and harmful effects in people and domestic animals (Bischoff 2016).

Structurally, glucosinolates consist of a β-D-glycopyranose with a sulfonated oxime and an R-group (Tripathi and Mishra 2007; Dinkova-Kostova and Kostov 2012; Vig et al. 2009). The R-group is an amino acid (Collett et al. 2014). While the glucosinolates themselves are minimally biologically active, their metabolites are bioactive (Tripathi and Mishra 2007; Vig et al. 2009). Indole glucosinolates are metabolized to isothiocyanates, which are associated with bitter flavors (Halkier and Gershenzon 2006). The bitter flavors can decrease palatability when *Brassica* spp. are used in livestock feed, which can be associated with decreased gain (Woyengo et al. 2014). Additionally, dietary glucosinolates have been associated with off-flavors in milk and eggs (Bell 1984; Burrows and Tyrl 2013; Tripathi and Mishra 2007). Other glucosinolates disrupt iodine metabolism and thyroid function or have hepatotoxic or nephrotoxic effects (Tripathi and Mishra 2007).

Forage and by-products of the oil seed industries are the most common sources of glucosinolates in livestock diets

K. Bischoff (✉)
New York State Animal Health Diagnostic Center, Cornell University, Ithaca, NY, USA
e-mail: KLB72@cornell.edu

R. C. Gupta et al. (eds.), *Nutraceuticals in Veterinary Medicine*, https://doi.org/10.1007/978-3-030-04624-8_9

(Tripathi and Mishra 2007). Oil seed meals, such as canola, are a good source of protein, but the glucosinolate concentrations limit the amount that can be added to livestock diets. Pennycress is a common weed in North America that originated from Europe and Asia. Because it is about 36% oil, it has potential for use in biodiesel production, and high protein oil meals and cakes from pennycress have been proposed for use in animal feeds (Alhotan et al. 2017).

Varieties of oilseeds were bred to decrease glucosinolate concentrations. Low and very low glucosinolate varieties of canola (known as double-zero or double-low) produce a meal that can have less than a quarter of the glucosinolate concentrations seen in older varieties (Flachowsky et al. 2014; Tripathi and Mishra 2007; Olukosi et al. 2017). Additionally, higher protein, lower fiber canola meals have been developed to make them more suitable for animal feeds (Pedersen et al. 2016). Oil seed cakes have the advantage of higher residual oil than meals, making them suitable feed for some livestock species (Drażbo et al. 2018). Glucosinolate concentrations in oil seed meals vary based on extraction processes (Tripathi and Mishra 2007). Heat treatment decreases glucosinolate in seed meals, and wet heat and pressure decrease glucosinolate concentrations more than dry heating. Heat treatment also reduced the activity of plant myrosinase, the enzyme that metabolizes glucosinolates to more bioactive products (Alhotan et al. 2017). Storage conditions can also affect glucosinolate concentrations in cakes and meals, which can increase in the short-term but decrease in the long-term (Cavaiuolo and Ferrante 2014). Fermentation is documented to decrease glucosinolate concentrations in conventional canola meal and cake used for animal feed (Shi et al. 2015; Drażbo et al. 2018). Fermentation for 10 days can decrease glucosinolate concentrations by more than 40%, and fermentation for 30 days decreases glucosinolate concentrations tenfold in *Brassica napus* (Burrows and Tyrl 2013; Drażbo et al. 2018). This is most likely due to the utilization of sulfur groups in glucosinolates by microbial enzymes (Tripathi and Mishra 2007; Drażbo et al. 2018). Fermentation also increases the protein availability and promotes vitamin and antioxidant production (Drażbo et al. 2018).

2 Metabolism of Glucosinolates

Glucosinolates are bound to a sugar moiety and thus are glycosides. While glucosinolates in and of themselves are minimally biologically active, the aglycone metabolites (metabolites with the sugar removed) are unstable and often do have biological properties. The parent compounds are stored within cell vacuoles in the plant (Cavaiuolo and Ferrante 2014). Idioblasts, structures within plant cells, contain the enzyme myrosinase (Burel et al. 2000; Cavaiuolo and Ferrante 2014; Collett et al. 2014; Halkier and Gershenzon 2006; Vig et al. 2009). Damage to the cell releases the myrosinase which, at a neutral pH, causes hydrolysis of glucosinolates (Cavaiuolo and Ferrante 2014; Ahlin et al. 1994). This system has an important role in plant defense against fungi, bacteria, and insect pests and also prevents spoilage of harvested plants (Vig et al. 2009). Glucosinolate metabolism by plant myrosinase can occur in the rumen of ruminants and the intestinal tract of birds and other monogastric animals (Burrows and Tyrl 2013; Pekel et al. 2015; Tripathi and Mishra 2007). Additionally, common organisms of the gastrointestinal microflora can produce their own myrosinase (Tripathi and Mishra 2007; Kiebooms et al. 2014; Velayudhan et al. 2017). Consequently, antibiotic treatment in humans has been reported to decrease metabolism of glucosinolates (Dinkova-Kostova and Kostov 2012). Metabolites of glucosinolates include isothiocyanates, thiocyanates, nitriles, sulfates, and goitrins (Burel et al. 2000; Cavaiuolo and Ferrante 2014; Burrows and Tyrl 2013; Wight et al. 1987).

Glucosinolates and their metabolites are not known to accumulate in animal tissue, but can be found in milk and eggs, where they produce off-flavors (Bell 1984; Burrows and Tyrl 2013).

3 Important Glucosinolates

A few examples of relatively well-characterized glucosinolates are listed below. Metabolites of these include reactive isothiocyanates or mustard oils, which impart the pungent mustard or garlic-like odors associated with mustard and horseradish, and also include thiocyanates, nitriles, sulfates, and goitrins (Burrows and Tyrl 2013).

- *Glucobrassicin* (3-indolylmethyl glucosinolate), found throughout the Brassicaceae family, is hydrolyzed to an isothiocyanate. One metabolite is indole-3-carbinol, which inhibits nuclear factor κβ (Wagner et al. 2013).
- *Glucoraphanin* (4-methylsulfinylbutyl glucosinolate), found in broccoli and other vegetables, is hydrolyzed to an isothiocyanate, specifically sulforaphane (4-methylsulfonylbutyl isothiocyanate), which has tumor preventive and other properties. Sulforaphane blocks the cell cycle and promotes apoptosis (Halkier and Gershenzon 2006; Fimognari et al. 2012). Sulforaphane also induces mammalian cytoprotective proteins through the Keap1-NrF2-ARE pathway (Dinkova-Kostova and Kostov 2012).
- *Progoitrin*, found in rapeseed and a number of vegetables, is hydrolyzed to thiocyanate and goitrin (L-5-vinyl-2-thioöxazolidine), which is named for its antithyroid effects. Long-term ingestion is associated with goiter

formation. Thiocyanate inhibits sodium-iodide symporter, the transport molecule responsible for iodine uptake by the thyroid, mammary gland, and some other tissues (Flachowsky et al. 2014). Goitrin blocks tyrosine iodination and inhibits T4 formation (Burrows and Tyrl 2013).

- *Sinigrin* (prop-3-enyl glucosinolates), found in a number of vegetables and seeds, is hydrolyzed to allyl isothiocyanate, which is believed to inhibit expression of microRNA and activate the transcription factor Nrf2 (Fimognari et al. 2012; Wagner et al. 2013; Alhotan et al. 2017).

4 Effects of Glucosinolates

Glucosinolates are metabolized to isothiocyanates and related compounds. The effects of these metabolites are through interactions with cellular proteins, such as those involved in intracellular signaling (Fimognari et al. 2012). These biologically active compounds have a variety of known beneficial and detrimental effects.

Benefits Metabolites of glucosinolates protect the plant itself and prevent spoilage of harvested plants. They can have a variety of beneficial biological activities in humans and other animals (Vig et al. 2009).

Anti-inflammatory and immunomodulatory effects of glucosinolates have been reported. Isothiocyanates are able to inhibit cyclooxygenase-2, preventing inflammatory prostaglandin production (Fimognari et al. 2012). Isothiocyanates can downregulate vascular endothelial growth factor (VEGF), and sulforaphane can inhibit endothelial cell response to VEGF. These compounds can also downregulate proinflammatory cytokines such as interleukin (IL)-1β, IL6, granulocyte-macrophage colony-stimulating factor, tumor necrosis factor α, and macrophage migratory inhibitory factor (Dinkova-Kostova and Kostov 2012; Fimognari et al. 2012). Certain isothiocyanates are known to inhibit activity of the transcription regulator NF-κB, which is involved in stress response and inflammation (Wagner et al. 2013). Some activate nuclear factor (erythroid-derived 2)-like 2 factor (NFE2L2), which promotes synthesis of the regulatory protein Nrf2, important in phase II microsomal metabolism (Wagner et al. 2013; Cavaiuolo and Ferrante 2014; Vig et al. 2009).

Glucosinolates and isothiocyanates are considered indirect antioxidants (Vig et al. 2009). They can inhibit phase I metabolic (cytochrome P450) enzymes, which act on xenobiotics and endogenous compounds and generate reactive molecules. Sulforaphane and other isothiocyanates induce phase II metabolic enzymes, which deactivate the products of phase I metabolism and promote excretion, by increasing transcription of the antioxidant response element (ARE) sequence in the genome (Vig et al. 2009). Sulforaphane also induces other antioxidant enzymes via the Nrf-w pathway, including catalase, superoxide dismutase, glutathione peroxidase, glutathione reductase, glutathione *S*-transferase, and glutathione (Dinkova-Kostova and Kostov 2012; Fimognari et al. 2012). Sulforaphane also inhibited production of matrix metalloproteinases and increased antioxidant activity associated with osteoarthritis in vitro (Fimognari et al. 2012).

Antioxidant activities and phase II enzyme are one mechanism responsible for the anticarcinogenicity of glucosinolates and their metabolites (Vig et al. 2009). Isothiocyanates have been protective against carcinogenesis in rodent models, and protection was not organ specific (Dinkova-Kostova and Kostov 2012). There is a decreased risk of bladder cancer related to increased intake of raw cruciferous vegetables in humans (Dinkova-Kostova and Kostov 2012). There is also evidence that dietary cruciferous vegetables are protective against lung, colorectal, breast, prostate, and pancreatic cancer (Fimognari et al. 2012). Isothiocyanate inhibition of phase I enzymes prevents activation of procarcinogens (Dinkova-Kostova and Kostov 2012; Fimognari et al. 2012). Intake of glycoraphanin was associated with enhanced excretion of glutathione conjugates of aflatoxin and certain airborne pollutants in people (Dinkova-Kostova and Kostov 2012). Isothiocyanates are also reported to induce apoptosis in cancer cells, possibly through covalent binding to structural proteins leading to cell cycle arrest (Dinkova-Kostova and Kostov 2012; Fimognari et al. 2012). Epigenetic effects of isothiocyanates include inhibition of histone deacetylase, inhibition of DNA methylation, and inhibition of microRNA expression (Dinkova-Kostova and Kostov 2012; Wagner et al. 2013).

Isothiocyanates have direct antimicrobial effects (Cavaiuolo and Ferrante 2014). The antifungal function of isothiocyanates appears to be due to uncoupling of oxidative photophosphorylation in fungal mitochondria (Vig et al. 2009).

Isothiocyanates have antibacterial activity, though gram-negative bacteria are less susceptible than gram-positives (Vig et al. 2009). Borges et al. (2015) postulated that antibacterial effects are due to interactions with bacterial cell membrane molecules, leading to changes in membrane potential and hydrophobicity, and eventually to membrane disruption. A metabolite of sinigrin, 2-propenyl(allyl) isothiocyanate, has some antimicrobial activity in vitro against *E. coli* 0157:H7. Both benzyl isothiocyanate, a metabolite of glucotropaeolin (benzylglucosinolate), and 2-phenylethylisothiocyanate, a metabolite of gluconasturtiin (2-pheylethylglucosinoate), are effective, in vitro, against gram-positive and gram-negative bacteria at low concentrations. However the combination of 2-propenyl (allyl)isothiocyanate, benzyl isothiocyanate, and phenylethyl isothiocyanate is more effective than any one of the

isothiocyanates alone (Saavedra et al. 2012). Saavedra et al. (2012) also found that some of the metabolites of glucobrassicin, including indole-3-carbinol and indole-3-acetonitrile, had in vitro antimicrobial activity against gram-negative organisms, and ascorbigen had activity against gram-positive organisms, but only at high concentrations.

Sulforaphane has in vitro bacteriostatic and bacteriocidal activity against *Helicobacter pylori* (Dinkova-Kostova and Kostov 2012; Vig et al. 2009). In patients with chronic obstructive pulmonary disease and in animal models, sulforaphane enhanced resistance to pulmonary bacterial pathogens (Fimognari et al. 2012). Glucosinolates have been implicated in improvements in intestinal microflora in birds, as well as improved antioxidant status (Drażbo et al. 2018).

Isothiocyanates and nitriles are toxic to insects and some other invertebrates, which offer protection to growing plants. Proposed insecticide and anti-nematode mechanisms include uncoupling oxidative phosphorylation, binding to thiol groups, and alkylating DNA (Vig et al. 2009). Nitriles can act as insect growth regulators (Vig et al. 2009). Work in Atlantic salmon determined that sea lice infestations could be reduced by about 25% when fish fed diets containing an unspecified glucosinolate-containing plant from the Brassicaceae family (Holm et al. 2016). Possible causes of decreased parasite burden include direct toxic effects of glucosinolates on invertebrates, modulation of host immunity, and changes in the host's skin mucus, making it less attractive or less nutritious to the lice.

Glucosinolates have recently been investigated for their antimicrobial properties due to the increased concern about antibiotic resistance in zoonotic and food-borne pathogens, and some glucosinolate metabolites do have antimicrobial properties. Unfortunately, their cytotoxicity could preclude their use as antimicrobials in vivo (Borges et al. 2015). Isothiocyanate metabolites can react with sulfhydryl groups, for example, from glutathione, to produce dithiocarbamates, which promote oxidation (Nobel et al. 1997). These metabolites can react with sulfhydryl groups on proteins, leading to enzyme inhibition and cell death (Saavedra et al. 2012). Metabolites can react with amino acids to form reactive thiocyanate radicals (Borges et al. 2015). Borges et al. (2015) postulated that antibacterial effects are due to interactions with bacterial cell membrane molecules, leading to changes in membrane potential and hydrophobicity, and eventually to membrane disruption.

A metabolite of sinigrin, 2-propenyl(allyl)isothiocyanate, has some antimicrobial activity in vitro against *E. coli* 0157: H7. Both benzylisothiocyanate, a metabolite of glucotropaeolin (benzylglucosinolate), and 2-phenylethylisothiocyanate, a metabolite of gluconasturtiin (2-pheylethylglucosinoate) are effective, in vitro, against gram positive and gram negative bacteria at low concentrations. However the combination of 2-propenyl(allyl)isothiocyanate, benzylisothiocyanate, and phenylethylisothiocyanate is more effective than any one of the isothiocyanates alone (Saavedra et al. 2012). Saavedra et al. (2012) also found that some of the metabolites of glucobrassicin, including indol-3-carbinol and indole-3-acetonitrile, had in vitro antimicrobial activity against gram negative organisms, and ascorbigen had activity against gram positive organisms, but only at high concentrations.

Recent work suggests that, when used as livestock forage, lambs fed canola had faster growth rates compared to sheep on ryegrass, produced less methane, and their fecal and urinary waste produced less nitrogen dioxide pollution (Luo et al. 2014; Sun et al. 2015). Methane and nitrogen dioxide are important greenhouse gases, and ruminants are a significant contributor to total anthropogenic greenhouse gas production (Sun et al. 2015; Durge et al. 2016). The reduced methane production could be due to the rapid fermentation and increased digestibility of *Brassica* spp.-based feed concentrates, increased rumen pH, and consequent shift in microflora to propionate-forming bacteria, in lambs fed canola forage (Sun et al. 2015; Durge et al. 2016).

Adverse Effects Unfortunately, feeding an excess of *Brassica* vegetables and seed products is associated with toxic effects. Glucosinolates stimulate appetite at low concentrations and enhance the palatability of some foods, such as horseradish, mustard, and black pepper, to people (Burrows and Tyrl 2013). However, glucosinolates have also been associated with reduced palatability and feed consumption in livestock and, consequently, decreased growth rate and reproductive performance. Additionally, endocrine effects of glucosinolates are well known, and nitriles from *Brassica* can cause hepatic and renal dysfunctions (Tripathi and Mishra 2007; Pearson et al. 1983). Increasing dietary iodine and copper can decrease some of the adverse effects of dietary glucosinolates.

As potent electrophiles, isothiocyanates can be genotoxic (Fimognari et al. 2012). Bacterial assays with crude extracts of cruciferous vegetables have found genotoxic effects caused by glucosinolates (Fimognari et al. 2012).

Thyroid enlargement can occur with prolonged exposure to goitrin specifically, but thiocyanates can form thioureas with amino acids which affect thyroid uptake of iodide (Saavedra et al. 2012). Antithyroid effects of glucosinolates can result in goiter, but subclinical effects can include poor reproductive performance and decreased growth (Taljaard 1993). Rodent studies found fetal deaths associated with fetal thyroid follicular epithelial hypertrophy with decreased colloid present (Burrows and Tyrl 2013). Hypothyroidism was also reported in fish (Burel et al. 2000).

Brassicaceae overfeeding in livestock is commonly associated with Heinz body anemia, though the mechanism is slightly different between horses and ruminants. Glucosinolates directly cause Heinz body formation in horses, but the adapted rumen microflora is better able to degrade glucosinolates, decreasing ruminant susceptibility to direct Heinz body hemolysis (Burrows and Tyrl 2013; Cox-Ganser et al. 1994). However, dimethyl sulfoxides produced in the rumen by metabolism of Brassicaceae can also cause Heinz body formation and hemolysis (Taljaard 1993). Poultry are also susceptible to anemia caused by Brassicaceae in the diet (Woyengo et al. 2011).

Nitriles are hepatotoxic and have been associated with lesions including megalocytosis, hepatocyte necrosis, bile duct hyperplasia, and fibrosis (Collett et al. 2014). Serum chemistry in affected livestock will be consistent hepatocellular damage and bile stasis. Hepatic congestion and hemorrhage were reported in laying hens fed rapeseed meal (Wight et al. 1987). The hemorrhage most likely causes pressure necrosis of hepatic parenchyma (Martland et al. 1984). Megalocytosis has been reported in the kidneys (Collett et al. 2014).

The high concentration of sulfur in kale and other brassicas inhibits uptake of copper and selenium and causes deficiency in these trace minerals (Taljaard 1993). Excessive sulfur in ruminants is reported as a cause of polioencephalomalacia (Burrows and Tyrl 2013; Taljaard 1993).

Erucic acid is a monounsaturated fatty acid present in rapeseed, mustards (*B. napus*, *B. campestris*, *B. juncea*, *B. sarson*) and some related plants. Erucic acid is reported to cause cardiac injury in cattle and, experimentally, in rodents (Burrows and Tyrl 2013). Canola oil contains less than 5% erucic acid, but canola meal can contain high concentrations depending on the extraction method used in production (Burrows and Tyrl 2013; Woyengo et al. 2011).

Some other species-specific effects of *Brassica* spp. in the ration are noted below.

- *Swine*: Pigs are considered to be highly sensitive to dietary glucosinolates, and younger pigs are more susceptible than older swine (Tripathi and Mishra 2007). Glucosinolates in swine diets reduce gain in weanling pigs due to reduced palatability and thyroid effects (Woyengo et al. 2014). Currently, it is recommended that total glucosinolate concentrations in swine diets be below 2 μmol/g, dry matter (Kim et al. 2017; Tripathi and Mishra 2007). Higher dietary glucosinolate concentrations have sometimes been associated with thyroid hypertrophy due to reduced ability to uptake iodine (Pedersen et al. 2016). It is recommended that iodine be supplemented at a rate of 1000 μg/kg when using glucosinolate-containing feeds in the ration (Tripathi and Mishra 2007). Anemia and hepatic and renal damage have also been reported in swine fed glucosinolates (Velayudhan et al. 2017).
  It was discovered in Europe that thiouracil, a drug forbidden in meat-producing animals due to possible carcinogenic and teratogenic effects and increasing the water content of edible tissues, was detected in livestock fed canola or cabbage (Kiebooms et al. 2014). Thiouracil is produced from certain glycosinolates by the mature intestinal flora and occurs more commonly in older sows than in feeder pigs (Kiebooms et al. 2014).
- *Poultry*: Laying hens and turkeys are more susceptible to the adverse effects of *Brassica* spp. in the diet than broilers, and clinical effects included decreased growth and egg production and increased mortality (Tripathi and Mishra 2007). Decreased growth and reduced egg production are due to decreased feed intake and antinutritive factors including glucosinolates (Woyengo et al. 2011; Martland et al. 1984; Wight et al. 1987; Olukosi et al. 2017). Reduced growth in birds fed a diet containing 30% canola meal was more remarkable in chicks less than 3 weeks old compared to older broilers and was attributed to lack of thyroid development in young birds (Mushtaq et al. 2007). Pennycress cake containing 59 to 68 μmol/g sinigrin and 1.62–1.73% erucic acid caused reduced growth in chicks when fed at a rate of 5% to chicks less than 10 days of age or 8.5% of the diet for older chicks (Alhotan et al. 2017). There is some decrease in growth reported at feed glucosinolate concentrations greater than 2 μmol/g, and severe depression in growth is reported at dietary glucosinolate concentrations of 8 μmol/g and greater (Tripathi and Mishra 2007). Thyroid hypertrophy due to glucosinolates has been reported in poultry (Martland et al. 1984; Wight et al. 1987). When turkeys were fed conventional canola cake at 15% of the diet, those fed an unfermented cake had lower T4 concentrations than those fed a fermented cake (Drażbo et al. 2018). Hepatic enlargement has also been reported in broilers fed diets with high glucosinolate concentrations (Velayudhan et al. 2017). It is recommended that poultry feed contain less than 2.5 μmol glucosinolates per gram (Mushtaq et al. 2007; Pekel et al. 2015; Drażbo et al. 2018).
  Conventional canola meal was fed to ducks at a little more than 6% of the diet without adverse effects, but high glucosinolate products (7.57 μmol glucosinolates/g diet) had adverse effects on growth at lower concentrations (Qin et al. 2017). Adverse effects reported in ducks fed higher glucosinolate concentrations in the diet included decreased growth, impairment of feather growth, increased thyroid weight, and evidence of hepatic protein synthesis (Qin et al. 2017).
- *Horses*: Allyl isothiocyanates are direct gastrointestinal tract irritants, causing potentially lethal colic in horses

(Taljaard 1993; Mason and Lucas 1983; Woyengo et al. 2011).

- *Ruminants*: Ruminants are less sensitive to glucosinolates than other species due to ruminal digestion (Tripathi and Mishra 2007). Young animals are more sensitive than mature animals. Long-term use of glucosinolate-containing feeds, however, has been associated with goiter, decreased plasma thyroxin, poor reproductive performance in both cows and bulls, and decreased milk production in dairy cows (Tripathi and Mishra 2007). Goitrogen and thiocyanates from glucosinolates can also reduce milk iodine concentrations in dairy cattle through decreased iodide uptake by the mammary gland (Flachowsky et al. 2014). Conventional canola meal in dairy rations decreased the milk iodine concentration by half to three quarters, and this decrease was not prevented by adding more iodine to the diet. Glucosinolates in diets of dairy cattle also produced off-flavored milk (Tripathi and Mishra 2007). Though carcass quality is unaffected in ruminants fed glucosinolates, off-flavors have been reported in lambs fed canola meal (Tripathi and Mishra 2007).

  Severe irritant effects due to high dietary glucosinolates have been reported and can include sloughing of the ruminal epithelium. Symptomatic treatment, including pain control and a clean diet containing no *Brassica*, is the treatment for sever acute gastrointestinal signs (Burrows and Tyrl 2013).

  Interestingly, thiouracil, an illegal contaminant of animal products, was detected in cattle fed *Brassica* spp. Similar to swine, gastrointestinal microflora in cattle produced a myrosinase-like enzyme that leads to the formation of thiouracil from glucosinolates (Kiebooms et al. 2014).

  Ruminants can tolerate up to 4.22 μmol glucosinolates per gram of diet, though lambs fed glucosinolates at this rate developed evidence of iodine deficiency (Tripathi and Mishra 2007). Iodine supplementation can increase glucosinolate tolerance.
- *Rabbits*: Rabbits fed mustard meal had decreased feed intake and growth, and liver hypertrophy. Rabbits could tolerate less than 8 μmol glucosinolates per gram of feed, but feed concentrations above 17.6 μmol/g were associated with increased mortality (Tripathi and Mishra 2007). Decreased hematocrit was also reported in rabbits fed high glucosinolate diets (Velayudhan et al. 2017).
- *Fish*: Low dietary concentrations of glucosinolates were associated with reduced growth and thyroid lesions in rainbow trout, and the recommended maximum glucosinolate concentration in feed for fish is 1.4 μmol/g, which can still be associated with goiter (Tripathi and Mishra 2007).

5 Concluding Remarks and Future Directions

As more research is being conducted on the myriad of bioactive compounds in plants from the Brassicaceae family, more potential benefits are found. However, these compounds can also have adverse effects and lead to food taint when used in livestock rations. Interactions between glucosinolates and other plant compounds and biological processes in domestic animals are extremely complex and difficult to predict; thus more research is needed to determine the most beneficial ways to use glucosinolate-containing feeds in livestock diets to promote health and minimize risk.

References

Ahlin KA, Emanuelson M, Wiktorsson H (1994) Rapeseed products from double-low cultivars as feed for dairy cows: effects of long-term feeding on thyroid function, fertility and animal health. Acta Vet Scand 35(1):37–53

Alhotan RA, Wang RL, Holser RA et al (2017) Nutritive value for maximum inclusion level of pennycress meal for broiler chickens. Poult Sci 96:2281–2293

Bell JM (1984) Nutrients and toxicants in rapeseed meal: a review. J Anim Sci 58(4):996–1010

Bischoff KL (2016) Glucosinolates. In: Gupta RC (ed) Nutraceuticals: efficacy, safety and toxicity. Academic, Amsterdam, pp 551–554

Borges A, Abreu AC, Ferreira C et al (2015) Antibacterial activity and mode of action of selected glucosinolate hydrolysis products against bacterial pathogens. J Food Sci Technol 52(8):4737–4748

Burel C, Boujard T, Escaffre AM et al (2000) Dietary low-glucosinolate rapeseed meal affects thyroid status and nutrient utilization in rainbow trout (*Oncorhynchus mykiss*). Br J Nutr 83(6):653–664

Burrows GE, Tyrl RJ (2013) Toxic plants of North America, 2nd edn. Wiley-Blackwell, Ames, IA

Cavaiuolo M, Ferrante A (2014) Nitrates and glucosinolates as strong determinants of the nutritional quality in rocket leafy salads. Nutrients 6(4):1519–1538

Collett MG, Stegelmeier BL, Tapper BA (2014) Could nitrile derivatives of turnip (*Brassica rapa*) glucosinolates be hepato- and/or cholangiotoxic in cattle? J Agric Food Chem 62 (30):7370–7375

Cox-Ganser JM, Jung GA, Pushkin R et al (1994) Evaluation of Brassicas in grazing systems for sheep: II. Blood composition and nutrient status. J Anim Sci 72(7):1832–1841

Dinkova-Kostova AB, Kostov RV (2012) Glucosinolates and isothiocyanates in health and disease. Trends Mol Med 18 (6):337–348

Drażbo A, Ognik K, Zaworska A et al (2018) The effect of raw and fermented rapeseed cake on the metabolic parameters, immune status, and intestinal morphology of turkeys. Poult Sci 97 (11):3910–3920. https://doi.org/10.3382/ps/pey250

Durge SM, Tripathy MK, Dutta N (2016) *In-vitro* fermentation characteristics and methane reduction potential of mustard cake (*Brassica juncea* L.). Vet World 9(10):1141–1146

Fimognari C, Turrini E, Furruzzi L et al (2012) Natural isothiocyanates: genotoxic potential *versus* chemoprevention. Mutat Res 750:107–131

Flachowsky F, Franke K, Meyer U, Leiterer M, Schöne F (2014) Influencing factors on iodine content of cow milk. Eur J Nutr 53(2):351–365

Halkier BA, Gershenzon J (2006) Biology and biochemistry of glucosinolates. Annu Rev Plant Biol 57:303–333

Holm HJ, Wadsworth S, Bjelland A-K et al (2016) Dietary phytochemicals modulate skin gene expression profiles and result in reduced lice counts after experimental infection in Atlantic salmon. Parasites Vectors 9:271

Kiebooms JAL, Wauters J, Vamden Bussche J et al (2014) Thiouracil-forming bacteria identified and characterized upon porcine *in vitro* digestion of *Brassicaceae* feed. Appl Environ Microbiol 80 (23):7433–7442

Kim JW, Koo B, Nyachoti CM (2017) Digestible, metabolizable, and net energy of camelina cake fed to growing pigs and additivity of energy in mixed diets. J Anim Sci 95:4037–4044

Luo J, Sun XZ, Pacheco D et al (2014) Nitrous oxide emission factors for urine and dung from sheep fed either fresh forage rape (*Brassica napus* L.) or fresh perennial ryegrass (*Lolium perenne* L.). Animal 9 (3):534–543

Martland MF, Butler EJ, Fenwick GR (1984) Rapeseed induced liver haemorrhage, reticulolysis and biochemical changes in laying hens: the effects of feeding high and low glucosinolate meals. Res Veter Sci 36(3):298–309

Mason RW, Lucas P (1983) Acute poisoning in cattle after eating old non-viable seed of chou moellier (*Brassica oleracea* convar. acephala). Aust Vet J 60(9):272–273

Mushtaq T, Sarwar M, Ahmed G et al (2007) Influence of canola meal-based diets supplemented with exogenous enzyme and digestible lysine on performance, digestability, carcass, and immunity responses of broiler chickens. Poult Sci 86(10):2144–2151

Nobel CS, Burgess DH, Zhivotovski B et al (1997) Mechanism of dithiocarbamate inhibition of apoptosis: thiol oxidation by dithiocarbamate disulfides directly inhibits processing of the caspase-3 pro-enzyme. Chem Res Toxicol 10(6):636–643

Olukosi OA, Kasprzak MM, Kightley S et al (2017) Investigations of the nutritive value of meals of double-low rapeseed and its influence on growth performance of broiler chickens. Poult Sci 96:3338–3350

Pearson AW, Greenwood NM, Butler EJ et al (1983) Biochemical changes in layer and broiler chickens when fed on a high-glucosinolate rapeseed meal. Br Poult Sci 24(3):417–427

Pedersen TF, Liu Y, Stein HH (2016) Effects of diet energy concentration and an exogenous carbohydrase on growth performance of weanling pigs fed diets containing canola meal produced from high protein or conventional canola seeds. J Anim Sci 94:5206–5218

Pekel AY, Kim JI, Chapple C et al (2015) Nutritional characteristics of camelina meal for 3-week-old broiler chickens. Poult Sci 94:371–378

Qin S, Tian G, Zhang K et al (2017) Influence of dietary rapeseed meal levels on growth performance, organ health and standardized ileal amino acid digestibility in meat ducks from 15 to 35 days of age. J Anim Physiol Anim Nutr 101:1297–1306

Saavedra MJ, Dias CSP, Martinez-Murcia A et al (2012) Antibacterial effects of glycosinolate-derived hydrolysis products against *Enterobacteraiceae* and *Enterocci* isolated from pig ileum segments. Foodborne Pathog Dis 9(4):338–345

Shi C, He J, Yu J et al (2015) Amino acids, phosphorus, and energy digestibility of *Aspergillus niger* fermented rapeseed meal fed to growing pigs. J Anim Sci 93:2916–2925

Sun X, Henderson G, Cox F et al (2015) Lambs fed fresh winter forage rape (*Brassica napus* L.) emit less methane than those fed perennial ryegrass (*Lolium perenne* L.), and possible mechanisms behind the difference. PLoS One 10(3):e0119697

Taljaard TL (1993) Cabbage poisoning in ruminants. J S Afr Vet Assoc 64(2):96–100

Tripathi MK, Mishra AS (2007) Glucosinolates in animal nutrition: a review. Anim Feed Sci Technol 132:1–27

Velayudhan DE, Schuh K, Woyengo TA et al (2017) Effects of expeller extracted canola meal on growth performance, organ weights, and blood parameters of growing pigs. J Anim Sci 95:302–307

Vig AP, Rampal G, Thind TS et al (2009) Bio-protective effects of glucosinolates – a review. LWT – Food Sci Technol 42: 1561–1572

Wagner AE, Terschluesen AM, Rimbach G (2013) Health promoting effects of brassica-derived phytochemicals: from chemopreventive and anti-inflammatory activities to epigenetic regulation. Oxid Med Cell Longev [Online] 2013:964539. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3885109&tool=pmcentrez&rendertype=abstract. Accessed 22 Apr 2014

Wight PA, Scougall RK, Shannon DW et al (1987) Role of glucosinolates in the causation of liver haemorrhages in laying hens fed water-extracted or heat-treated rapeseed cakes. Res Vet Sci 43(3):313–319

Woyengo TA, Beltenena E, Ziglstra RT (2014) Nonruminant nutrition symposium: controlling feed costs by including alternative ingredients into pig diets: a review. J Anim Sci 72:1293–1305

Woyengo TA, Kiarie E, Nyachoti CM (2011) Growth performance, organ weights, and blood parameters of broilers fed diets containing expeller-extracted canola meal. Poult Sci 90(11):2520–2527

Cannabis in Veterinary Medicine: Cannabinoid Therapies for Animals

Joshua A. Hartsel, Kyle Boyar, Andrew Pham, Robert J. Silver, and Alexandros Makriyannis

Abstract

The use of cannabis for animal species is an area of growing interest, largely due to the therapeutic benefits being observed for humans and animals in the era of cannabis legalization. The close relationship humans have with their pets and other veterinary species has led to a renewed interest in the possibility and promise of cannabis to treat similar health issues in the animal community. This chapter explores the literature available on cannabis, its interactions with the endocannabinoid system, and how animal species interact with various formulations and cannabis treatments. A brief overview of the biology, chemistry, and history of cannabis is discussed with the relevance to veterinary species in mind. The pharmacologically active components are discussed with both anecdotal and objective, evidence-based, and clinical data.

Keywords

Cannabis · Cannabidiol · CBD · Cannabinoids · Nutritional supplement · Nutraceutical · Veterinary medicine · Animals · Veterinarian

J. A. Hartsel (✉)
Delta-9 Technologies, LLC, Lake Forest, CA, USA
e-mail: jhartsel@delta9technologies.com; http://www.delta9technologies.com

K. Boyar
Medicinal Genomics, Woburn, MA, USA
e-mail: kyle.boyar@medicinalgenomics.com

A. Pham
BelCosta Labs, Long Beach, CA, USA
e-mail: andrew@belcostalabs.com

R. J. Silver
RX Vitamins, Elmsford, NY, USA
e-mail: rsilver@drsilverdvm.com; http://www.rxvitamins.com

A. Makriyannis
Northeastern University, Boston, MA, USA
e-mail: a.makriyannis@neu.edu

1 Introduction

The endocannabinoid system (ECS) has been identified in nearly all animals, from complex mammals like primates to phylogenetically primitive animals such as the cnidarians. The near universal presence and early emergence of the ECS, evolutionarily, is a strong indicator of its biological importance. Cannabinoid receptors are expressed in most animals, including vertebrates (mammals, birds, reptiles, and fish) and invertebrates (sea urchins, leeches, mussels, nematodes, and others). The most primitive animal an ECS has been observed in is the *Hydra* (*H. vulgaris*), a cnidarian in the class Hydrozoa, which is the first animal to develop a neural network. De Petrocellis et al. (1999) determined the major function of the ECS in the *Hydra* is to control the feeding response. It is evident from this data that all veterinary species contain an ECS. Therefore, an understanding of the ECS in these species is critical to the development of clinical applications for endocannabinoids and the phytocannabinoids derived primarily from *Cannabis sativa* L.

Clinical trials detailing the benefits and safety of phytocannabinoids in companion animals are finally being performed at academic institutions, after years of suppression of research due to the controversial status of cannabis. Public and commercial interest in this exciting and newly emerging therapy for animals has resulted in a number of studies and clinical trials being published or nearing publication. This chapter reports the findings of these studies that either have already been published, are in press, or are in their earlier stages; this chapter will provide personal communications with the principal investigators reporting on the results to date of their ongoing studies.

A thorough review of the literature reveals no published clinical trials prior to the studies reported in this chapter involving phytocannabinoids in dogs, cats, or horses. There are, however, quite a few studies in a laboratory setting using laboratory species to study the effects of phytocannabinoids, or to measure aspects of the ECS in a specific species.

R. C. Gupta et al. (eds.), *Nutraceuticals in Veterinary Medicine*, https://doi.org/10.1007/978-3-030-04624-8_10

In spite of the paucity of published controlled studies in veterinary species, animal caregivers have been using cannabis for their dogs, cats, and horses since before the legalization of medical marijuana in 1996 and likely much earlier. Observed benefits from the use of cannabis include the reduction of anxiety; relief of pain; improvement of mobility in animals with osteoarthritis; reduction in tumor size; improved appetite; improved control of type 2 diabetes, inflammatory conditions, and digestive issues; and improved control of epileptic seizures. These benefits have not been universal, and successful treatments are based on dosages relative to the type of condition, severity of condition, size and metabolism, and factors related to biochemical diversity and density and distribution of the ECS among members of the same species.

Veterinarians, veterinary researchers, and animal caregivers are all eager to adopt cannabis-based therapies and have been looking for the evidence to support the safe and effective use of cannabinoid therapies for their pets or their patients. We will focus on providing that data in this chapter.

2 *Cannabis sativa* L.: Food, Herbal Medicine, Pharmaceutical, and Nutraceutical

Many of the constituents of *Cannabis sativa* can be classified as either a pharmaceutical ingredient, a nutrient, a nutraceutical, or an herbal product. Nutraceutical is a term that was created in 1989 by DeFelice by joining the two words, "nutrition" and "pharmaceutical" (Kalra 2003). It has no legal definition but refers to those compounds that are neither nutrients nor pharmaceuticals. The North American Veterinary Nutraceutical Council in 1996 defined a veterinary nutraceutical as a "[non-drug] substance which is produced in a purified or extracted form and administered orally to a patient to provide agents required for normal body structure and function and administered with the intent of improving the health and well-being of animals (Booth 2009)."

2.1 Regulatory and Legal Considerations

The Center for Veterinary Medicine of the Food and Drug Administration (FDA-CVM) states that the Dietary Supplement Health and Education Act (1994), which regulates dietary supplements for humans, does not pertain to animals. This means that legally there are only two choices for regulating products which are similar to human dietary supplements under the law; based on the intended use and contents, products are either regulated as animal food or animal drugs. There is no other choice. The marketing of these animal food/feeds or animal remedy products may also occur on a state-by-state basis and as such may be subjected to regulatory approval by local State Feed Control Officials (FCOs).

The Association of American Feed Control Officials (AAFCO) has no regulatory powers, but it provides guidelines for individual states regarding animal nutrition products. Their guidelines are followed in most states although interpretations, especially of allowable claims, vary from state to state and individual to individual. If a nutritional product contains ingredients that are not approved as food ingredients by the FDA-CVM, or the claims are violative, the local State FCO can recommend a product not be sold or actually issue a stop sale notice or confiscation of the product from the supplier and retail outlets.

In 2000, Iowa FCOs began to pull nutritional products that contained unapproved ingredients from the shelves of veterinary offices because they did not have FDA-CVM approval. At that time a trade group was formed, the National Animal Supplement Council (www.NASC.cc) to work with the regulators at both the state and federal levels to develop workable guidelines for suppliers to follow that would satisfy the regulators' concerns about these products that were commonly referred to as nutraceuticals.

As a result of NASC's efforts, both products for health purposes are referred to as "dosage-form animal health products" and may be marketed under enforcement discretion provided companies act responsibly. The guidelines for company responsible behavior include but are not limited to labeling products properly; making claims that are not in violation of Sect. 201(g)(1)(b) of the Federal Food, Drug, and Cosmetic Act (FFDCA); following Good Manufacturing Practice Standards and implementing effective risk monitoring/management systems; ensuring product safety; and protecting both the health of animals and people which are always the most important consideration for any regulatory official (Fig. 1).

The FDA-CVM developed confidence in the work of the NASC when this trade group established a website for

Fig. 1 *NASC is an organization that* regulates the quality of animal-based nutritional supplements

veterinary nutraceutical adverse event reporting. This database, NAERS™, now contains risk management data, product labels, and statistical analysis on hundreds of nutraceutical and nutritional products and ingredients. NAERS contains many millions of recorded administrations and can accurately give the percent and type of adverse events recorded for a given material over this large number of animal administrations. The NASC Adverse Event Reporting System (NAERS™) is currently the most advanced data base in the world for dosage-form products for dogs, cats, and horses.

The NASC started with 18 members in 2001 and as of 2018 now has well over 175 members. Member companies must comply with cGMP standards, follow label template guidelines, and not make medical claims, but can use structure and function statements in their marketing materials and labels. Members must undergo regular audits by a third party that include random testing of their products to verify they meet label claims. Member companies who pass the audit are allowed to display the NASC seal on their bottles, or other marketing materials, which, for the consumer, has become a sign of quality, and many pet owners will select only those products that display this seal.

The FDA-CVM tightly regulates what ingredients are approved for animal feed. Hemp is currently not an approved ingredient for animal feed according to the FDA-CVM and the AAFCO (Association of American Feed Control Officials; www.AAFCO.org). Additionally, according to the FDA, in order for a material to be considered a supplement (for humans) versus a pharmaceutical, it needs to have been in use as a supplement prior to October 1994 when DSHEA was passed, or the company must file an NDI (New Dietary Ingredient) application. Currently, the FDA claims that there was no recognized use of hemp as a nutraceutical before GW Pharmaceuticals filed their IND for Epidiolex, their recently FDA-approved, CBD-containing drug to treat resistant pediatric epilepsy due to Dravet's syndrome. The limits surrounding this issue are currently being evaluated and will likely be argued in court.

The Drug Enforcement Agency has persisted in its controlled substance classification of the non-THC resins found in the cannabis plant, whether it's from the state-legal medical cannabis with high THC (aka: marijuana) or the federally legal low THC (aka: hemp). This is in spite of the fact that non-THC phytocannabinoids meet none of the requirements for a controlled substance that is a Schedule I. This scheduling category states that materials from this category have no medical applications and a great potential for abuse and/or toxicity. This Schedule I classification is in spite of the fact that the Federal Government holds a patent for the medicinal applications of CBD for neuroprotection (Hampson et al. 2003; US Patent #6630507). This classification of the non-THC resins in the hemp as a Schedule I plant needs to be changed to a nonscheduled classification in order for the nascent and burgeoning hemp industry to fully develop economically. As of Summer 2018, there is a bill introduced into Congress to remove the controlled substance status for hemp and all of its derivatives as a part of the 2018 Farm Bill.

The FDA-CVM, in a public statement in April 2018 about the medical use of cannabis in animals, points to the fact that currently there are no FDA-approved drugs for animals that contain cannabis, and there is no scientific evidence, as of Spring 2018, that supports the use of cannabis and/or CBD in animals. However, with a number of university veterinary studies now showing both safety and efficacy, that may change. The FDA has now approved Epidiolex™ as of June 2018 but has yet to be rescheduled by the DEA. The use of the term "CBD" or "cannabidiol" on a label or in the marketing of a hemp product for people or pets now has become a medical claim since CBD is in an approved drug, and this may leave the product open to enforcement by the FDA. Companies need to avoid using these two words and must choose other language when describing the contents of a product and its intended activity to the end user. Currently there is no established limit for naturally occurring CBD in hemp products, although it has been considered by regulatory agencies to keep the CBD content at or below that which is naturally occurring in the hemp plant, which in some cultivars may average 18% in the flowers. This potency limit has not yet been established, though.

The 2018 US Farm Bill and the Controlled Substance Act of 1970

Sect. 7606 of the 2013 US Farm Bill allowed for research into hemp cultivation and market research into the commercialization of hemp on a state-by-state basis, for each state that has passed legislation regarding hemp cultivation and commercialization in that state. Currently there are 34 states that have legislation on the books allowing for hemp cultivation, with Kentucky and Colorado the largest producers.

The 2018 Farm Bill contains the McConnell amendment which removes the controlled substance status of legally grown hemp, again, on a state-by-state basis. Each state can decide how they choose to regulate hemp within their state according to this amendment. Once the Farm Bill is passed with this amendment, it will remove any legal impediment to the commercialization of hemp and hemp derivatives in the United States.

This amendment is necessary because the Drug Enforcement Administration (DEA) has scheduled all cannabis (both hemp and marijuana) as a Schedule I controlled substance, which means it has no medical applications and is potentially toxic and/or addictive. Controlled substances cannot be transported across state lines, so many companies (especially the larger ones) that would normally be marketing a popular supplement like CBD will not get involved with commercializing hemp until this last barrier to trade is removed.

2.2 Hemp in Animal Feed

Hemp is becoming an agricultural commodity again in the United States, as the stigmas and restrictions on research and commercialization are gradually being removed as a result of the US Farm Bill of 2013, Section 7606. This landmark *omnibus* spending bill established the legal status of growing hemp in the United States, and as a result, an interest in the use of hemp for animal feed has developed.

The Colorado legislature passed a bill forming a stakeholders committee under the guidance of the Colorado Department of Agriculture to examine the use of hemp and hemp derivatives for animal feed for both companion animals and food animals. The final report of that stakeholders commission was published on December 29, 2017 (Glenn 2017).

To Summarize the Findings of This Stakeholders' Committee Report

The FDA and AAFCO both stated they would be receptive to a Feed Additive Petition (FAP) submitted to the FDA for the use of the noncontrolled substance part of the plant, the grain or sterilized seed, including the oil and its protein cake. This FDA approval is sought for dogs, cats, horses, and food animals. In animals destined to enter the human food chain, there will need to be studies demonstrating conclusively the safety to our food supply to feed a food that contains cannabinoids, even trace amounts of the non-psychoactive phytocannabinoids.

The Hemp Industries Association of Colorado has formed a steering committee to create a Feed Additive Petition for Hemp Seed to submit to the FDA-CVM for approval of hemp seed as an animal feed ingredient. Currently, hemp is an unapproved nutritional ingredient according to the FDA, and as such when contained in animal feed is considered to be adulterated, and may be subject to regulatory action when marketed as a food. Following a literature review, 6-month safety feeding studies will need to be performed before the FDA would agree to approve hemp as a feed ingredient. This process usually takes 2–3 years and a considerable amount of resources.

Currently, any animal food products that contain hemp that are labeled as nutritional products with a guaranteed analysis and hemp labeled as a nutrient will be considered adulterated until hemp is an approved feed ingredient. For now, hemp will have to be included in a product as a nutraceutical and have the label language reflect the support that hemp affords to the healthy structure and function of the body and be marketed as a dosage-form animal health product. This is provided the product meets the minimum limits for THC and does not contain phytocannabinoid concentrations that are greater than what would be normally found in the whole hemp plant on average.

The Nutritional Value of *Cannabis sativa* L.

Cannabis seed, also known as "grain," contains both valuable fatty acids and high-quality protein. The seed is particularly nutritious and is often consumed whole or used in food preparations. A protein cake made from the seed has been used for animal feed in Europe. Whole hemp seed contains approximately 20–25% protein, 20–30% carbohydrates, and 10–15% insoluble fiber (Callaway 2004; Deferne and Pate 1996). In addition, it contains a mixture of the saturated fatty acids palmitic and stearic acid as well as oleic acid. Hemp seed oil is an extremely rich source of unsaturated fatty acids, especially the essential fatty acids linoleic acid (LA) and alpha-linolenic acid (ALA) (Callaway 2004; Leizer et al. 2000).

Essential fatty acids (EFAs) cannot be produced naturally by an animal's body and must be sourced from the diet. LA and ALA are omega-6 and omega-3 fatty acids, respectively, and, in the dog, are considered to be essential, since the dog cannot synthesize them. In cats these two fatty acids are also essential; however, due to their liver function, cats cannot desaturate and elongate linoleic acid to form arachidonic acid (Hand et al. 2010).

Hemp seed oil also tends to contain high amounts of gamma-linolenic acid (GLA) and stearidonic acid (SDA), which are metabolites of LA and ALA (Callaway 2004). Since these metabolites are produced by the breakdown of dietary LA and ALA, they are not considered EFAs. However, supplementation in the diet can be extremely beneficial. Many chronic diseases of modern society, including cancer, diabetes, heart disease, arthritis, atopy, Alzheimer's disease, and others, have an inflammatory component (Kapoor and Huang 2006). Diets enriched in GLA have been shown to reduce inflammation (Tate et al. 1989), and therefore the nutritional value of GLA from hempseed oil is clear.

Cannabis seed oil has the proper ratio (4:1) of omega-6 to omega-3 fatty acids for optimal health. The pro-inflammatory eicosanoid cascade is promoted by a high omega-6/omega-3 fatty acid ratio. This results in increased *prostaglandin* 2a ($PG_2\alpha$), which is a pro-inflammatory eicosanoid. Diets rich in omega-3 s and poor in omega-6 s have a less pro-inflammatory nutrient profile, which can be anti-inflammatory with sufficiently high levels of long-chain polyunsaturated omega-3 fatty acids (LCPUFA) and/or the anti-inflammatory omega-6 LCPUFA, gamma-linolenic acid (GLA). The Western diet, high in grain-fed meat and grains which are all high in omega-6 fatty acids and low in omega-3 fatty acids, results in a 20:1 ratio of omega-6/omega-3. It is this high omega-6/omega-3 ratio that shunts the eicosanoid cascade to the pro-inflammatory side and which dietary omega-6 fatty acids and GLA will serve to reduce over inflammatory trends in the body (Bauer 2011).

Three clinical examples where the lower omega-6/omega-3 ratio tends to a less inflammatory environment in the body

are the following: (1) a ratio of 4:1 omega-6/omega-3 was associated with a 70% decrease in total mortality from cardiovascular disease in human patients; (2) in colorectal cancer a decrease in rectal cell proliferation was observed with a lower ratio of 2.5:1; and (3) a ratio of 5:1 was found beneficial in asthma patients (Simopoulos 2002).

3 Modern History of Cannabinoid Pharmacology

The therapeutic properties of cannabis have been appreciated over several millennia and its pharmacological properties studied since the mid-1900s. This effort was enhanced in the 1960s with the isolation, characterization, and subsequent synthesis of its major psychoactive ingredient (−)-Δ^9-THC by Mechoulam and Gaoni, and significant attention was given to developing synthetic compounds with more potent and targeted therapeutic effects (Gaoni and Mechoulam 1964). Concurrent approaches by individual research laboratories and the pharmaceutical industry produced a series of structurally related new compounds, which were collectively called cannabinoids. One of these drug development projects by Eli Lilly led to the first synthetic cannabinoid, nabilone (Cesamet), which was used to treat nausea, pain, and reduced appetite associated with cancer chemotherapy (Makriyannis 2014; Pertwee 2008). The major breakthrough in understanding the mechanism of action of cannabis, and more specifically Δ^9-THC, came with the discovery of the first biological targets for this compound. Following a very successful research meeting in 1987 by the National Institute on Drug Abuse in Bethesda, Maryland, collaborative work among the participating laboratories led to the discovery of a new G-protein-coupled receptor (GPCR), which was named CB_1, to be followed 2 years later by a second GPCR named CB_2 (Makriyannis 2014). The CB_2 cannabinoid receptor is mostly found in immunity-related cells, and it is largely involved in regulating the immune system and inflammatory conditions. Although under normal "homeostatic" conditions the CB_2 receptors have a very low presence in neurons, in "non-homeostatic" situations such as inflammation, neurodegenerative diseases such as Alzheimer, Parkinson, ALS, as well as in cancers such as gliomas, its presence in the brain is dramatically increased in astrocytes as well as microglial and cerebromicrovascular endothelial cells. Recent published work has provided evidence that this receptor may also have a role in modulating addictive disorders (Xi et al. 2011).

Both the CB_1 and CB_2 receptors play important roles in many processes including neuronal plasticity, pain, anxiety, neuroinflammation, immune function, metabolic regulation, reward, craving, and bone growth (Mackie 2006).

4 Endocannabinoid (eCB) Chemistry

The next discovery on the ECS was a group of compounds found in mammalian tissues, which were named endocannabinoids (eCBs). The most representative of which were arachidonoyl ethanolamide or anandamide (AEA, circa 1992), a long-chain fatty acid amide, and 2-arachidonoyl glycerol (2-AG circa 1995), the respective ester (Devane et al. 1992; Mechoulam et al. 1995; Pertwee 2000). These new substances are capable of activating both CB_1 and CB_2 receptors and, when tested in animals, produced biological effects paralleling those of Δ^9-THC (Fig. 2).

eCBs are produced on demand by a series of enzymes that are present within the cell membrane and are activated by elevated levels of calcium ions. eCB levels, also referred to as the "endocannabinoid tone," are tissue dependent and are regulated by another set of enzymes, the most prominent of which are fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL), whose role is to deactivate AEA and 2-AG and their congeners, respectively. An additional feature in the regulation of eCB tone is a transport mechanism that is involved in transporting released eCBs into the cell (Fig. 3) (Vemuri and Makriyannis 2015).

Anandamide is synthesized from its membrane precursor *N*-arachidonyl phosphatidylethanolamine (NAPE) through cleavage by a phospholipase D (NAPE-PLD). In contrast, anandamide is degraded by the enzyme fatty acid amide hydrolase (FAAH) into arachidonic acid (AA) and ethanolamine. 2-AG is produced through the activities of either diacylglycerol lipase (DAGL) or phospholipase C (PLC) using an AA precursor. 2-AG is then subsequently degraded by monoacylglycerol lipase (MAGL) into glycerol and AA (Hartsel et al. 2016)

eCBs are released from the postsynaptic neuron and act on cannabinoid receptors on the presynaptic neuron. The eCBs inhibit the influx of calcium intracellularly resulting in inhibition of neurotransmitter release. The actions of eCBs are relatively short lived as they undergo rapid reuptake by the cell and are then degraded. The production of eCBs can be

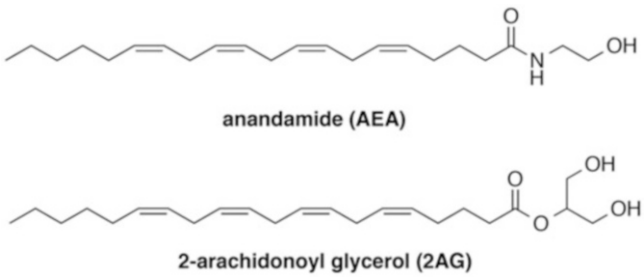


Fig. 2 Endocannabinoids AEA and 2-AG bind to the CB receptors that are endogenously produced inside the body

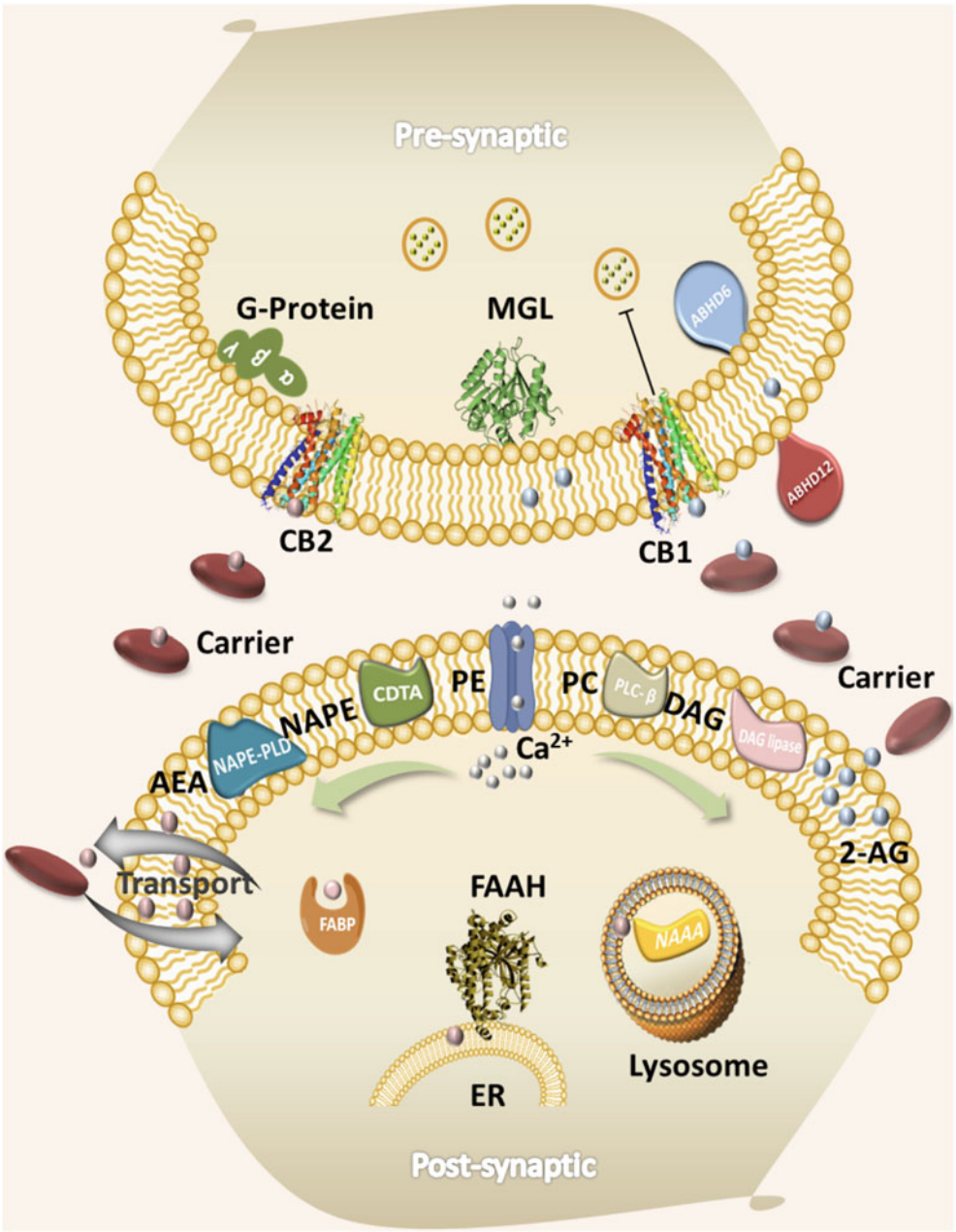


Fig. 3 The feedback loop for the endocannabinoid signaling system is depicted above. *CB1* cannabinoid receptor 1, *CB2* cannabinoid receptor 2, *FAAH* fatty acid amide hydrolase, *MGL* monoacylglycerol lipase, *ABHD6* α-β-hydrolase domain-containing protein 6, *ABHD12* α-β-hydrolase domain-containing protein 12, *NAPE* *N*-arachidonoyl phosphatidylethanolamine, *PE* phosphatidylethanolamine, *PC* phospholipase C, *PD* phospholipase D, *DGL* diacylglycerol lipase, *FABP* fatty acid-binding protein, *AEA* arachidonoyl ethanolamide, *2-AG* 2-arachidonoylglycerol, *ER* endoplasmic reticulum. Source*:* Adapted from Vemuri and Makriyannis (2015)

stimulated in a variety of ways such as neuronal injury or excessive neuronal firing (Basavarajappa 2007).

ECS signaling comes in two forms—tonic and phasic. Tonic signaling establishes eCB tone or a basal level of signaling, while phasic signaling involves temporal perturbations in eCB levels. Researchers have demonstrated that omega-3 fatty acids are essential for the proper regulation of the ECS tone, as these polyunsaturated fatty acids feed directly into the eCB signaling pathways (Lafourcade et al. 2011).

These new discoveries opened the door for characterizing the two cannabinoid receptors, the proteins that modulate their function and the eCB family of compounds that encompass the ECS system. The ECS plays a major role in the regulation of many aspects of human physiology. Today we know that the CB_1 cannabinoid receptor is the most abundant

GPCR in the brain but is also present in many other organs such as the heart, blood vessels, liver, lungs, and the digestive system, as well as fat and sperm cells (Mackie 2008).

4.1 The ECS Explained

The CB_1 receptor belongs to the Class A rhodopsin-like family of GPCRs. It is primarily found in the central nervous system being enriched in the cortex, hippocampus, outflow of the basal ganglia, and cerebellum. There can be both intra- and interspecies differences in the anatomical location of cannabinoid receptors in the ECS. It's important to note that CB_1 in humans is not prevalent in the brain stem or medulla oblongata, the organs responsible for controlling vital autonomic functions such as breathing and heartbeat. This is a strong contributing factor to the safety profile of cannabinoids in humans and the main reason that it is nearly impossible to overdose on THC. However, for dogs this is not true. This receptor is also found to a lesser extent in the periphery of cardiovascular, immune, gastrointestinal, and reproductive tissues. CB_2 receptors are located primarily in immune cells, among them leukocytes and those of the spleen and tonsils (Pertwee 2001). The CB_1 and CB_2 receptors share a significant degree of homology despite being located primarily in the CNS and immune system, respectively. One of the functions of cannabinoid receptors in the immune system is modulation of cytokine release. Activation of B- and T-cell CB_2 receptors by cannabinoids leads to inhibition of adenylyl cyclase in these cells and to a reduced response to immune challenge (Condie et al. 1996). Both CB_1 and CB_2 are coupled to Gi/o proteins and cause a decrease in adenylyl cyclase activity and the cAMP pathway. They also stimulate mitogen-activated protein kinase (MAPK) cascades, modulate ion channels, and modify intracellular calcium levels and hence neurotransmitter release (Howlett and Fleming 1984; Howlett 2002; Marcu and Schechter 2016; Pertwee 2005). Inwardly rectifying potassium channels can also serve as a signaling mechanism for the CB_2 receptor (Griffin et al. 1999; Ho et al. 1999).

Cannabinoid action is not limited to signaling outside of the cell. Fatty acid-binding proteins (FABP) have been demonstrated to be essential for the transport of cannabinoids into the cell where they can interact with cannabinoid receptors on the mitochondrial membrane or they recruit different transcription factors and are translocated into the nucleus where they modify gene expression (Elmes et al. 2015). Additionally there are other proteins that modify cannabinoid signaling such as CRIP1a which inhibits constitutive eCB signaling (Smith et al. 2015).

The cannabinoid receptor CB_1 has also been found intracellularly where it localizes to the mitochondrial membrane and regulates neuronal energy metabolism (Bénard et al. 2012). Mitochondrial CB_1 modifies cellular respiration through its inhibitory actions on soluble adenylyl cyclase and reduced complex I activity in the electron transport chain. Additionally, these mitochondrial receptors may also play a role in the pro-apoptotic mechanisms of cannabinoids in cancer cells.

4.2 Non-cannabinoid Receptor Interactions and Dimerization

Cannabinoids do not solely target CB_1 and CB_2 and can act through other receptor systems. Knockout mice for both of these cannabinoid receptors have been created; however, these mice still exhibit behavioral, biochemical, and electrophysiological responses when cannabinoids are applied, suggesting the presence of other cannabinoid receptor subtypes. For example, recently cannabidiol (CBD) has been shown to act as an inverse agonist at GPR3 and GPR6 (Laun and Song 2017). There are currently a few other GPCRs that are under investigation as potential cannabinoid receptors. One such receptor is GPR18 which has been shown to be activated by the eCB ligand *N*-arachidonoylglycine (NAGly). This receptor is thought to play a key role in microglial activation and hence response to neuronal injury (McHugh et al. 2010).

GPR55 is another putative cannabinoid receptor activated by lysophosphatidylinositol (LPI) and antagonized by CBD that has been shown to play a role in the regulation of bone physiology by regulating osteoclast number and function (Whyte et al. 2009). Similarly to GPR18 it also appears to play a role in the microglial function specifically mediating some of their neuroprotective activities (Kallendrusch et al. 2013). GPR119 is thought to be another component of the ECS. Specifically, this receptor regulates various physiological processes that improve glucose homeostasis, including glucose-dependent insulin secretion from pancreatic β-cells, gastrointestinal incretin hormone secretion, appetite control, epithelial electrolyte homeostasis, gastric emptying, and β-cell proliferation and cytoprotection. These properties make this receptor an attractive candidate as a drug target for metabolic conditions such as diabetes (Mo et al. 2014).

Other potential biological targets interacting with cannabinoids include PPARγ where CBD has been shown to attenuate a-beta inflammation and enhance hippocampal neurogenesis through actions at this site (Esposito et al. 2006). The pain receptor TRPV1 famous for its interactions with the capsaicin found in chili peppers also interacts with cannabinoids to reduce nociception. This target also appears to be implicated in cannabinoid hyperemesis syndrome (CHS) which has recently gained media attention. Treatment of CHS patients with topical capsaicin, a known TRPV1 agonist as well as alternating hot and cold showers, tends to resolve symptoms (Moon et al. 2018).

Opiate-Cannabinoid Receptor Dimerization

CB_1 has been shown to dimerize with both mu and delta opioid receptors (Fig. 4). The mu opioid receptor heterodimer has interesting properties, and when each individual component is activated, eCB signaling is enhanced. However, when both are activated simultaneously, this results in a decrease in signaling. In the case of delta opioid heterodimers, these receptors tend to antagonize each other if one is missing. For example, if the delta opioid receptor is missing from this complex CB_1, signaling increases and vice versa (Fujita et al. 2014).

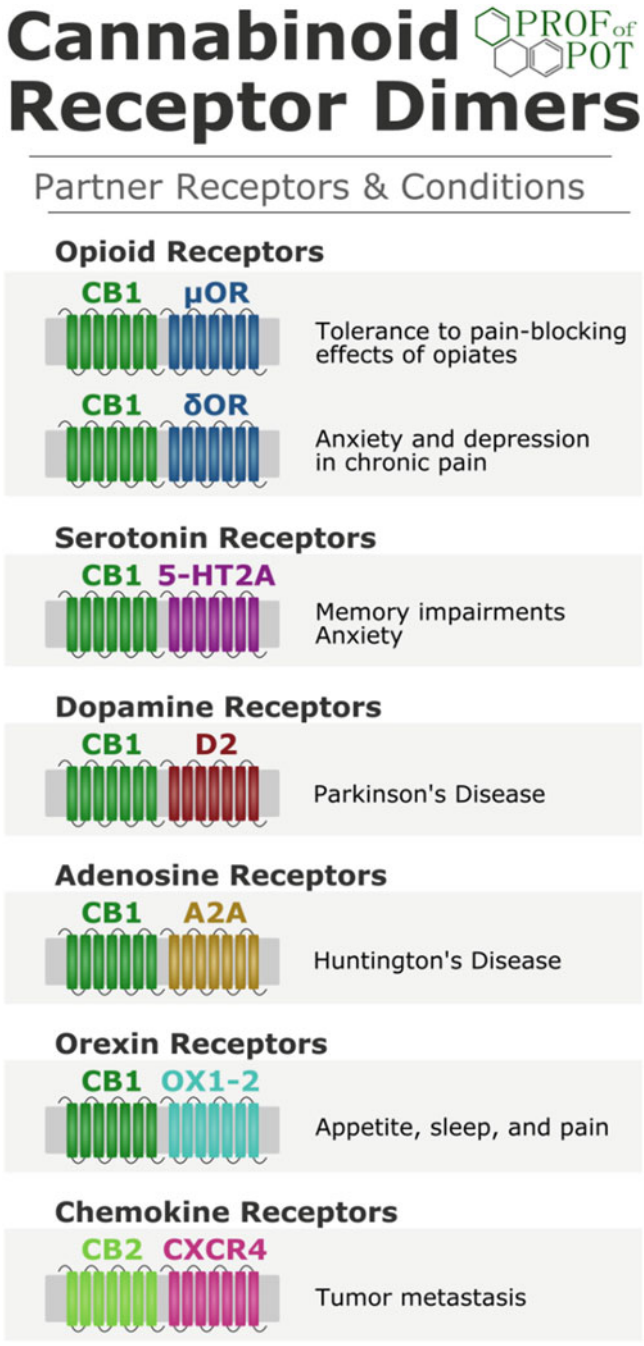


Fig. 4 Cannabinoid receptors have been shown to form dimers with other types of opioid, serotonin, dopamine, adenosine, orexin, and chemokine receptors. With permission from Professor of Pot

Serotonergic System and Cannabinoid Receptor Dimerization

CBD has been shown to produce agonist activity at the serotonin receptor 5-HT1A which may in part account for its anxiolytic properties. The cannabinoid receptor CB_1 has been shown to form heteromeric complexes with the serotonin receptor (5-HT2A). The activity of this receptor complex has been implicated in the memory augmenting properties of cannabinoids (Viñals et al. 2015). Interestingly the prevalence of this specific class of heteromeric complex increases in heavy users as they age and are implicated in certain conditions such as schizophrenia (Galindo et al. 2018). Additionally, cannabinoid agonists also inhibit signaling at the 5-HT3 receptor where they produce antiemetic effects (Fan 1995).

Other Receptor Systems

For an excellent review on this topic, see Prof of Pot's article on "Cannabinoid Receptor Dimerization" from which this figure was taken. See http://profofpot.com/cannabinoid-receptor-dimerization/

4.3 ECS in Health and Disease

Most of what we know about the medical and health benefits of cannabis relates to humans and not animals. Later in the chapter, we will focus on what we do know about cannabis as it relates to animals, but many of the biological interactions occur across the animal species. Below, we highlight many areas that cannabis can be helpful and acts as a starting point to evaluate cannabis as a therapeutic agent in veterinary species.

4.3.1 Sleep

Early studies from the 1970s indicate that THC causes a drastic increase in the production of melatonin, up to 4000% in some instances (Lissoni et al. 1986). It should be no surprise then that it has a profound effect on sleep (Babson et al. 2017). Additionally, cannabinoids affect the different stages of sleep and in particular act as a suppressor of REM sleep (Roehrs and Roth 2017).

4.3.2 Anxiety and Stress

Deficiencies in eCB signaling have been implicated in the etiology of a variety of conditions including PTSD, migraine, and fibromyalgia. In particular circulating levels of eCBs are markedly decreased in all of these disorders. This decline in circulating eCBs also tends to be correlated with anxiety-like

behaviors. A wide variety of studies have demonstrated that chronic environmental stress reliably leads to a down-regulation of CB_1 receptors and reduced levels of AEA increasing levels of 2-AG (Morena et al. 2016).

When tested in animal models, CBD was shown to have anxiolytic properties (Zuardi and Karniol 1983), as confirmed in a number of studies in humans. In an early study using healthy human volunteers subjected to a stressful public speaking test (SPST), a 300 mg dose reduced the volunteers' subjective anxiety to a level comparable to the standard anxiolytic diazepam (Zuardi et al. 1993). Neuroimaging confirmed these effects in follow-up studies (Bergamaschi et al. 2011; Crippa et al. 2009). CBD has also been shown to ameliorate some of the undesirable effects of THC and may be responsible for the improved properties of smoked cannabis compared to THC administration.

4.3.3 Obesity and Metabolic Diseases

Hypothalamic pro-opiomelanocortin neurons are responsible for feelings of satiety. CB_1 receptor activation on these neurons induces activity that causes the effects of these neurons to be silenced. This reduction in satiety can be attributed to the inhibitory effects of cannabinoids on the release of α-melanocyte-stimulating hormone (α-MSH) which normally produces appetite-suppressing effects. Recent studies have also demonstrated an inverse correlation between levels of orexin-A and α-MSH, which led to the discovery that orexin-A induces hyperphagia by increasing levels of the eCB 2-AG. Blockade of the OX-A receptor type 1 mitigated the impairment of α-MSH signaling which may serve as a target for controlling hunger responses (Morello et al. 2016). Because both GABAergic and glutamatergic presynaptic terminals on POMC neurons express CB_1, it is reasonable to assume that the bimodal effect of cannabinoids on feeding is due to the differential sensitivity of GABAergic versus glutamatergic axons to CB_1 activation (Koch et al. 2015).

Cannabis users who are prone to the munchies are generally thought to eat in greater quantities than their cannabis-naive counterparts. On average, users of cannabis display a slimmer waistline and lower BMI than nonusers. While seemingly paradoxical, Le Foll et al. (2013) have found that the prevalence of obesity is lower in regular cannabis users compared to nonusers, even after adjusting for important variables such as age, sex, and tobacco smoking status. A study in 3 T3-L1 adipocytes gives some insight into one possible mechanism by which cannabis users keep their weight in check and shows that treatment of these cells with CBD causes changes in the expression of certain signaling cues that lead to the browning of these cells (Parray and Yun 2016). In addition, cannabis users also display lower levels of fasting insulin and display better insulin sensitivity than their nonusing counterparts (Muniyappa et al. 2013).

Certain cannabinoids have demonstrated effects that may be useful for obesity treatment and prevention. The use of the cannabinoid THCV has correlated with weight loss, as well as decreased body fat and serum leptin concentrations in obese mice (Riedel et al. 2009; Wargent et al. 2013). Additional support for this idea comes from a recent paper by Silvestri et al., in which THCV and CBD were both shown to reduce accumulated lipid levels in adipocytes and in a model of hepatosteatosis (Silvestri et al. 2015).

4.3.4 Cancer

The ECS plays a key role in modulating cell differentiation, cell proliferation, and cell death. Additionally, cannabinoids such as THC stimulate appetite and reduce the emetic responses seen in chemotherapy. These qualities make the ECS an attractive target for use in cancer therapy. Cannabinoids can also be beneficial in certain cancers through their modulation of gene expression. For example, in lung cancers the application of CBD results in an upregulation of the expression of the intracellular adhesion molecules (ICAM) which in turn prevents the metastasis of cancerous cells beyond the tumor site (Haustein et al. 2014). In gliomas, the administration of CBD results in a dose-dependent reduction in the expression of pro-angiogenic factors (Vaccani et al. 2005). THC, when administered in conjunction with CBD, provides synergistic effects in the inhibition of human glioblastoma cell proliferation and survival. Another example of such changes in gene expression occurs in breast cancer cells where McAllister et al. demonstrated that the application of this cannabinoid downregulates the expression of ID-1, which is a large contributor to breast cancer metastasis (McAllister et al. 2007).

4.3.5 Inflammatory Conditions

Inflammation is a highly prevalent condition that contributes to the development and progression of many diseases and health conditions. The ECS has been shown both in vivo and in vitro to be involved in regulating the immune system through its immunomodulatory properties. Cannabinoids have been tested in several experimental models of autoimmune disorders such as multiple sclerosis, rheumatoid arthritis, colitis, and hepatitis.

As the cannabinoid receptor CB_2 is primarily found on the surface of immune cells, it should come as no surprise that these receptors modify the inflammatory response. These receptors have been shown to protect the host from the pathogenesis of these conditions through induction of multiple anti-inflammatory pathways such as suppression of T-cell-mediated immune responses by primarily inducing apoptosis and suppressing inflammatory cytokines and chemokines. However, some studies have shown both pro-inflammatory and anti-inflammatory effects, suggesting different thresholds for different cell populations.

Compounds found in cannabis that reduce inflammation are abundant and diverse. The most abundant phytocannabinoids in cannabis, THC and CBD, both have strong anti-inflammatory properties, while CBC, CBG, and THCV have also demonstrated anti-inflammatory properties. Cannabinoids act as anti-inflammatory agents by inducing apoptosis, inhibiting of cell proliferation, suppressing cytokine production, and inducing T regulatory cells. Apoptotic mechanisms induced by cannabinoids in immune cells include activation of CD95 to induce Bcl-2 and caspase cascades in immune cells. Cannabinoids have also been demonstrated to promote the production of anti-inflammatory interleukins such as IL-10 while inhibiting the production of pro-inflammatory cytokines such as TNF-α in a CB_1-dependent fashion (Klein et al. 2000).

Veterinarians and physicians alike might find it intriguing that nonsteroidal anti-inflammatory drugs (NSAIDs) produce their anti-inflammatory response through interactions with the ECS. Specifically, in the case of acetaminophen, its metabolism by the liver leads to the production of *N*-arachidonoylphenolamine (AM-404) which acts as both a cannabinoid receptor agonist and eCB reuptake inhibitor (Ottani et al. 2006). These interactions also block the conversion of arachidonic acid into inflammation- and pain-promoting prostaglandins (Saliba et al. 2017).

Additionally, certain terpenes also display anti-inflammatory activity. Among the terpenes, α-pinene, β-myrcene, and β-caryophyllene appear to act through prostaglandin receptors (PGE_1 and/or PGE_2) to have an anti-inflammatory effect. Additionally, beta caryophyllene is the only terpene currently known to bind to cannabinoid receptors attenuating inflammation in a CB_2 receptor-dependent fashion (Klauke et al. 2014). Hydrogenated terpenes and cannabinoids often display different characteristics as well and can exist in cannabinoid extracts at low levels as a result of the variety of distillation and refinement techniques being employed (USP #20160324909) (Scialdone 2017)

4.3.6 Pulmonary Effects

Studies performed in the 1970s at the University of California,—Los Angeles, by Donald Tashkin have shown that both inhaled and orally ingested THC produce bronchodilation for up to 2 hours after administration (Tashkin et al. 1974). Further investigations by the Respiratory Pharmacology Laboratory in Paris have shown that CB_1 receptor activation inhibits cholinergic contraction in a concentration-dependent fashion, offering a possible mechanism for the inhibiting of bronchospasms. Additional studies performed at the University of Sao Paulo using CBD have also shown some potential for improving the symptoms of COPD. They found decreased pulmonary inflammation and improvements in lung function in murine models of inflammatory lung disease using the inflammatory agent lipopolysaccharide (Ribeiro et al. 2015). Other findings using murine models have shown that intraperitoneal administration of THC results in a reduction of allergen-induced mucus production (Reddy et al. 2012).

4.3.7 Cardiovascular Effects

Cannabinoids can both increase and decrease blood pressure and heart rate, depending upon the specific context in which they are being used. These effects are only recently becoming better understood as more studies are being conducted regarding these effects (Dewey 1986; Wagner et al. 1998; Niederhoffer and Szabo 1999, 2000).

CBD specifically has been found to have direct effects on arteries, influencing vaso-relaxation, which is clinically observed as a mild hypotensive effect when CBD is administered. CBD has a protective effect against the vascular damage caused by hyperglycemia, as with type 2 diabetes, diabetic angiopathies, and systemic inflammatory processes. It is the antioxidant and anti-inflammatory effects of CBD that mediate these benefits (Stanley et al. 2013).

CBD has been found to have anti-arrhythmic effects in an in vivo rat model of coronary artery occlusion which may not be mediated through the CB_1 receptors found on myocardial cell membranes, but may have other non-receptor-mediated pathways that allow its control over cardiac rhythm (Hepburn et al. 2011).

It was determined in spontaneously hypertensive rats (SHR) that CB_1 antagonists increased blood pressure and left ventricular contractile performance. Additionally, by preventing the degradation of the endocannabinoid anandamide by fatty acid amide hydrolase (FAAH), as is found with CBD, reductions in blood pressure, cardiac contractility, and vascular resistance were found in normotensive rats. These effects were inhibited by CB_1 antagonists. It was found that CB_1 antagonists lower blood pressure much more in the SHR than in normotensive Wistar-Kyoto rats. Additionally, CB_1 receptors are upregulated in the heart and aortic endothelium in SHR versus the normotensive rat cohort (Bátkai et al. 2004).

4.3.8 Antioxidants and Neuroprotection

The NIH holds a patent on cannabinoids as antioxidants and neuroprotectants (Hampson et al. 2003; USP: 6630507). THC, CBD, and CBG have been shown to have antioxidant properties (Hampson et al. 1998; Borrelli et al. 2013). Through their actions as antioxidants, cannabinoids can neutralize reactive oxygen species. THC and CBD were both found to reduce ROS in vitro, with similar potency to known antioxidants such as ascorbate and butylated hydroxytoluene. Additionally, by inhibiting voltage-gated calcium channels, cannabinoids can inhibit the release of glutamate, hence curbing excitotoxicity due to excessive neuronal firing. During periods of ischemia and other traumatic brain events, the

neurotransmitter glutamate is released. Glutamate itself is toxic in excess and can lead to neuronal cell death in a process known as excitotoxic stress. Compounds with antioxidant properties are often neuroprotective, which is believed to be through the reduction of toxic ROS produced during ischemic metabolism. The neuroprotective effect of these compounds was found to be independent of their CB receptor binding activity. CBD in particular has been shown to protect against cerebral ischemic injury (Hayakawa et al. 2008) and also attenuates Alzheimer's-related neuroinflammation in animal models (Esposito et al. 2007).

Plant antioxidants such as ascorbic acid and tocopherols, polyphenolic compounds, terpenes, and numerous mono- and sesquiterpenes are important for human health. Cannabis terpenes with demonstrated antioxidant properties include β-caryophyllene (Calleja et al. 2013), limonene, and β-myrcene (Ciftci et al. 2011). In a mouse study of cerebral ischemia (Ciftci et al. 2011), β-myrcene protected against oxidative stress and histological damage induced by ischemia-reperfusion and is thus an effective neuroprotectant. Further, the compound is suggested as a good candidate for treatment of ischemic stroke.

The ECS is implicated in the etiology of a variety of neurodegenerative conditions, and cannabinoids have been shown to have neuroprotective qualities and the ability to attenuate neuroinflammation and promote neurogenesis (Jiang et al. 2005; Saito et al. 2012; Van der Stelt et al. 2001). In the case of Alzheimer's disease (AD), cannabinoids have shown promise for their ability to clear the toxic beta amyloid (Aβ) plaques that accumulate in the brain in patients with the disease. Considering patients treated with cannabinoids demonstrate improvement in symptoms, it should come as no surprise that a precursor to this debilitating disease is a loss of the body's natural production of eCBs. CBD has also been shown to reduce the expression of genes implicated in the phosphorylation of the tau protein, the hyperphosphorylation of which leads to the formation of neurofibrillary tangles that further contributes to the progression of the disease (Esposito et al. 2006). Furthermore, cannabinoids have been demonstrated to enhance the clearance of Aβ from the brain as well as prevent the inflammatory cascade that is produced by the accumulation of these misfolded proteins intracellularly (Eubanks et al. 2006).

4.3.9 Cannabis, Memory, and the ECS

Michael Pollan in his book, *The Botany of Desire: A Plant's-Eye View of the World*, addresses the short-term memory issues associated with the use of THC and especially the value of cannabis in treating post-traumatic stress disorder (PTSD) (Pollan 2001). Not all memories need to be retained. Some can be traumatic and toxic when recalled, as with PTSD. Pollan poses a question to the reader: "Do you really want to remember all the faces you saw on the subway this morning?"

The ECS acts as a filter for memory, promoting the retention of useful memories and removal of unnecessary ones. This ability to forget is critical for survival and goes awry in certain conditions such as PTSD. The ability of THC to enhance this process of forgetting is in part due to alterations in long-term depression (LTD) in the CA1-CA3 circuit of the hippocampus which is specifically dependent on astroglial CB_1 rather than neuronal cells—suggesting the cleanup of the synapse is impaired (Han et al. 2012). Newer research demonstrates that memory deficits from cannabis are also associated with increased COX-2 activity in the hippocampus (Chen et al. 2013).

It has also been suggested that chronic cannabis use is associated with structural changes in the hippocampus, such as reduced gray matter and alterations in shape. One particular study showed that long-term cannabis users had a 12% reduction in hippocampal volume on average. However, these findings may be flawed since there was a very small sample size used ($n = 15$) (Demirakca et al. 2011; Solowij et al. 2013; Yücel et al. 2008). If these changes are valid, they could have an influence on memory formation in cannabis users. However newer research refutes these findings, showing that daily cannabis use does not cause alterations in adolescents or adults. This study also used a much greater sample size than previous studies, comparing the brain morphology of 29 adult users and nonusers, as well as 50 adolescent users and nonusers using high-resolution MRI scans (Weiland et al. 2015). Other follow-up studies shed some light on the reasons behind these discrepancies by demonstrating that these changes in hippocampal volume and morphology are ablated when co-administered with CBD, displaying a protective effect of this cannabinoid against THC-induced changes (Yücel et al. 2016). Beale et al. examined this effect further and found that its restorative effects were limited to only certain hippocampal regions, specifically the left subicular complex (parasubiculum, presubiculum, and subiculum) (Beale et al. 2018).

4.3.10 Clinical Endocannabinoid Deficiency (CECD)

Deficiencies in eCB signaling have been implicated in the etiology of a variety of conditions including PTSD, migraine, and fibromyalgia (Russo 2016a, b). There are known mutations in ECS genes that contribute to such deficiencies. For example, patients with mutations in CNR1 and DAGLA exhibit CECD phenotypes (Smith et al. 2017). Another example of this is seen in IBS patients where the variant rs806378 *CT/TT* in the CNR1 gene showed a statistically

significant association with rates of colonic transit (Camilleri et al. 2013). Specifically, in the context of PTSD, fear extinction is impaired in patients who were homozygous for the CNR1 variant rs2180619 (Heitland et al. 2012).

In particular circulating levels of eCBs are markedly decreased in all of these disorders. This decline in circulating eCBs also tends to be correlated with anxiety like behaviors. A wide variety of studies have demonstrated that chronic environmental stress reliably leads to a downregulation of CB_1 receptors and reduced levels of 2-AG and AEA (Hill et al. 2009). Equally as predictable is a transient increase in 2-AG in the amygdala following chronic stress showing increases after 30 minutes that return to baseline after 60 min (Patel et al. 2009).

4.3.11 Limitations of the ECS as a Therapeutic Target

The involvement of the ECS in such a wide variety of physiological processes makes it enticing from a practitioner's perspective to understand how to manipulate this system. While the ECS can be viewed as a panacea for the treatment of various conditions, it is not without limitations. In particular, cannabinoid receptor agonists such as THC carry psychoactive effects which can be undesirable in certain populations. Additionally, persistent activation of the cannabinoid receptors results in receptor desensitization which ultimately leads to tolerance and the need for higher dosages, which also increases the likelihood of adverse responses. Unintended consequences of ECS stimulation include memory deficits and the development of withdrawal symptoms. One drawback of prolonged ECS stimulation that has recently gained attention is the development of cannabinoid hyperemesis syndrome (Chang and Windish 2009). This disorder only develops in certain subsets of the population and results in cyclical vomiting that can only be absolved by the administration of alternating hot and cold showers or a TRPV1 agonist such as capsaicin. One such focus area of research was that of appetite and metabolism and attempts to harness this avenue took form as a drug called SR141716A (Rimonabant), a CB_1 receptor inverse agonist. The use of rimonabant had negative outcomes, causing some to have negative thoughts and ideations of suicide, with some patients actually carrying it out. It was later discovered that such issues were attributed with effects on the central nervous system; therefore researchers are now working on developing CB_1 inverse agonists that are restricted to the periphery. There is also the possibility of synthesis issues producing off-target by-products which is still currently under investigation by the FDA.

4.4 Veterinary ECS: Our Current State of Knowledge

The CB_1 receptor is highly conserved across all mammalian species, but there are significant primary sequence differences that have been discovered between the human and rat cannabinoid CB_2 receptors and the newly cloned canine cannabinoid receptor, CB_2. It was found that the binding affinities for canine CB_2 receptor were 30 times less than those measured for human and rat CB_2 receptors. The functional properties of the cannabinoid CB_2 receptor were found to be highly dependent upon the receptor expression level and the nature of the signaling pathway selected (Ndong et al. 2011).

4.4.1 Anatomical Localization of Cannabinoid Receptors in the Dog

Cannabinoid Receptor 1

A recent study used immunohistochemistry to anatomically localize the CB_1 receptor in the normal canine nervous system. Nervous systems were examined from a healthy 4-week-old puppy, three 6-month-old dogs, and one 10-year-old dog. Strong "dot-like immunoreactivity" was found in the neutrophils of the cerebral cortex, *cornu ammonis* (CA), and dentate gyrus of the hippocampus, midbrain, cerebellum, medulla oblongata, and gray matter of the spinal cord. Dense CB_1 expression was found in fibers of the globus pallidus and substantia nigra surrounding immunonegative neurons. Astrocytes were consistently positive in all examined regions. In the PNS, CB_1 immunohistochemistry stained neurons and satellite cells of the dorsal root ganglia and myelinating Schwann cells in the PNS.

The younger dog examined had a lower general CB_1 expression in the brain, showing that the density of receptor expression was less than observed in human fetal and neonatal brain tissue. Lower CB_1 expression has been found in aged rats in specific regions, most prominent being the cerebellum, cerebral cortex, and basal ganglia and less prominent in the hippocampus. This reduction in CB_1 density with age in these rats was consistent with the findings in the older dog examined in this study (Freundt-Revilla et al. 2017). Previous studies have identified CB_1 receptors in salivary glands (Dall'Aglio et al. 2010), hair follicles (Mercati et al. 2012), skin, and hippocampus in dogs (Campora et al. 2012).

Immunohistochemistry was used to study the localization of CB_1 receptors on developing canine embryo (30 days old) with a commercially available antibody. CB_1 receptor immunoreactivity was found primarily in epithelial tissues and included most structures of the central and peripheral nervous system, inner ear, olfactory epithelium and related structures, eye, and thyroid gland (Pirone et al. 2015).

Canine CB_1 Receptor Localization
- Cytoplasm of basal and suprabasal layer cells.
- Hair follicle inner epithelial root sheaths and arrector pili muscles.
- Undifferentiated sebocytes at the periphery of sebaceous glands.
- Secretory and ductal cells of sweat glands.
- Mast cells and fibroblasts.
- Upregulated in atopic dermatitis.

Cannabinoid Receptor 2

As compared to human skin samples, clinically normal dogs have a homogeneous distribution of CB_1 and CB_2 receptors in all epidermal layers. In human, CB_1 is mainly detected in epidermal spinosum and granulosum layers, and CB_2 is detected mainly in basal keratocytes. Both CB_1 and CB_2 receptors have been found in the skin of healthy dogs and dogs with atopic dermatitis. The epidermis of dogs is thinner than that of humans (2–3 nucleated layers in the dog versus 6–7 in the human), which might account for this difference. In dogs with atopic dermatitis, hyperplastic epidermal changes were found, with strong CB_1 and CB_2 immunoreactivity in suprabasal keratinocytes and weak CB_1 and strong CB_2 immunoreactivity in basal keratinocytes indicating upregulation of these receptors during inflammation. CB_1 and CB_2 agonists decrease mast cell degranulation (Campora et al. 2012). To summarize, cannabinoid receptor localization on the skin of the dog was found in cytoplasm of epidermal and follicular keratinocytes, sweat and sebaceous gland epithelial cells, and the mesenchymal dermal cells

Canine CB_2 Receptor Localization
- Epidermis.
- Cytoplasm of cells in the basal and suprabasal layers.
- Hair follicles in the basal and suprabasal cells of the outer and inner epithelial root sheaths.
- Mild immunoreactivity in cells of arrector pili muscles and secretory and ductal cells of sweat glands.
- Sebaceous glands in the cytoplasm and peripheral reserve cells.
- Mast cells, fibroblasts, and endothelial cells.
- Lymph nodes.
- Strong B-cell zone immunoreactivity mainly in germinal centers of secondary follicles.
- Upregulated in atopic dermatitis.

4.4.2 Invertebrate ECS

The two cannabinoid receptors, CB_1 and CB_2, have been found in mammals, birds, reptiles, and fish. In a study of seven representative species of invertebrates, McPartland used tritiated ligand binding assays to characterize the cannabinoid receptors in *Ciona intestinalis* (Deuterostomia), *Lumbricus terrestris* (Lophotrochozoa), *Peripatoides novaezealandiae* (Onychophora), *Jasus edwardi* (Crustacea), *Panagrellus redivivus* (Nematoda) [the beer mat nematode], *Actinothoe albocincta* [white striped anemone] (Cnidaria), and *Tethya aurantium* (Porifera) [Orange Puffball sponge] (McPartland et al. 2006).

Cannabinoid binding was detected in all species studied except for the sea anemone (*A. albocincta*) and sponge (*T. aurantium*). The receptors were consistent with CB_1 receptors but not CB_2 receptors. Three of the organisms tested, earthworm (*L. terrestris*), velvet worm (*P. novaezealandiae*), and mat nematode (*P. redivivus*), were compared to a standard CB_1 ortholog in rat cerebellar tissue. A high affinity binding interaction was observed at various concentrations characteristic of CB_1 receptors.

The authors of this study hypothesize that cannabinoid receptors evolved in the last common ancestor of bilaterians, with secondary loss in insects and other clades. After conducting a systematic literature review, the authors found that cannabinoid receptors have been identified in sea urchins, leeches, earthworms, hydra, lobster (*H. americanus and J. edwardi*), and the beer mat nematode (*P. redivivus*), but not the nematode (*C. elegans*). No binding was observed in sponges (Porifera).

In a separate study, McPartland found that insects (*Apis mellifera* [western honey bee], *Drosophila melanogaster* [common fruit fly], *Gerris marginatus* [water strider], *Spodoptera frugiperda* [fall armyworm moth larva], and *Zophobas atratus* [darkling beetle]) are devoid of cannabinoid receptors. This loss of CB receptors is unique to comparative neurobiology, in that no other known mammalian neuroreceptor has been found to be missing in insects (Ecdysozoa). The authors suggest that the lack of cannabinoid receptors in insects is due to their lack of ligands, in that insects produce little or no arachidonic acid, the precursor to the biosynthesis of endocannabinoids (McPartland et al. 2001).

5 *Cannabis sativa* Chemistry

The fascinating effects that cannabis plant constituents have on the human experience have led to the discovery of 113 phytocannabinoids, each with a unique pharmacological profile (*C. sativa*. Pertwee *Handbook of Cannabis* 2014; Radwan et al. 2008). There is an emerging interest in another pharmacologically active group of 140 terpenes that have been identified in cannabis.

Terpenes have been shown to synergistically modulate the therapeutic effects of cannabinoids and also define the aromatic scent profile of the differing cultivars (Brenneisen and ElSohly 1988). The strain-specific effects are attributed to the unique chemotaxonomy of cannabinoids and terpenes (Russo 2011). Over 750 naturally produced compounds have been

found in cannabis (Upton et al. 2014), which may be divided into many different chemical classes. Covering each component of *C. sativa* is beyond the scope of this chapter and not relevant to the therapeutic activity of cannabis. An excellent review of *C. sativa* chemistry can be found in the body of work compiled in Table 1 (Brenneisen and ElSohly 1988; Callaway 2004; ElSohly and Slade 2005; Turner et al. 1980; Upton et al. 2014).

5.1 Phytocannabinoids

Plant-derived cannabinoids, or phytocannabinoids, refer to chemicals derived from botanical sources that interact with the ECS. The term is often synonymous with pharmacologically active compounds isolated from *Cannabis sativa*. Phytocannabinoids differ from eCBs with regard to their long-lived pharmacokinetic profile when compared to the relatively fast acting, short, and intermittent lifetime of the eCBs.

Of the 113 cannabinoids identified in *C. sativa*, the majority can be catalogued as analogs of Δ^9-tetrahydrocannabinol (Δ^9-THC), CBD, cannabichromene (CBC), cannabigerol (CBG), cannabinol (CBN), cannabicyclol (CBL), cannabielsoin (CBE), and cannabitriol (CBT). There are common points of variability on the phytocannabinoid scaffold that are highlighted in Fig. 5. The points of structural variability existing across the phytocannabinoid classes follow some general trends and quickly add up to over 100 potential cannabinoids when repeated across the common analogs shown in Fig. 5. The length of the alkyl side chain is a common site of variability and can range from one to five carbons in length (e.g., R^1, CBD, Fig. 5). The most recognizable and abundant cannabinoids contain *n*-pentyl side chains ($n = 5$), but *n*-butyl, *n*-propyl, ethyl, and methyl side chains have also been identified at lower concentrations (ElSohly and Slade 2005; Turner et al. 1980). Cannabivarins contain a 3-carbon side chain and are also commonly observed. The presence or absence of a carboxylic acid functional group is another point of common structural variability. Though THC and CBD are the most well-known cannabinoids, it is important to note that they are not synthesized in the cannabis flower directly. Rather, they are synthesized in their acidic forms in the plant, which are tetrahydrocannabinolic acid (THCA, R = COOH, R^1 = *n*-pentyl, R^2 = H) and cannabidiolic acid (CBDA, R = COOH, R^1 = *n*-pentyl). These compounds can be converted into THC and CBD by heating. The application of increased heat speeds up the reaction in which a CO_2 molecule is released from THCA to form THC in a process termed decarboxylation. Though some people refer to this reaction as "activating" the cannabinoids, it is a bit of a misnomer, as the acidic versions of these compounds also have been shown to induce medical benefits themselves, most notably anti-inflammation properties (Veress et al. 1990). Microbial hydroxylated forms also exist at low levels and remain relatively unstudied (Rashidi et al. 2009). The choice to decarboxylate can have important effects on the therapeutic application.

Table 1 Chemical components identified in *Cannabis sativa*

Chemical class	Identified compounds
Terpenes	140
Cannabinoids	113
Hydrocarbons	50
Sugars and related compounds	34
Nitrogenous compounds	27
Non-cannabinoid phenols	25
Fatty acids	23
Flavonoids	23
Simple acids	20
Simple ketones	13
Simple ester and lactones	13
Simple aldehydes	12
Proteins, enzymes, and glycoproteins	11
Steroids	11
Elements	9
Simple alcohols	7
Vitamins	3
Pigments	2

Tetrahydrocannabinol, or Δ^9-THC, is the main cannabinoid responsible for the characteristic psychoactive effects of cannabis with which most people are familiar. The binding activity of THC to the CB_1 receptor can cause downstream physiological changes such as mental euphoria, increased appetite, and slower cognitive functioning. A synthetic isolated version of THC called Marinol was approved by the FDA in the late 1990s for its antiemetic effects and prescribed to cancer patients undergoing chemotherapy.

Cannabidiol, or CBD, has received a surge of interest in the last decade by many interested parties, ranging from academic researchers to pharmaceutical drug developers to even nutritional supplement manufacturers. Part of this is due to the well-studied medical benefits. At the time of writing of this chapter, a CBD-based pharmaceutical drug (Epidiolex) has just been approved as a drug by the FDA on June 25, 2018.

Starting in the 1970s, investigators identified CBD's anticonvulsant properties in rats (Consroe and Wolkin 1977) and conducted an initial testing in humans in small groups (Carlini and Cunha 1981). This was followed by a larger human study using 900–1200 mg/kg daily which showed that CBD was effective in treating seizure states (Trumbly 1990). Overall, CBD exhibits the most reliable anticonvulsant effects of cannabis constituents. In very recent reports describing its use in children experiencing epileptiform

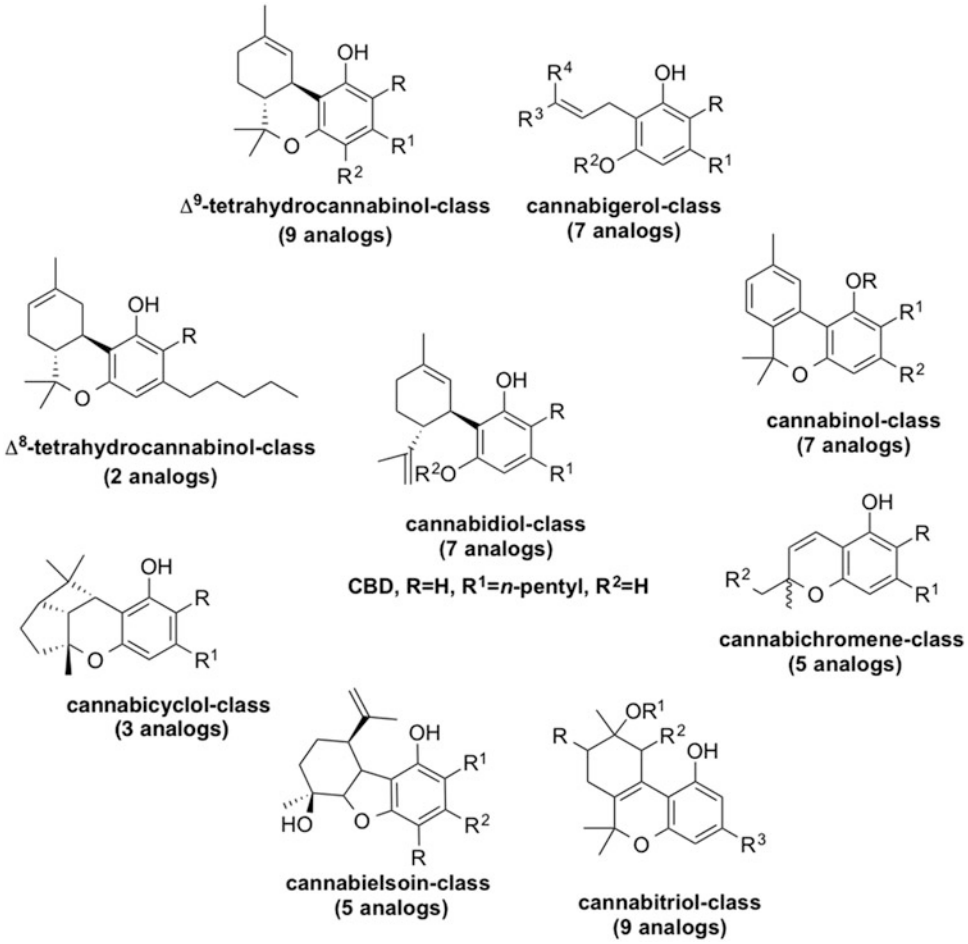


Fig. 5 Common cannabinoid analogs found in *Cannabis sativa*. This figure illustrates the common points of variability within each class and how many analogs have been discovered

conditions, CBD was shown to greatly ameliorate these effects. Also, initial results from a broad clinical evaluation of CBD in a seizure-associated condition in children are very promising. In epileptic patients there is a dysregulation of neuronal firing resulting in seizures and excitotoxicity. Increased levels of AEA have been found in the cerebrospinal fluid of dogs suffering from idiopathic epilepsy as compared to healthy dogs (Gesell et al. 2013).

Ligresti et al. (2006) examined the antitumor effects of a variety of cannabinoids, including both neutral and acidic forms. While CBD was determined to be the most potent antitumor cannabinoid tested, CBD-A was the least. The activity of CBD was due to its capability of inducing apoptosis via direct or indirect activation of CB_2 and potential vanilloid type 1 receptors (Ligresti et al. 2006) (Fig. 6).

A key structural distinction between THC and CBD is the freely rotatable bridge between the aromatic and nonaromatic cyclic systems that only exists in CBD. THC and CBD both have the same empirical formula as well as molecular weight. However, THC is conformationally constrained in a way that doesn't allow this region of the molecule to "flop" around. They are different in three-dimensional space, but CBD also has an additional free hydroxyl group that results in distinct

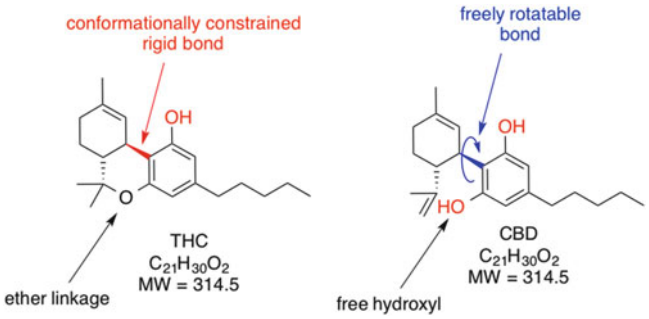


Fig. 6 Illustrates that CBD and THC have the same empirical formula and molecular weight. However, THC is conformationally constrained through an ether linkage and interacts with the receptors in a slightly different way compared to CBD

interactions with CB receptors and other biologically active targets.

(−) Δ^9-Tetrahydrocannabivarin (THCV) is the propyl analog of THC in which the 5-carbon side chain is shortened by two methylene units. Its pharmacological properties have been studied since the early 1970s when it was recognized that it behaves as a significantly weaker agonist (approximate fivefold) compared to THC.

Thus, as with THC, THCV produces analgesic but also cataleptic effects in animals (Hill et al. 2012; Pertwee 2008). These effects can be antagonized by synthetic CB_1 antagonists such as SR1716A or AM251. This property of being a weaker agonist than THC is consistent with extensive medicinal chemistry work showing the decreased potency of the cannabivarin family relative to the longer side chain analogs. Maximum potency is observed with side chains of 7–8 carbons (Makriyannis 2014).

Although THCV can behave as a CB_1 agonist in vitro, it can also act as a CB_1 antagonist capable of blocking the effects of more potent synthetic CB_1 agonists as well as THC and the eCBs AEA and 2-AG. For this reason, Δ^9-THCV can act as an in vivo CB_1 antagonist. At the CB_2 receptor, Δ^9-THCV behaves as a partial agonist and is capable of activating this receptor, although only to a limited extent. CB_2 receptor activation has been shown to be associated with attenuation of inflammation. The antiepileptic effects of this compound are being tested in humans currently.

Cannabigerol (CBG), in its acid form (CBGA), is the principal precursor and convergent intermediate of all phytocannabinoids. CBG is non-psychoactive and is a relatively weak partial agonist for both CB_1 and CB_2. Because of its low cannabinoid receptor potency, it can functionally antagonize the CB_1 effects of THC. It has been shown to relieve intraocular pressure, potentially useful in the treatment of glaucoma. Additionally, its antioxidant and anti-inflammatory properties make it a potential candidate for inflammatory bowel disease. Recent evidence identifies CBG as a potential candidate for the treatment of colon cancer (Ligresti et al. 2006).

There is some information on *tetrahydrocannabinolic acid (Δ^9-THCA)* and *cannabidiolic acid (CBDA)*, the two naturally non-psychotropic precursors of Δ^9-THC and CBD, respectively, that shows that their therapeutic value is derived from mechanisms other than classical CB_1/CB_2 receptor binding (Ahmed et al. 2008). THCA in vitro is capable of modulating the functions of two TRP channel receptors acting as a potent TRPA1 agonist and TRPM8 antagonist, inhibiting both cyclooxygenase enzymes, COX-1 and COX-2. When tested in rat models of nausea, THCA appears to be a better alternative for treating nausea and vomiting associated with cancer chemotherapy (Rock et al. 2013, 2014). THCA has also been shown to reduce levels of TNF-α in vitro, suggesting a mechanism for immune modulation (Ligresti et al. 2006). This reduction in TNF-α has been demonstrated in culture supernatants from U937 macrophages and peripheral blood macrophages after stimulation with LPS in a dose-dependent manner (Verhoeckx et al. 2006). Additionally, THCA has also been demonstrated to have potent activity as a PPARγ agonist (Nadal et al. 2017). However, these findings may only have implications for the periphery as there is some evidence to suggest that THCA's access to the central nervous system is restricted due to interaction with the BBB (Moreno-Sanz 2016). CBDA was shown to be a selective inhibitor of COX-2 (Takeda et al. 2008), implying anti-inflammatory activity. It also has been demonstrated to be a potent agonist at 5-HT1A (Bolognini et al. 2013). Unlike its congener, CBDA effectively reduced anticipatory nausea and may be useful against acute nausea induced by chemotherapy (Rock and Parker 2013).

Cannabinol (CBN) is a degradation product of Δ^9-THC caused by oxidation; therefore, it isn't surprising that it was the first phytocannabinoid to be isolated in pure form. CBN can activate both CB_1 and CB_2 receptors with a potency approximately ten-fold lower than Δ^9-THC. It can thus be viewed as a weak Δ^9-THC relative (Izzo et al. 2009). Less is known about the pharmacology of the diverse array of low abundance phytocannabinoids found in cannabis. Research on rare phytocannabinoids is rapidly expanding and an active area of investigation (Hartsel et al. 2016).

5.2 Chemotaxonomy of *C. sativa*

Chemical phenotypes (chemotypes) can be useful to classify *C. sativa* as drug- or fiber-type varieties. The United Nations Office on Drugs and Crime categorizes *C. sativa* into three chemotypes based on the proportion of THC and CBN relative to CBD [Eq. (1)] (Drugs 2009). Chemotype I (drug-type) cultivars are characterized by X values greater than one, while values less than one are representative of chemotype III cultivars (fiber-type). CBD-rich cultivars containing low levels of THC are regarded as fiber-type, or chemotype III cultivars. The evidence suggests that drug-type or chemotype I *C. sativa* cultivars with high levels of Δ^9-THC originated from below the 30°N latitude (Hillig and Mahlberg 2004). Equivalent levels of Δ^9-THC and CBD are known as chemotype III and are typically found above the 30 °N latitude. Hillig and Mahlberg (2004) used a statistical approach to define chemotaxonomic trends in *C. sativa* and found that most cultivars fell outside of the arbitrary values set by the United Nations Office on Drugs and Crime. They demonstrated that most cultivars clustered into either chemotype I ($X > 10$), chemotype II ($0.2 < X < 10$), or chemotype III ($X < 0.2$). The relative cannabinoid levels in *C. sativa* remain constant from the seedling stage throughout the plant life cycle (Broséus et al. 2010), making it possible to determine the chemotype at early development stages prior to flowering (Barni-Comparini et al. 1984; Vogelmann et al.

Equation 1 Chemotaxonomy is calculated by dividing the sum of THC and CBN concentration by the CBD concentration

$$X = \frac{[\mathrm{THC}] + [\mathrm{CBN}]}{[\mathrm{CBD}]}$$

1988). To date, 52 hemp cultivars that fall below the 0.3% legal limit for hemp have been approved for commercial use by the European Union (Directive 2013). These registered varieties originate from high latitude European nations, which are not acclimated for equatorial regions of the world. Little is known about the viability of the European cultivars in the United States, and this is currently being examined by agronomists. Several high CBD strains have come online in the United States in recent years that are being legally cultivated under the Farm Bill. Some of these strains have CBD contents above 15% and render traditional European cultivars obsolete for oil and resin production.

Over recent decades the cannabis gene pool has undergone selective pressure to maximize THC production at the expense of CBD and other minor or non-psychoactive cannabinoids. The observation that different high THC cannabis cultivars produce a variety of effects prompted researchers to look further into the other chemical constituents that may be impacting the therapeutic effects. It turns out that there is a wide spectrum of effects encountered when different terpene profiles are administered with THC. This has been a major focus of research as of late, and there are numerous publications that demonstrate the variability and unique signatures of terpene profiles in differing cultivars (Fischedick et al. 2010; Henry 2017; Lewis et al. 2018).

5.3 Cannabimimetics: Cannabinoids from non-*C. sativa* Species

Aside from cannabis, there are a variety of other plants that produce compounds that interact with the body's ECS. These compounds are classified as cannabimimetics or cannabinoid-mimicking compounds. Some of the plants that produce cannabimimetics are widely used in herbal medicine, and some are even part of our regular diet.

There is a class of lipid compounds that is widespread in the plant kingdom called *N*-acylethanolamines (NAE). Rather than directly binding to the body's cannabinoid receptors, these compounds inhibit the actions of the eCB-degrading enzymes such as the fatty acid amide hydrolase (FAAH) (Gertsch 2017). There is also a triterpenoid compound called pristimerin found in a family of shrubs called Celastraceae that reversibly inhibits another eCB-degrading enzyme, monoacylglycerol lipase (MAGL) (King et al. 2009). These compounds are significant because this type of activity indicates that their presence can modify eCB tone. Newer research on compounds with similar structural components suggests that *N*-alkylamides and their derivatives could also interact with the putative third cannabinoid receptor GPR55 (Dalle Carbonare et al. 2008).

Studies on *black truffles* have shown that eCBs are not just unique to mammals. The enzymes required for producing anandamide as well as the compound itself are found in certain species of truffles (Pacioni et al. 2015).

Researchers out of Zurich have been investigating a class of compounds in *Echinacea* called *N*-alkylamides, which possess immunomodulatory properties (Gertsch et al. 2008b; Raduner et al. 2006). In particular, *Echinacea* roots produce endocannabinoid-like molecules that have been shown to bind to cannabinoid receptors in rodents (Woelkart and Bauer 2007). Specifically, *N*-isobutylamide has been shown to bind to both cannabinoid receptors but shows preference for CB_2. Its actions there have been shown to cause a decrease in the expression of the pro-inflammatory mediator TNF-α (Gertsch et al. 2004).

South African *Helichrysum umbraculigerum* has been shown to contain CBGA, CBG, and other prenylated dibenzyls similar to cannabinoids in *C. sativa*. Cannabinoid-like molecules derived from *Helichrysum* and other Asteracea genera generally have a phenethyl group at the *n*-pentyl position and have been used in traditional medicine to treat a host of inflammation and infections (Pollastro et al. 2017).

Piper methysticum also known as *kava kava* contains yangonin, a kavalactone that displays significant binding affinity for CB_1 (Russo 2016a, b).

Liverwort (*Radula marginata*) has also been shown to produce a compound called perrottetinenic acid, which closely resembles Δ^9-THC (Toyota et al. 2002).

The Chinese *Rhododendron has* also been shown to produce compounds that are structurally similar to the cannabinoids CBC, CBL, and cannabicitran, although their pharmacological activities at cannabinoid receptors have yet to be explored (Iwata and Kitanaka 2011).

5.4 Cannabis Terpenes

Terpenes are the aromatic compounds in cannabis that contribute to its unique scents and aromas. Over 100 different mono-, di-, and sesquiterpene compounds are produced within the resinous trichomes of cannabis flowers. Terpenes are produced alongside cannabinoids, which share common biosynthetic pathways and intermediates (Fig. 7).

Terpenes are thought to modulate the therapeutic effects of the cannabinoids and are termed the ensemble effect, a reference in literature to a large body of evidence suggesting that whole plant cannabis is more effective than isolated cannabinoid compounds by themselves. The complex aromatic profile of terpenes is often a distinguishing factor between cannabis strains and contribute the pluripotent physiological effects. Despite the fact that the terpene profile can

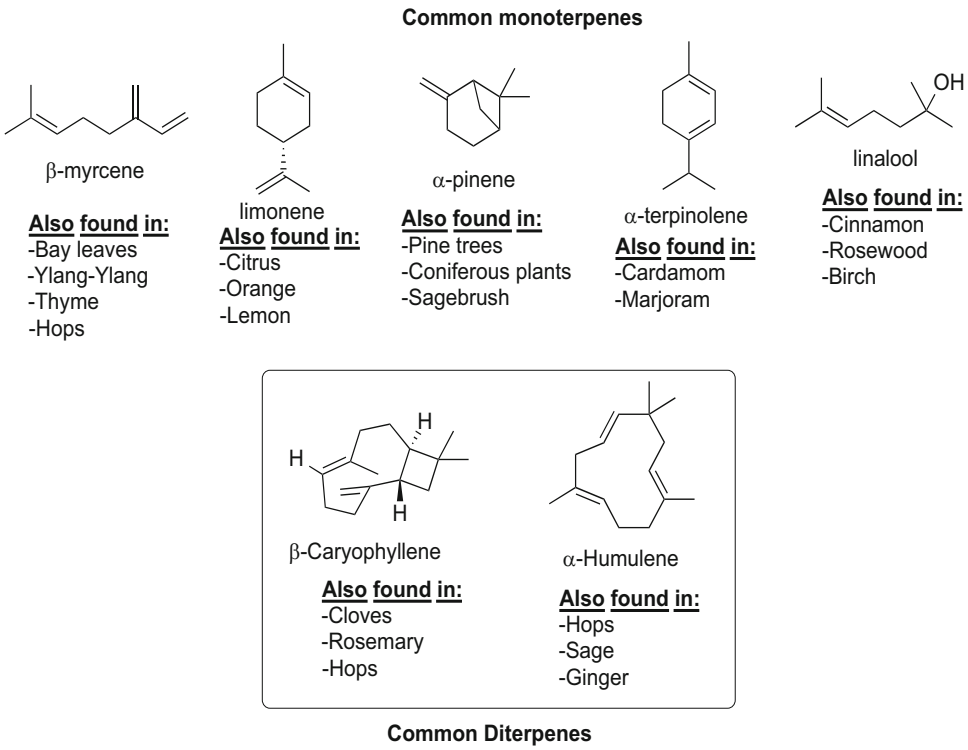


Fig. 7 Showcases five common monoterpenes and two diterpenes found in *Cannabis sativa*

make a cannabis strain unique, terpenes themselves are commonly found in other plants. In fact, many of these terpenes have been used in aromatherapy for millennia. In this section, we will cover five of the most common monoterpenes and two common diterpenes.

β-Caryophyllene is the primary sesquiterpene in black pepper that contributes to its spicy flavor. It is also a major constituent of cloves, hops, rosemary, and, of course, cannabis. Certain strains of cannabis such as Girl Scout Cookies can have levels of β-caryophyllene as high as 5% by mass. It is unique in that it has been shown to directly interact and bind with the CB_2 receptor, making it one of the most ubiquitous non-cannabis cannabinoids found in nature. CB_2, particularly in peripheral tissues in the body, is a therapeutic target for treatment of inflammation, pain, atherosclerosis, and osteoporosis (Gertsch et al. 2008a, b). β-Caryophyllene itself has shown promising results in animal models for colitis, osteoarthritis, diabetes, anxiety, depression, liver fibrosis, and cerebral ischemia. In support of the entourage effect hypothesis, co-administration of β-caryophyllene with the chemotherapy drug Paclitaxel on in vitro cancer cells stimulated increased cancer cell death and decreased tumor growth. β-Caryophyllene has received GRAS status by the FDA, meaning it is generally recognized as safe for oral consumption in humans.

Myrcene is known as the active sedating principle of hops, lemon grass, basil, and mangoes. *Myrcia sphaerocarpa*, from which myrcene is derived, is a medicinal shrub from Brazil traditionally used to treat diabetes, diarrhea, dysentery, and hypertension (Ulbricht 2011). Myrcene's earthy, fruity, and clove-like aroma is pungent in higher concentrations and commonly used in culinary and perfume preparations. Myrcene has been shown to enhance transdermal absorption (Schmitt et al. 2009). It also has a significant analgesic effect, which is blocked by the action of naloxone, an opioid antagonist, suggesting a mechanism of action through the opioid receptor (Rao et al. 1990). Myrcene lacks affinity for opioid receptors pointing to alpha 2-adrenoceptor-stimulated release of endogenous opiates. No tolerance was observed after repeated dosing in rats, which is in contrast to morphine (Lorenzetti et al. 1991). Myrcene was a sedative comparable to phenobarbital at very high doses in rats (Do Vale et al. 2002), an effect increased by simultaneous administration of citral, a mixture of other terpenes. Myrcene was also shown to improve glucose tolerance in alloxan diabetic rats comparable to metformin (Al-Omari 2007), without an effect on glucose levels in normal rats. Further, myrcene showed powerful anti-inflammatory and anti-catabolic effects in a human chondrocyte model of osteoarthritis (Rufino et al. 2015). Myrcene is the subject of a broad array of current research given that inflammation is the underlying cause of numerous diseases.

Limonene is one of the most abundant terpenes in cannabis and has been reported at concentrations up to 16% of the essential oil fraction. Limonene is a monoterpene commonly found in citrus rind and used in perfumes, household cleaners, food, and medicines. Limonene has numerous potential medicinal benefits demonstrated in human and

animal studies. Limonene's antioxidant and anticancer properties make it an excellent dietary source for cancer prevention (Aggarwal and Shishodia 2006). Multiple modes of anticancer activity and chemoprevention were observed for limonene (Crowell and Gould 1994). Perillyl alcohol is a metabolic product or limonene, which is also a subject of numerous cancer-related studies (Prates Ong et al. 2012). Anti-inflammatory effects in models of osteoarthritis (Rufino et al. 2015) and asthma have also been observed (Hirota et al. 2012). Anxiolytic effects in a mouse maze model were not antagonized by flumazenil and comparable to diazepam, implying a non-benzodiazepine biological target (Lima et al. 2013). Earlier results which also demonstrated antidepressant activity via the 5-HT_{1A} receptor pathway (Komiya et al. 2006) are in contrast to anxiolytic effects in the mouse maze model.

Humulene is the characteristic terpene of hops, *Humulus lupulus*, that is also abundant in cannabis. It is also found in sage and ginseng among other plant species. Humulene, also known as α-caryophyllene, is a ring-opened isomer of β-caryophyllene, which lacks in the CB_2 activity of the latter. β-Caryophyllene and humulene have been shown to interact in a synergistic manner in one study (Legault and Pichette 2007). Humulene also possesses powerful anti-inflammatory activity, equal to dexamethasone in an animal model (Fernandes et al. 2007). Both topical and systemic anti-inflammatory properties (Chaves et al. 2008) as well as topical, oral, and aerosol delivery methods as an analgesic have also been observed for humulene (Rogerio et al. 2009). It also has the potential to aid in wound healing (Satsu et al. 2004) from the increase secretion of IL-8, a chemokine with various functions, including promoting angiogenesis.

α-Pinene is a terpene commonly found in pine needles, and its pharmacological properties have been well studied. In particular, α-pinene acts as an acetylcholinesterase (AChE) inhibitor which may aid in memory function (Miyazawa and Yamafuji 2005). It also exhibits anti-inflammatory properties through numerous pathways decreasing levels of interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-α). It also reduces prostaglandin synthesis through its potent inhibition of prostaglandin E_1 (PGE_1) and like many terpenes also exhibits antimicrobial effects (Nissen et al. 2010; Russo 2011).

Terpinolene carries a woody and floral odor and is commonly found in "sativa" varieties of cannabis. Studies in mice carried out using *Microtoena patchoulii* which has terpinolene as a primary active constituent show that it exhibits sedating properties (Ito and Ito 2011). It also exhibits anticancer properties in the brain cells of rats (Aydin et al. 2013).

Linalool is a terpene commonly found in lavender that produces antianxiety and antidepressant effects. Studies performed in mice suggest that these properties are primarily due to its action on monoaminergic pathways in particular the serotonin receptor 5-HT1A (Chioca et al. 2013; Guzmán-Gutiérrez et al. 2015).

5.5 The Entourage/Ensemble Effect in Cannabis: Why It Matters

The idea of an entourage or ensemble effect in eCB signaling was first proposed by Ben-Shabat in 1998. Since then there have been numerous studies that support this theory. Ben-Shabat's initial work demonstrated this phenomenon with typically inactive fatty acids in the presence of eCBs. A few years later in 2001, McPartland and Russo published a paper titled "Cannabis and Cannabis Extracts: Greater Than the Sum of Their Parts" which extended this entourage concept presenting early evidence that terpenes enhanced the effects of the cannabinoids (McPartland et al. 2001). This work was further refined later by Russo in August of 2011 when he published "Taming THC: Potential cannabis synergy and phytocannabinoid terpene entourage effects" in the *British Journal of Pharmacology* (Russo 2011). This paper proposed that the terpenes in cannabis were capable of modulating the effects of cannabinoids and offered many possible mechanisms by which terpenes could induce this action.

Terpenoids are potent modulators of consciousness and have been used for thousands of years in aromatherapy for a number of conditions. Recent work using essential oils derived from cannabis void of any cannabinoids illustrates such effects where results revealed decreased diastolic blood pressure, increased heart rate, and significant increased skin temperature. There were also changes in the amplitude of alpha, delta, and theta brain waves (Gulluni et al. 2018).

More recent work confirms the concept of an ensemble effect. One such study demonstrates that CBD administered in a whole plant extract rather than an isolated form allows for the bell-shaped dose-response curve to be overcome and results in greatly enhanced therapeutic efficacy in the context of TNF-α production (Gallily et al. 2015).

A number of studies have found that although there is definite clinical potency to the isolated phytocannabinoids such as THC or CBD, when they are provided in a whole plant extract containing terpenes, flavonoids, and other cannabinoids, they have a greater effect. Romano et al. (2014) found that a standardized cannabis extract with a high content of CBD was able to inhibit colon carcinogenesis both in vitro and in vivo. It was found that the whole plant extract reduced cell proliferation in cancerous but not in healthy cells. Pure CBD reduced cell proliferation in a CB_1-sensitive antagonist manner only. In vivo, plant-based CBD reduced the carcinogen azoxymethane (AOM)-induced preneoplastic lesions and polyps as well as tumor growth in

the xenograft model of colon cancer. The authors concluded that plant-based CBD in the whole plant context attenuates colon carcinogenesis and inhibits colorectal cancer cell proliferation via CB_1 and CB_2 receptor activation (Romano et al. 2014).

6 Animal Studies and Veterinary Clinical Trials

For legal reasons, although cannabis for medical uses has been applied in an uncontrolled (and possibly illegal) fashion to pets and farm animals for many years, going back as far as the mid-1800s, there has been no basic, fundamental research into the safety, efficacy, or pharmacokinetics of phytocannabinoids, and specifically CBD, in our domestic or wild species of animals. With the federal legalization of hemp in the 2014 Farm Bill, as mentioned previously, there has been a loosening of the restrictions on research in states that have legal hemp cultivation and commercialization.

As a result of this legal loosening of cannabis research, several veterinary academic institutions have completed or nearly completed studies into the safety of high-dose, long-term use (6 weeks) of CBD in the dog, the relative pharmacokinetics of three different avenues of administration (transmucosal, oral, and transdermal), and the efficacy of CBD use for osteoarthritic pain and refractory epilepsy at a specific dosage. The efficacy studies are prospective, double-blind, placebo-controlled, randomized clinical trials. Additionally, several pet owner surveys/studies have been completed. One has been published in a peer-reviewed journal, and the second has been reviewed and is scheduled for publication later in 2018 by the same peer-reviewed journal. These surveys are from Colorado State University's College of Veterinary Medicine. A summary of the study results from the CSU comparative pharmacokinetics study is reported here. The safety and efficacy studies for epilepsy and osteoarthritis will be published later in 2018. The preliminary data from the two studies are reported here based on presentation of this data from conference proceedings.

Cornell University's College of Veterinary Medicine has just released a pharmacokinetics, safety, and efficacy study of CBD used in dogs with osteoarthritis. This document was presented at the American College of Veterinary Sports Medicine and Rehabilitation track at the World Rehabilitation Summit (IAVRPT) in Knoxville, TN, July 31, 2018. This study's results are reported in this chapter as well. Both the Cornell and CSU studies used "macro-dosages," which are substantially higher (4–5 times) than have been reported as effective by veterinarians and pet owners based on anecdotal and observational data.

6.1 Pet Owner Experiences with Hemp Products

Kogan et al. (2015, 2018) from Colorado State University conducted a survey of pet owners who had visited an e-commerce site to purchase animal hemp products. The survey was intended to inform future research of the best veterinary applications for low THC cannabis, otherwise known as "industrial hemp." This study was designed to determine what hemp products pet owners are purchasing, reasons for their purchases, and the perceived value of these products on their pet's health. An anonymous survey was given to pet owners who bought products from a single online hemp company. A total of 632 responses were recorded, and 58.8% of these indicated they currently use a hemp product for their dog. Most dog owners (77.6%) indicated they use the product for an illness or condition diagnosed by a veterinarian, with the most common conditions including seizures, cancer, anxiety, and arthritis.

Of the total responses, fewer participants indicated they currently use the hemp products for their cat (11.93%), with 81.8% indicating they use the product for an illness or condition diagnosed by a veterinarian. The most common conditions being addressed by the hemp products included cancer, anxiety, and arthritis. This study provides a guide to researchers seeking to perform clinical studies on hemp in terms of its best areas of efficacy and potential adverse outcomes with its use. The authors suggest, for this first evidence-based publication in the veterinary realm of the clinical effects of cannabinoids, to consider controlled clinical trials in areas that include pain management, behavioral interventions for sleep disorders and anxiety for dogs, inflammation reduction, and improvement in sleep patterns for cats (Kogan et al. 2015).

6.2 Demographics and Dog Owner Perceptions of Cannabis

In a follow-up questionnaire-based study to the 2015 publication cited above, Kogan et al. created a second online survey to assess US perception regarding hemp and marijuana products for their dogs. The survey originated from Colorado State University in collaboration with investigators at North Carolina State University. Survey participants were recruited via social media in late 2017. The data was collected anonymously. Dog owners residing in the United States were the only inclusion factors used in this study. Survey participants were asked for their demographics; the state they lived in was compared to states that currently have medical marijuana laws permitting legal use of marijuana for

medical and/or recreational purposes. Further questioning determined if they had given their pets a marijuana product or not and the reasons for use or nonuse of it. Finally, survey participants were asked for what reasons they had given the marijuana products to their pets.

A total of 1196 responses were collected, and after eliminating non-dog owners or non-US residents, there were 1068 responses. Eighty-four percent were female, the remainder, male. Eighty percent of respondents had used cannabis for their dogs. Those that did not use cannabis cited no medical reasons to do so or weren't aware that it could help. Pain relief, reduction of anxiety, and reduction of inflammation were the most common reasons for the use of cannabis in dogs in this study. Other reasons (total 36%) less commonly selected were for epilepsy (11.5%), cancer (9.4%), arthritis (1.9%), and allergies (1.3%)

The type of cannabis product most commonly used in the study were capsules or pills marketed for animals (57%), as compared to capsules or pills marketed for humans (3.9%). For edibles this same trend was seen, with more animal-labeled products being used in dogs than edibles labeled for people. No mention was made in this study of the most commonly used format which is the liquid oil infusion, also known as the "tincture." Side effects associated with the use of cannabis in dogs were reported only by a minority of dog owners (<5%), with the most common adverse effects being sedation, xerostomia, and associated polydipsia.

6.3 Safety of High-Dose Long-Term Exposure to CBD in Dogs

A study evaluating the safety of cannabidiol in the dog was published in the Fall of 2018. It was conducted at Colorado State University's College of Veterinary Medicine, Department of Neurology. The principal investigator, Dr. Stephanie McGrath, who is a veterinary neurologist and Assistant Professor at CSU's Veterinary Teaching Hospital, conducted a 6-week high-dose evaluation of the tolerability of two high doses of CBD in healthy beagle dogs. A sample population of 30 healthy beagle dogs were randomly assigned to receive 1 of 3 formulations: microencapsulated oil beads, CBD-infused oil, or CBD-infused transdermal cream for 6 weeks. Two dosage tiers were evaluated in this study, 10 mg/kg/day and 20 mg/kg/day. These dosages far exceed the dosages used in the two efficacy studies that followed this by a factor of 2× and 4× greater. The two efficacy studies evaluated the use of CBD for refractory epilepsy and osteoarthritis in the dog.

Complete blood counts, chemistry panels, urinalysis, and pre- and postprandial bile acids were performed at 0, 2, 4, and 6 weeks. Elevations in alkaline phosphatase double the high end of the reference range (140 IU/L) were observed in some dogs (11/30:36%) after being on the CBD for 4 weeks, although it did elevate in some dogs at 2 weeks, especially at the higher dosing tier. Long-term liver toxicity was not evaluated in this study, although bile acids and liver enzymes remained normal for all dogs throughout the study. None of the dogs receiving the transdermal formulation developed elevated alkaline phosphatase values. All dogs experienced mild diarrhea, although there was no correlation with formulation or dose. Six out of the 30 dogs developed vomiting, but there was no significant difference between the occurrence of vomiting and CBD dose or formulation.

Erythematous pinnae were the next most commonly reported clinical sign in this study. These otic changes were seen in 36% of dogs with the otic changes becoming more severe after 2 weeks in the 10 mg/kg/day dosage group for all three formulations. The transdermal cream had more incidences of otic changes than either the transmucosal or oral routes of administration, which is understandable since the transdermal crème was applied to the inside of the pinna. Less common findings included ocular discharge in 10/30 dogs (33%) and nasal discharge in 10/30 dogs (33%). Five dogs (17%) had salivary staining on their feet and occasionally on their ventral abdomen. Two dogs had spontaneous prolapsed glands of the nictitans. One dog had a transient elevated body temperature (104.2 °F). It was also observed that some dogs would salivate following administration of the CBD-infused oil formulations at both doses. The study concludes that CBD seems to be well tolerated in the dog at these high dosages but emphasizes that a larger and longer in duration safety study is needed to evaluate the very long-term effect of CBD on the liver and its association with diarrhea (McGrath et al. 2018).

6.4 Pharmacokinetics, Safety, and Clinical Efficacy of CBD Treatment in Osteoarthritic Dogs

The objectives of a recent Cornell study were to determine the oral pharmacokinetics and safety, as well as analgesic efficacy, of using CBD in dogs with osteoarthritis (OA). Single-dose pharmacokinetics were performed using two different doses of 2 mg/kg and 8 mg/kg of CBD in a carrier oil. From this data, a prospective, randomized, placebo-controlled, double-blind crossover study was conducted using 16 client-owned dogs with radiographically confirmed evidence of osteoarthritis who were enrolled and who completed this study. Dogs were randomized to receive either 2 mg/kg q 12 h orally of CBD oil or a placebo consisting of olive oil with a benign herbal extract at a similar volume q 12 h for 4 weeks. Subjects were given a 2-week washout period, and then the treatments were crossed over, and each subject received the other treatment twice daily for 4 weeks.

Veterinary assessment of lameness, movement, and response to manipulation, owner questionnaires (Canine Brief Pain Inventory (CBPI), Hudson activity scale), objective kinetic analysis on a pressure-sensitive walkway, hematology, and chemistry analysis were obtained at weeks 0, 2, and 4 for both oils. Statistical analysis was performed on the results, with a $p < 0.05$ considered to be significant.

Pharmacokinetics showed a half-life of elimination of 4–5 h at both doses and no observable side effects. Median maximum concentration of CBD oil was 102 ng/ml (61–132 ng/ml), and this peak was reached at 90 min following administration of the single dose of 2 mg/kg. The investigators on this study decided that since the pharmacokinetics of the 2 mg and 8 mg doses were so similar, they would use the lower of the two doses for the efficacy wing of this study.

Assessment of pain and mobility showed a significant decrease in pain and increase in activity ($p < 0.001$) at weeks 2 and 4 during CBD treatment as compared to baseline at each bi-weekly evaluation. It was found that the CBD oil resulted in reduced pain scores when compared to baseline on both bi-weekly examinations ($p = 0.03$). No side effects were reported by owners, but serum chemistry demonstrated an increase in serum alkaline phosphatase (9/16 dogs, 56%) while receiving the CBD oil, which reached significance at week 4 ($p < 0.005$).

The authors of this study conclude that the dogs with OA who received 2 mg/kg q 12 h were found to be more comfortable and active with very few undesirable side effects compared to placebo. The authors note that the CBD oil used in this study was a "strain-specific" extract and other products that do not have this same strain specificity may not have the same efficacy as measured for this proprietary product (Gamble et al. 2018).

6.5 Evaluation of Trends in Marijuana Toxicosis in Dogs Living in a State with Legalized Medical Marijuana: 125 Dogs (2005–2010)

This study correlated the number of medical marijuana licenses issued in the state of Colorado between 2005 and 2010 and the number of admissions to the ER for marijuana toxicosis. This is a retrospective case series from January 1, 2005, to October 1, 2010. A total of 125 client-owned dogs presented for known or suspected marijuana toxicosis with or without a urine drug screening test (UDST).

A significant correlation was found between the number of medical marijuana licenses and marijuana toxicosis cases seen in two veterinary hospitals in Colorado. Ingestion of baked goods made with high potency THC butter extractions resulted in two recorded deaths. The authors note that due to the difference in urine metabolites in the dog as compared to the human, the UDST test may not be valid in dogs. It is also important to note here that there have been no recorded deaths in the many hundreds of years of human use of marijuana. These two canine deaths were due to the toxicity of chocolate in dogs combined with the cardiovascular effects of THC on the myocardium. In each case, brownies or chocolate chip cookies were made with very large amounts of THC butter, and the two dogs, each rather small, ate very large quantities of the chocolate with cannabis. The authors were not able to attribute both deaths to the THC alone and felt there was a major influence of the chocolate contributing to their deaths (Meola et al. 2012).

6.6 Comparative Pharmacokinetic Study of Three Routes of Administration of CBD in the Beagle Dog

This study was designed to determine the pharmacokinetics of CBD in healthy dogs. A sample population of 30 healthy research dogs were assigned to receive 1 of 3 different formulations at a dose of 75 or 150 mg q12 h for 6 weeks. The dosage formats were (1) liquid oil infusion administered to the oral mucosa; (2) oral capsules with microencapsulated oil beads; and (3) transdermal application. Serial CBD plasma concentrations were measured over the first 12 hours and repeated at 2, 4, and 6 weeks. Greater plasma concentrations were measured with the oral CBD oil-infused formulation. The plasma half-life of CBD administered via this route after 75 mg and 150 mg doses, respectively, was 199.7 +/− 55.0 and 127.5 +/− 32.2 min. This study found that blood levels are dose proportional, as expected, and the oral liquid CBD absorbed transmucosally was the superior formulation of the three formulations tested, with orally administered microencapsulated beads the second-best formulation in terms of pharmacokinetic profile (Bartner et al. 2018).

6.7 Clinical Efficacy of CBD for Treating Osteoarthritis and Refractory Epilepsy in the Dog: A Pilot Study

The osteoarthritis (OA) wing of this efficacy study consisted of 24 client-owned dogs with clinical evidence of OA radiographically and who had an identifiable lameness. A double-blinded, randomized, placebo-controlled, study design was utilized, with each study group receiving medication for 6 weeks and a placebo for 6 weeks. The treatment group received 2.5 mg of CBD oil q 12 h. Gait analysis and an

activity monitor were used to gain objective data, and a behavioral questionnaire was given to the dog owners which provided subjective information. The study results for OA were not yet available at the time of this publication (McGrath 2018).

The epilepsy segment of the study consisted of 16 client-owned dogs who were diagnosed with idiopathic refractory epilepsy, having 2 or more breakthrough seizures per month while receiving conventional anticonvulsant therapies. Inclusion criteria included a normal neurologic exam and a normal epilepsy workup with an MRI and CSF analysis. Nine dogs were randomly assigned to the treatment group and seven to the control (placebo) group. The treatment group received 2.5 mg/kg CBD oil q 12 hours by mouth. The control group received placebo oil for 12 weeks. Study subjects were required to stay on their standard anticonvulsant drugs (AED). Routine blood work and CBD levels were determined every 4 weeks. AED levels were measured at the conclusion of the trial.

Sixty-seven percent (6/9) of the dogs in the treatment group experienced a greater than 40% reduction in average monthly seizures during the study, whereas only 29% (2/7) of the dogs in the control group had a greater than 40% reduction in average monthly seizures.

Elevations in alkaline phosphatase (ALP) were recorded for the treatment group and one dog in the control group. The single control dog had previously measured elevations in ALP, so this elevation was not considered to be relevant to the study. Six dogs (67%) in the treatment group had elevations in ALP measured at the end of the study. The mean ALP value was 619 IU/L (range 15–140 IU/L).

AED concentrations in the treatment group for phenobarbital decreased in 2/7 dogs (29%) and increased in 5/7 dogs (71%). In the control group, phenobarbital levels decreased in 3/5 dogs (60%) and increased in 2/5 dogs (40%); there was no significant change in either group. This is an interesting finding to note, because there has been a concern that CBD, which is metabolized through the P450 group of cytochromes, might interfere with the drug disposition of pharmaceuticals that also are metabolized through that pathway. From the results of this pilot study, that effect is not apparent, at least with respect to phenobarbital levels.

Potassium bromide (KBR) levels in the treatment group decreased in 2/3 dogs (67%) and increased in 1/3 dogs (33%). In the control group, KBR levels decreased in one out of two dogs (50%) and increased in one out of two dogs (50%). There was no significant change in either group, although the total number of study subjects was low in this pilot study. This research and the osteoarthritis section of this study have not yet been published, pending the results of the plasma analysis of cannabinoid levels that were measured at 0, 4, 8, and 12 weeks and the completion of the efficacy study of the effects of CBD on osteoarthritis.

The American Kennel Club Canine Health Foundation has granted nearly $400,000 in funding to this research group at CSU for a larger, expanded study with 60 dogs, as a result of the positive results of this pilot work, with respect to the use of CBD oil to address refractory epilepsy. This study will also be looking at uncontrolled epileptics having two or more seizures per month while receiving standard therapy. In this expanded study, which will use a crossover design, each subject will receive 12 weeks of treatment or placebo with a 4-week washout period between treatments. This study began in January 2018 and is currently enrolling patients (McGrath 2018).

7 Guidance on Veterinary Cannabis Products in the US Market

Cannabis preparations have historically been used for home remedies, as medicine, as a functional food, and as a source of nutrition, primarily for humans. However, there have been reports of cannabis products being used in animals dating back to the 1800s, when cannabis had become a popular herbal remedy at a time prior to the development of the pharmaceutical industry. Patent medicines containing cannabis, usually in an alcohol tincture, were sold to horse owners for colic and other equine ailments, and topical liniments were used externally for joint and lameness problems.

As cannabis became vilified and stigmatized in the 1930s, and as pharmaceutical medicines became more available as substitutes for cannabis products, the use of cannabis in the horse became less common to nonexistent. For our companion animal species, dogs, cats, and horses, the use of cannabis products did not gain popularity until the state-by-state legalization of cannabis for medicinal purposes began in the mid-1990s. Pet owners, learning of the many benefits that cannabis has created for people, naturally began to explore its use in their pets for the medical problems that were not easily solved by conventional veterinary medicine. These are problems such as epilepsy that are resistant to pharmaceutical remediation; pain that is poorly responsive to opiates, steroids, and nonsteroidal anti-inflammatory drugs; cancer treatment side effects such as nausea and vomiting; and to treat cancer. Some veterinary patients respond well to NSAIDs for osteoarthritis, but the drugs themselves can cause toxicity in sensitive patients. The use of cannabis as an alternative means of remediating the pain of arthritis can substitute for these drugs or allow for lower and safer dosages of the pharmaceuticals. Several veterinary randomized placebo-controlled clinical trials that have been conducted have demonstrated objectively the benefit of cannabis for osteoarthritic pain in the dog (McGrath 2018; Gamble et al. 2018).

7.1 Cannabis Product Formats and Delivery Methods

There are several formats of cannabis products for companion animals, currently in the marketplace, and several up-and-coming formats currently undergoing research and development efforts by forward-thinking companies. The route of administration is a primary contributing factor in the effectiveness of any medicine, natural or otherwise. Choosing the appropriate delivery method for the patient can greatly affect medical outcomes. The associated bioavailability of the drug should be balanced with the practical needs of the patient.

Dogs have proven to be highly sensitive to the adverse neurological effects of the psychoactive decarboxylated THC. They can benefit from the use of the non-decarboxylated non-psychoactive THCA, without any fear of a visit to the veterinary ER. THCA, although not psychoactive, is still a Schedule I controlled substance according to the Drug Enforcement Agency. Although the THCA does not bind to CB_1 receptors in the brain, which is why it is psychoactive, it is regulated through many other entourage non-cannabinoid receptor-mediated pathways.

Juicing of cannabis leaves is gaining popularity as a method of preparation and consumption. The cold extraction of raw cannabis preserves the heat-sensitive acidic cannabinoids THCA and CBDA that readily undergo degradation through the decarboxylation pathway. Decarboxylation typically occurs at temperatures above 100 °C that are common for combustion (smoking), vaporization, or hot extraction. THCA has a significantly lower affinity for the endogenous cannabinoid receptor CB_1 and does not have the same psychoactive effects as preparations containing "activated" of "decarboxylated" THC. Consumption of unheated cannabis preparations allows for the administration of significantly higher doses of acidic cannabinoids, without the psychoactive effects of THC. Juicing of raw cannabis would have the advantage of retaining volatile terpenes, which also have important medicinal properties. Original terpene profiles are generally altered in extraction methods involving heat.

7.1.1 Oral Route of Administration

Ingested route is very common and still quite effective, in spite of its poor bioavailability. Pharmacokinetic (pK) studies documenting the bioavailability of oral cannabis extracts, specifically the major cannabinoids THC and CBD in both humans and dogs, demonstrate that only 5–10% of the lipophilic drug reaches its intended active site. In spite of this relatively poor bioavailability, good clinical responses have been documented in both species. pK studies documenting the bioavailability of cannabinoids in the equine species remain to be performed, as well as in other veterinary species. The majority of products currently available for animal species are targeted toward ingestible formulated products. Some examples of these formulations are highlighted below.

Capsules—The first animal companion cannabis product to be launched in the United States, shortly after the federal legalization of low THC cannabis ("hemp"), contained powdered hemp seed meal infused with the lipophilic extracts of the cannabis plant and was manufactured into capsules. Capsules contain a fixed volume of active materials and are limited in efficacy to specific weight ranges of patients. This limitation necessitates manufacturing and packaging multiple capsule sizes to accommodate the many different sizes of veterinary species, Chihuahua to Great Dane to draft horse. Capsules are convenient to keep and administer and are a dosage-form that is familiar to most consumers. They can be hidden in a small amount of food to facilitate absorption on an empty stomach, but they are also subjected to rapid first pass liver metabolism and, based on the published pK study from Colorado State University, are less bioavailable than a lipophilic liquid absorbed through the oral mucosa (Bartner et al. 2018).

Tinctures—The second cannabis product format introduced to the US animal health market was a product that has become commonly known in the cannabis industry as a "tincture." These are oil infusions of lipophilic extracts of the cannabis plant and can contain either high THC cannabis ("marijuana") or low THC cannabis ("hemp"). Carrier oils can be any oil, but most commonly grape seed oil, hemp seed oil, and medium-chain triglycerides are used in these formulations. It's worth mentioning that the word "tincture" was derived from botanical medicine and in that context denotes an ethanolic extract of a botanical material.

Tinctures can contain just cannabis or can be compounded with additional terpenes and other botanicals and nutraceuticals or even pharmaceuticals for a more targeted effect. Tincture labels should include the potency of the formulation in total milligrams of CBD and THC and other measurable levels of cannabinoids, as well as listing the other amounts of the active ingredients in the bottle, and any preservatives that have been added to improve shelf life. Tinctures have the advantage of being scalable in terms of being able to dose different sizes of animals, since all that is needed for a larger or smaller patient would be more or less volume, respectively. Tinctures can also be added or mixed with a small amount of tasty food to facilitate administration but are most efficiently absorbed transmucosally from the oral cavity.

A pharmacokinetic study comparing the bioavailability of transmucosal, oral, and transdermal routes of administration for CBD was recently completed (Bartner et al. 2018). It was

found that transmucosal administration of a lipophilic liquid produced the highest C_{max} values in this study. Thus, the oil-based proprietary tincture in this study, applied transmucosally to the oral mucous membranes, gums, inner lips, tongue, and buccal pouch, would be the most bioavailable means of administration compared to the other two materials evaluated in this study.

Extended stability studies of cannabis products are needed to determine the shelf life of the formulation, which generally ranges from 12 to 24 months. This "outdating" should be expressed on the label as a manufacturing date or a "best by" date so as to inform the consumer of how recently the product has been manufactured. This dating of the formulation guides the consumer in selecting a product where its potency should meet label claims based on its aging over time and the subsequent gradual deterioration of the delicate active materials contained therein. For a patient who doesn't need much extract on a daily basis, a bottle containing a large amount of material could conceivably expire before it is finished.

Dosage-form animal health products are commonly used to administer pharmaceuticals and nutraceuticals. Treats that contain nutraceuticals or pharmaceuticals, aka "dosage-form treats," have become very popular among pet-owning consumers. Dosage-form treats need to have the intent, and be labeled, as nutraceuticals, not as nutritional compounds. Neither cannabis nor hemp are approved feed ingredients by the FDA-CVM and AAFCO. This means that the guaranteed analysis (GA) and a nutritional statement on the label and/or product package with total calories and calories from fat is not appropriate for a dosage-form treat and could cause a "stop sale" letter to be issued by the FDA-CVM for selling an adulterated nutritional product.

The label of dosage-form treats, based on NASC and FDA-CVM guidelines, needs to contain a listing in the order of decreasing weights of the materials that are the "active ingredients" and the "inactive ingredients." This second category would include the nutritional components of the treat on the label.

Hard biscuits and soft-chews currently are the most popular dosage-form treats available in the marketplace. Like capsules, these products can contain only a fixed amount of the active ingredients, thus limiting the weight ranges addressed by a single dosage-form treat size. The advantages of dosage-form treats are the relative ease of their administration. The potential disadvantages could be patient hypersensitivity to the ingredients in the treat, the need for multiple treats at a time to treat a larger weight patient, the reduced shelf life, and the potential that the manufacturing process that involves baking or heating and extruding the material could cause heat adulteration of the product. Postproduction product analyses are critical to ensure that product potency has not been compromised by the manufacturing process.

Pellets and powders are scalable for dosing a wide variety of target species weights. There are a few powders made from low THC cannabis ("hemp") available. These powders can be manufactured from the powdered plant material or from the pharmaceutical modification of the lipophilic extract of the plant material into a powder format or may use an inert powder that is infused with the lipophilic extract of the cannabis plant. For most of the applications for animals, the low THC variety of cannabis is the preferred raw material for manufacturing this powder due to the potential for problems with high THC cannabis both in regard to the patient response and as regards the stricter regulatory environment for high THC cannabis.

Powders can be contained in wide-mouthed bottles with a measuring scoop in them that can be used to estimate effective dosages. Standard kitchen measuring spoons can also be used to scoop out the appropriate dosage for the size and condition of the patient. Powders can be packaged into stand-up pouches, as well as sticks, sachets, and, of course, capsules. Individual serving sizes make sticks, sachets, and capsules more convenient for dosing but remove the scalability benefit of powder over dosage-form treats.

Pellets are pressed from dried cannabis plant material, most commonly using the low THC cannabis variety ("hemp"). These pellets can be given to horses, goats, sheep, and other farm animals, as well as poultry, swine, zoo animals, caged birds, and pocket pets. A big concern with giving horses cannabis, even low THC cannabis, is that there would be enough THC even in low THC cannabis that the horse would test positive for THC on drug testing and be disqualified from whatever event it was being tested for.

Water-soluble cannabinoids apply existing technology using lipid emulsifiers and reducing the size of the oil droplets to as small a size as possible, usually in the range of nanometers. Water solubility increases the plasma concentrations and helps the lipophilic drug reach the active site. Further, a rapid onset is observed with water-soluble formulations that do not have the same long-lasting effects. Although published pK studies comparing the bioavailability of lipophilic versus hydrophilic preparations of cannabinoids administered through the same route do not exist, there are credible pK studies using other lipophilic materials, such as Coenzyme Q10, which document the improved bioavailability to be as much as 5–8 times better than the lipophilic material with this approach. The impact of increased bioavailability on clinical outcomes remains to be measured as these water-soluble cannabinoids become more available; however, less cannabinoids are required in water-soluble formulations to achieve similar active site concentrations compared to oil-based solutions.

Transmucosal routes of administration can be very effective in most contexts, as it is fairly easy to apply the liquid cannabinoids to the oral mucous membranes. Bartner

measured the comparative bioavailability of transmucosal with oral and transdermal routes in 30 beagle dogs. The transdermal method of administration was 12–32% as bioavailable as the transmucosal approach between 75 mg and 150 mg/day, based on the C_{max} values. The oral approach was 55–68% as bioavailable as the transmucosal approach. It was concluded from this study that the most bioavailable administration avenue among these three dosage formats was the transmucosal approach (Bartner et al. 2018).

7.1.2 Topical Administration

Topical applications of cannabinoids have low systemic bioavailability but will penetrate locally to benefit the regional anatomy. For most veterinary species that have hair or feathers covering their bodies, "salves" and "balms" that are oil-based run the risk of matting the hair and interfering with the application to the epidermis where it will be absorbed locally. Products that are in solvents like alcohol or use emulsifiers to create a water-soluble cream or lotion have a better chance of penetrating the hair or feathers and ultimately penetrate the skin. These are termed "liniments" if they contain alcohol and "creams" or "lotions" if they are emulsified and water soluble.

Transdermal applications involve the use of a bipolar material such as a phospholipid to carry the cannabinoids into the local circulation and from that enter the systemic circulation. Transdermals can be creams or can be in "patches" that use a membrane to separate the transdermal solution from the skin. For veterinary species, transdermal liquids and patches will need to have a spot of hair clipped prior to application. Some anatomical sites that are hairless, such as the inside of the pinna and the ventral inguinal regions, do not need clipping and facilitate administration. It is recommended prior to reapplication of the transdermal cream to cleanse the site of application to improve absorption. Administration to the inside of the ear flap prevents the veterinary patient from licking the transdermal cream from an accessible area such as the inguinal region. Transdermal medication absorption is dependent upon the amount of local vascularity to carry the medication from the skin into the systemic circulation.

7.1.3 Inhalation

Inhalation is an impractical delivery method for veterinary species, except maybe primates or laboratory animals connected to breathing masks. As water-soluble versions of cannabinoids are developed, the possibility exists that a nebulization method could be employed to allow for absorption through the large surface area of the lungs. Inhalation methods are rapid onset and bypass first pass metabolism in the liver.

7.2 Zero-THC Hemp Extracts

Although low THC cannabis, which is called "hemp," already has less than 0.3% THC on a dry matter basis at the time of harvest, there are a number of situations where significantly reducing the amount of THC to undetectable levels has distinct advantages for veterinary species. Dogs are more sensitive to the adverse neurological effects of THC when they are naïve to exposure and have not developed tolerance. Some dogs are more sensitive than others, and anecdotal reports from veterinary clients (personal communication) indicate that there is a small percentage of dogs who react adversely to the small amount of THC present in hemp cultivars. For these dogs a zero-THC product would be advantageous.

Horses can have unpredictable responses to THC, potentially increasing the risk to the horse, the horse owner, and the veterinarian. Additionally, both horses and dogs in competitive events are commonly drug tested in order to qualify to compete. The use of a zero-THC product would make it less likely the animal would fail the drug test due to detectable levels of THC. Drug testing laboratories commonly test both urine and blood for many substances, including THC and CBD. Most venues will disqualify if any THC is detected (zero tolerance), but many do not disqualify for the detection of CBD. It is up to the individual event to decide what the disqualifying parameters are. The World Anti-Doping Agency (www.WADA-ama.org) has allowed the use of CBD in athletes since January 2018. It still disqualifies for THC, synthetic cannabinoids, and cannabimimetic agents. For this reason, Olympic athletes that are using a zero-THC source of CBD will not be disqualified, per the example of gold medal winner, Michael Phelps.

The veterinarian co-author of this chapter has distributed over 40,000 bottles of zero-THC tincture to veterinarians over the past 2 years. Reports from hundreds of these veterinarians have documented the clinical strength of the zero-THC formulation to reduce pain, improve mobility, bring some tumors into remission, and successfully address uncomplicated epilepsy, type 2 diabetes, and anxiety-related behavior problems such as noise phobias.

Prior to the discovery and use of all of the many non-THC cannabinoids in hemp, it was thought that THC was absolutely necessary for any clinical benefits. It is now known from these many cases that have successfully been treated with zero-THC extracts that the entourage effect does not need THC to be activated. In patients with reduced endocannabinoid tone, it may be necessary to use a small amount of THC to replace the endogenous ligand for better clinical response. Cases of severe pain and aggressive cancers may also benefit from THC, but for most other clinical cases, THC is not necessary.

7.3 Dosing Considerations and Strategies for Veterinary Species

The determination of evidence-based dosages through dosage-tiered Phase I studies in any veterinary species has yet to be conducted. Several of the randomized clinical trials recently published from veterinary academic institutions have used dosages extrapolated from published studies in human and laboratory animals, verified by single-dose pharmacokinetics to ensure detectable blood levels over a therapeutic period of time. These veterinary studies in dogs used a single dosage, which is quite high, but these high dosages did have statistically significant results with clinical improvement in response to clinical patients with OA and refractory epilepsy. However, there have not been any incremental dose-to-response studies performed to see if a lower dose might also benefit veterinary patients and be more cost-effective for the pet-owning public.

Dosages in the human patient have been derived both from published objective studies and from the clinical impressions of hundreds of physicians who have been trained in medical cannabis in the 29 states with medical cannabis legislation since 1996 when cannabis was first legalized for medical purposes in the United States (MacCallum and Russo 2018). Due to the legal considerations affecting veterinarians' clinical use of cannabis, there has not been this kind of hands-on practical empirical dosage-setting by veterinary clinicians. Instead, dosages have been empirically and anecdotally determined largely by pet owners and by a few veterinarians willing to risk their veterinary licenses to employ cannabinoids with their patients.

The data from veterinarians who have been recommending phytocannabinoids for their patients shows that veterinary species have a "biphasic response" to cannabis dosages in the same way that humans do. "Biphasic" means that a low dose (or "microdose") will generate one set of effects and address one set of clinical issues and a high dose (or "macrodose") will produce a second set of effects and address a second set of clinical issues.

Microdoses are considered to be less than 0.5 mg/kg BID of cannabinoids. Macrodoses would be greater than 2.0 mg/kg BID. Individual responses to dosage levels may vary. Based on anecdotal reports by veterinarians and pet owners who are using microdoses, the effective dosages used in dogs and cats have been reported to be as much as 40 times less than the macrodoses used in the CSU safety study or 5 times less than those used in the CSU efficacy study. There will be a small portion of animals that will develop the reported side effects of diarrhea, sedation, or elevated serum alkaline phosphatase, even with these lower doses.

McGrath evaluated the safety of administering high doses of CBD over a 6-week period. The results found that at the macrodose level (10–20 mg/kg/day), there were a greater frequency of adverse reactions to hemp extracts. Diarrhea, sedation, and serum alkaline phosphatase elevations were recorded in the subjects receiving 10 and 20 mg/kg/day (McGrath et al. 2018).

Tischler in his presentation at the Second Annual Conference at the Institute of Cannabis Research at Colorado State University in Pueblo discussed the value of microdoses in the human patient over the use of macrodoses. Microdosing serves to reduce or eliminate the psychotropic effect of THC through sub-psychotropic dosing and through the development of tolerance over a period of 2 weeks. Veterinary patients, for whom the use of THC is necessary and therapeutic (severe pain, appetite stimulation not effective with CBD, certain types of neoplasia), using the microdose strategy initially will facilitate the development of tolerance to the adverse effects of THC. Once this is achieved, THC dosages can be escalated to achieve the desired clinical benefit(s) (Tischler 2018).

In an unpublished study, the veterinarian co-author of this text gave 30 horses, in 3 different stables, dosages of 25–50 mg of CBD in a zero-THC hemp extract once or twice daily to address complaints of anxiety, gait abnormalities, mild to severe laminitis, and metabolic syndrome. Study subjects averaged around 1000 pounds. It was found that for anxiety and mild cases of lameness or gait abnormalities, administration of 25 mg once or twice daily was adequate to elicit a response with regard to anxiety from loading up into a trailer, or at events, or for mild gait abnormalities. In one stable the horses were only able to be given their dose once daily, yet that single dose still produced good clinical results.

For more severe conditions such as moderate laminitis, other sources of lameness, or metabolic syndrome, it was found that 50 mg once or twice daily was sufficient for clinical response. When horse owners were asked to discontinue giving the hemp extracts so as to determine withdrawal times for CBD, for situations in which the horses may be drug tested for an event, many refused to stop administration of the hemp, as they were very pleased with their horses' response to the hemp extracts. Horses have evolved to be very efficient in absorbing fats from their diet, as their natural diet of forage is very low in fats and oils. pK studies in the equine are very much needed to better understand dosing intervals and levels.

For most other animals, we use the dosage range established empirically through thousands of veterinary uses, starting with a low dosage and slowly increasing the dose over time. It is recommended to give a dose for 10–14 days to allow for the upregulation of CB receptors, and if after 2 weeks patient clinical response is inadequate, then increasing the dosage twofold greater than the starting dose may produce a better response in a given patient. Following through with dosage escalation, slowly over time, will yield the best clinical results. "Start low, go slow, stay low" is the mantra for dosing a patient whether human or veterinary,

and this protocol, combined with the safety and efficacy studies of McGrath, allows the practitioner to be able to provide a dosage as high as 20 mg/kg/day safely (MacCallum and Russo 2018; McGrath et al. 2018).

7.3.1 Ratio Dosing

Ratio dosing is the use of cannabis formulations that contain both THC and CBD in specific proportions to each other. The goal of this approach is twofold:

1. It allows for the development of tolerance to THC through the initial use of a high CBD to low THC ratio (THC:CBD/1:25) to avoid psychoactivity while tolerance to the adverse effects of THC is being established.
2. Once tolerance has been established, the dosage of the THC fraction in this combination formula can then be escalated safely. Escalation of the THC dose will also escalate the CBD dose administered, which may be the desired approach for a given patient's clinical needs.

If it is determined that a patient needs a higher amount of THC to provide better medical benefits but may not need as much CBD as escalating the THC dose in a low ratio formula would provide, then moving to a ratio formulation with an equal amount of THC:CBD may be more efficacious. Equivalent ratio formulations of THC:CBD (1:1) are being used empirically and effectively for moderate to severe pain and in oncology patients for whom the low THC ratio product does not provide sufficient antineoplastic activity or remediation of oncologic side effects from therapeutics or the disease itself.

In rare situations in veterinary species, it may be necessary to provide a high THC formulation for improved pain management or to better address the needs of the oncology patient. THC:CBD/4:1 is typically the ratio that is being used clinically. The need and use of this in veterinary patients has a higher potential for adverse effects than the lower THC ratio formulations. In all cases of using THC in veterinary species, it is important to develop tolerance first by using the low THC or THC-free products mentioned previously for the first 2 weeks of therapy to avoid adverse reactions or trips to the veterinary ER.

7.3.2 Adverse Events (AEs) and Dosage

McGrath reported that the CBD safety study determined that a dosage of 10 or 20 mg/kg/day of a full-spectrum CBD divided BID produced a greater incidence of adverse events than reported at lower dosages. Diarrhea and sedation were the two dominant AEs. Elevation of serum alkaline phosphatase levels was also observed to be significant events at these higher dosages. Other liver function tests, such as bile acids and ALT, were within normal limits (McGrath et al. 2018).

Sellers et al. (2013) found that the use of a 1:1 ratio of THC:CBD in their sublingual spray, Sativex™, was associated with significantly less AEs than the 100% pure synthetic THC capsule, Marinol™. This illustrates the harm reduction that comes with adding CBD to a formulation containing THC or a synthetic THC (Sellers et al. 2013).

Russo reported that the incidence of AE associated with the drug Sativex™, approved throughout the world for muscle spasticity with MS, was significantly less when the drug was gradually titrated to the patient's response. This study found that most AEs will be early and transient using a modification of the dosing strategy by starting at a lower dose increasing it slowly to achieve to clinical effect (Russo et al. 2011). In a later paper, MacCallum and Russo state, "Cannabis medicine doses must be individually determined, as (the effective dosage for a specific patient) . . .. depends on the endocannabinoid tone of that patient" (MacCallum and Russo 2018).

7.3.3 Drug Interactions with Cannabis

It is reported that in human medical marijuana patients, physicians have only rarely observed clinically significant drug interactions. MacCallum and Russo (2018) state, "there is no drug that cannot be used with cannabis, if necessary" (MacCallum and Russo 2018). Depending on the strength of the affinity of the metabolite for the metabolizing cytochrome, serum levels of cannabinoids or pharmaceuticals may increase with inhibitors or decrease with enzyme inducers. It is known that THC and CBD in human are oxidized by the p450 cytochromes (CYP2C9, 2C19, and 3A4). It is assumed that this is similar to the metabolic pathways in the dog and other veterinary species, but studies still need to be conducted in each species to detail possible interspecies variation.

In human patients the main AEs have been associated with the concomitant use of other CNS depressants with cannabis. High-dose CBD has been found in human to interfere with the metabolism of clobazam, thus potentially requiring a dose reduction of that drug when used with CBD (Devinsky et al. 2017). Drug interaction studies for each individual drug and cannabinoid are lacking, but the studies that currently exist have found no increased toxicity or loss of effect with concomitant medications. It is good medical practice, however, to continue to test for these interactions as individual responses may not be the same or predictable from population responses.

McGrath in her refractory epilepsy study measured both post-pill phenobarbital levels and potassium bromide (KBR) levels in her study subjects at the end of the 6-week study period. She found no statistical difference between the treatment and placebo groups for either anticonvulsant. This may indicate that, in spite of the theoretical potential for herb-drug interaction between CBD and P450-metabolized drugs, at

least in dogs on anticonvulsants, the interference may not be clinically relevant. Regardless, it is good medical practice to retest important serum drug levels 2 weeks following initiation of CBD therapy, especially if using macrodose protocols. McGrath mentioned the small study group size in this pilot work as possibly not giving an accurate indication of whether there really is herb-drug interaction or not, and she mentioned that she will analyze these levels not just at the end of her AKC-funded clinical trial but at regular intervals during the trial (McGrath 2018).

8 Current Good Manufacturing Practices (cGMP)

The National Animal Supplement Council (NASC.cc) in partnership with the FDA-CVM has established cGMP guidelines for animal nutraceuticals. These have recently been updated to include the recently enacted FDA Food Safety Modernization Act (FSMA). The NASC will provide third-party inspections of manufacturing facilities of its members to ensure that they meet cGMP standards. NASC member companies that pass their audits successfully can display the NASC seal on the product labels which can guide the consumer to locate products that meet the highest standards of quality and transparency.

Product labels need to contain information about the batch or lot number of the manufacturing process, outdating, company contact information, precautions and contraindications, and the intended use of the product, which, as a nutraceutical, cannot have any medical claims made in the label copy or marketing material or even in the name of the product. For instance, a product that contains the letters CBD, or the word "cannabidiol" in its name or on the label, runs the risk of enforcement by the FDA. This is because the FDA has recently approved CBD as a drug to treat resistant pediatric epilepsy. Medical claims are a common reason for FDA-CVM stop sale letters.

8.1 Third-Party Lab Testing

Quality control testing is a critical aspect of any manufacturing process. Without QC testing, manufacturers would not be able to manufacture products with standardized concentrations.

In addition, contamination can be introduced, and the only way to assure that the product remains unadulterated is through validated testing methods. Other important things to consider include organic cultivation methods, Good Agricultural Practices (GAP), and cGMP protocols.

The analytical laboratory performing the analysis must be validated for accuracy and precision in its measurements in order for the standardization of product potency to be effective. Companies that use in-house laboratories must have those results validated through the use of validated third-party analytical laboratories. It is also up to the labs to apply statistically valid sampling methods to obtain a representative sample of a batch in order to generate meaningful data.

There are a few major concerns that third-party testing can alleviate:

Cannabinoid and Terpene Potency Testing—This is absolutely essential in order to achieve effective and consistent dosing of a patient. An accurate analysis for each manufactured batch is needed to meet strict food and drug guidelines. The contents need to be listed on the product label and in marketing materials to allow the clinician to more accurately establish an effective dosing strategy for the patient. Further, it allows the pet-owning consumer to better select the appropriate potency of product for their size of animal and the potential duration of therapy needed for that product. Manufacturers can make label claims regarding the cannabinoid and terpene content of their products in order to mislead consumers and inflate perceived value of their products. This can pose a serious health risk if a patient is not getting the required dosage necessary to alleviate their conditions. Certificates of analysis on cannabis-derived products convey the potency and potential dosages of products.

Pesticide Contamination—The application of pesticides has long been a standard practice in cultivation of cannabis plants, despite market demand for organic products. The pesticides that are commonly sold in hydroponic stores can present a specific health risk to both animals and humans. For example, a commonly sold fungicide called Eagle 20 has a principal active ingredient called myclobutanil, a compound that when exposed to heat can generate hydrogen cyanide as a reactive by-product. Another fungicide and plant growth regulator called paclobutrazol has been shown to cause downstream kidney and liver damage in animal case studies, and the state of California has designated it as a Category I pesticide, meaning absolutely no detectable amount is allowed in a cannabis product. Be sure to ask for a robust multiresidue pesticide analysis from a third-party laboratory for any organic cannabis products.

Microbiological Contamination—Food testing has long set the standard for ensuring microorganisms are not present in products meant for consumption, but certain cannabis operations have yet to fully mitigate this risk. While it is fairly rare to find organisms like *E.coli* or *Salmonella* in cannabis, it is very common to find *Aspergillus*, a certain type of mold that is very prevalent in outdoor cannabis. *Aspergillus* poses a very specific health risk, especially for patients with a compromised immune system. This is due to a type of neurotoxic by-product produced in the spores collectively referred to as mycotoxins. If the veterinary patient has

any type of immune disorder, it is imperative that any products purchased come with a certificate of analysis certifying the absence of microorganisms.

Heavy Metal Contamination—Cannabis is known to be a bioaccumulator, meaning it will absorb contaminants from the soil. While this can be useful in some applications such as remediating poor soils, it can cause a huge risk if the hemp or cannabis plant was grown in contaminated soils. Arsenic, cadmium, lead, and mercury are the principal dangers, and heavy metal testing should be conducted on any plant-based products to ensure a clean product. Be especially wary if the plants were grown in a country with poor environmental regulations.

9 Concluding Remarks and Future Directions

Veterinary medicine has not seen the same advances compared to human medicine for objective, non-biased scientific evidence for the use of medical cannabis in veterinary species. This is due, in part, to the fact that the legalization statutes, state by state, do not provide for similar legal privileges for veterinarians and their patients as physicians have for recommending cannabis for their human patients.

Nonetheless, there are now several university-based, prospective, double-blinded, placebo-controlled, randomized clinical studies examining CBD from hemp extracts to measure its safety and pharmacokinetics. Included with these studies, once safety has been confirmed, is measuring the efficacy of hemp oil extracts over diagnosed veterinary conditions such as osteoarthritis and epilepsy. Some of these studies have already been published, some have been presented at professional conferences, and some are in press. These landmark studies have all been reported and summarized in this chapter.

Due to the nearly universal distribution of the endocannabinoid system in all chordates, and in many invertebrates, the same or similar benefits of cannabinoids found in humans also can be applied to most veterinary species. This chapter highlights what we know about cannabinoids and their interactions with the endocannabinoid system in animals. A thorough review of the scientific literature has been compiled here for veterinarians and veterinary scientists to better understand this fascinating and emerging therapy and, by understanding it, to be better able to deploy cannabinoid therapies for their patients and formulate more effective cannabinoid medications.

Acknowledgments The authors of this chapter would like to thank the many individuals and institutions who have had the courage and insight to work tirelessly in this field of cannabinoid therapeutics to contribute substantial understanding to the world scientific literature of the many benefits and few risks associated with the use of cannabis and its derivatives, in both human and veterinary species. In particular, the authors of this chapter want to express their gratitude to the veterinary clinical research teams from Colorado State (under the guidance of Stephanie McGrath) and Cornell Universities (under the guidance of Joe Wakslag) for having completed in the canine patient the first-ever safety studies, comparative pharmacokinetic studies, and efficacy studies to determine the ability of cannabinoids to reduce osteoarthritic discomfort and to help patients with refractory epilepsy. The authors would also like to extend their gratitude to Dr. Ethan Russo, Dr. Jahan Marcu, and Kevin McKernan for their thoughtful insights and review of the chapter. We look forward to the continued development of evidence supporting the clinical use of cannabis and its derivatives for many valuable applications in veterinary species. It is our fervent hope that the information presented in this chapter will help future research efforts bring more detailed data regarding the range of applications for cannabinoids in veterinary species.

References

Aggarwal BB, Shishodia S (2006) Molecular targets of dietary agents for prevention and therapy of cancer. Biochem Pharmacol 71:1397–1421

Ahmed SA, Ross SA, Slade D et al (2008) Cannabinoid ester constituents from high-potency *Cannabis sativa*. J Nat Prod 71:536–542

Al-Omari SM (2007) The effect of thujone and myrcene on diabetes mellitus in albino rats. Faculty of Graduate Studies University of Jordan

Aydin E, Türkez H, Taşdemir Ş (2013) Anticancer and antioxidant properties of terpinolene in rat brain cells. Arch Ind Hyg Toxicol 64:415–424

Babson KA, Sottile J, Morabito D (2017) Cannabis, cannabinoids, and sleep: A review of the literature. Curr Psychiatry Rep 19:23

Barni-Comparini I, Ferri S, Centini F (1984) Cannabinoid level in the leaves as a tool for the early discrimination of cannabis chemiovariants. Forensic Sci Int 24:37–42

Bartner LR, McGrath S, Rao S, Hyatt LK et al (2018) Pharmacokinetics of cannabidiol administered by three delivery methods at 2 different dosages to healthy dogs. Can J Vet Res 82:178–183

Basavarajappa BS (2007) Neuropharmacology of the endocannabinoid signaling system-molecular mechanisms, biological actions and synaptic plasticity. Curr Neuropharmacol 5:81–97

Bátkai S et al (2004) Endocannabinoids acting at cannabinoid-1 receptors regulate cardiovascular function in hypertension. Circulation 110:1996–2002

Bauer JE (2011) Therapeutic use of fish oils in companion animals. J Am Vet Med Assoc 239:1441–1451

Beale C, Broid SJ, Chye Y et al (2018) Prolonged cannabidiol treatment effects on hippocampal subfield volumes in current cannabis users. Cannabis Cannabinoid Res 3:94–107

Bénard G, Massa F, Puente N et al (2012) Mitochondrial CB 1 receptors regulate neuronal energy metabolism. Nat Neurosci 15:558–564

Bergamaschi MM, Queiroz RH, Chagas MH et al (2011) Cannabidiol reduces the anxiety induced by simulated public speaking in treatment-naive social phobia patients. Neuropsychopharmacology 36(6):1219–1226

Bolognini D et al (2013) Cannabidiolic acid prevents vomiting in suncus murinus and nausea-induced behaviour in rats by enhancing 5-HT1A receptor activation. Br J Pharmacol 168:1456–1470

Booth D (2009) Evaluating the quality of nutraceuticals to help improve your patient's quality of life. Paper presented at the Proceedings North American Veterinary Conference

Borrelli F, Fasolino I, Romano B et al (2013) Beneficial effect of the non-psychotropic plant cannabinoid cannabigerol on experimental inflammatory bowel disease. Biochem Pharmacol 85(9):1306–1316

Brenneisen R, ElSohly MA (1988) Chromatographic and spectroscopic profiles of cannabis of different origins: Part I. J Forensic Sci 33:1385–1404

Broséus J, Anglada F, Esseiva P (2010) The differentiation of fibre- and drug type cannabis seedlings by gas chromatography/mass spectrometry and chemometric tools. Forensic Sci Int 200:87–92

Callaway JC (2004) Hempseed as a nutritional resource: an overview. Euphytica 140:65–72

Calleja MA, Vieites JM, Montero-Meterdez T et al (2013) The antioxidant effect of β-caryophyllene protects rat liver from carbon tetrachloride-induced fibrosis by inhibiting hepatic stellate cell activation. Br J Nutr 109:394–401

Camilleri M, Kolar GJ, Vazquez-Roque MI et al (2013) Cannabinoid receptor 1 gene and irritable bowel syndrome: phenotype and quantitative traits. Am J Physiol Gastrointest Liver Physiol 304(5): G553–G560

Campora L, Miraqliotta V, Ricci E et al (2012) Cannabinoid receptor type 1 and 2 expression in the skin of healthy dogs and dogs with atopic dermatitis. Am J Vet Res 73:988–995

Carlini EA, Cunha JM (1981) Hypnotic and antiepileptic effects of cannabidiol. J Clin Pharmacol 21:417S–427S

Chang YH, Windish DM (2009) Cannabinoid hyperemesis relieved by compulsive bathing. In: Mayo Clinic Proceedings, vol 1. Elsevier, Amsterdam, pp 76–78

Chaves JS, Leal PC, Pianowisky L et al (2008) Pharmacokinetics and tissue distribution of the sesquiterpene α-humulene in mice. Planta Med 74:1678–1683

Chen R et al (2013) Δ9-THC-caused synaptic and memory impairments are mediated through COX-2 signaling. Cell 155:1154–1165

Chioca LR et al (2013) Anxiolytic-like effect of lavender essential oil inhalation in mice: participation of serotonergic but not GABAA/benzodiazepine neurotransmission. J Ethnopharmacol 147:412–418

Ciftci O, Ozdemir I, Tanyildizi S et al (2011) Antioxidative effects of curcumin, β-myrcene and 1, 8-cineole against 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin-induced oxidative stress in rats liver. Toxicol Ind Health 27:447–453

Condie R, Herring A, Koh WS et al (1996) Cannabinoid inhibition of adenylate cyclase-mediated signal transduction and interleukin 2 (IL-2) expression in the murine T-cell line, EL4. IL-2. J Biol Chem 271:13175–13183

Consroe P, Wolkin A (1977) Cannabidiol—antiepileptic drug comparisons and interactions in experimentally induced seizures in rats. J Pharmacol Exp Ther 201:26–32

Crippa JA, Zuardi AW, Martin-Santos R et al (2009) Cannabis and anxiety: a critical review of the evidence. Hum Psychopharmacol 24(7):515–523

Crowell PL, Gould MN (1994) Chemoprevention and therapy of cancer by d-limonene. Crit Rev Oncog 5(1):1–22

Dall'Aglio C, Mercati F, Pascucci L et al (2010) Immunohistochemical localization of CB1 receptor in canine salivary glands. Vet Res Commun 34:9–12

Dalle Carbonare M, Del Giudice E, Stecca A et al (2008) A saturated N-acylethanolamine other than N-palmitoyl ethanolamine with anti-inflammatory properties: a neglected story. J Neuroendocrinol 20:26–34

De Petrocellis L, Melck D, Bisogno T et al (1999) Finding of the endocannabinoid signalling system in Hydra, a very primitive organism: possible role in the feeding response. Neuroscience 92:377–387

Deferne JL, Pate DW (1996) Hemp seed oil: a source of valuable essential fatty acids. J Int Hemp Assoc 3(1):4–7

Demirakca T, Sartorius A, Ende G et al (2011) Diminished gray matter in the hippocampus of cannabis users: Possible protective effects of cannabidiol. Drug Alcohol Depend 114:242–245

Devane WA, Hanus L, Breuer A et al (1992) Isolation and structure of a brain constituent that binds to the cannabinoid receptor. Science 258:1946–1949

Devinsky O, Cross JH, Wright S (2017) Trial of cannabidiol for drug-resistant seizures in the Dravet syndrome. N Engl J Med 376 (21):2011–2020

Dewey WL (1986) Cannabinoid pharmacology. Pharmacol Rev 38 (2):151–178

Directive C (2013) Common catalogue of varieties of agricultural plant species. Off J Eur Union 379

Do Vale TG, Furtado EC, Santos J Jr et al (2002) Central effects of citral, myrcene and limonene, constituents of essential oil chemotypes from *Lippia alba* (Mill.) NE Brown. Phytomedicine 9:709–714

Drugs UNOo (2009) Recommended methods for the identification and analysis of cannabis and cannabis products. United Nations Publications, Vienna

Elmes MW, Kaczocha M, Berger ST et al (2015) Fatty acid-binding proteins (FABPs) are intracellular carriers for Δ9-tetrahydrocannabinol (THC) and cannabidiol (CBD). J Biol Chem 290:8711–8721

ElSohly MA, Slade D (2005) Chemical constituents of marijuana: the complex mixture of natural cannabinoids. Life Sci 78:539–548

Esposito G, De Filippis D, Carnuccio R et al (2006) The marijuana component cannabidiol inhibits β-amyloid-induced tau protein hyperphosphorylation through Wnt/β-catenin pathway rescue in PC12 cells. J Mol Med 84:253–258

Esposito G, Scuderi C, Savani C (2007) Cannabidiol *in vivo* blunts β-amyloid induced neuroinflammation by suppressing IL-1β and iNOS expression. Br J Pharmacol 151:1272–1279

Eubanks LM, Rogers CJ, Beuscher AE IV et al (2006) A molecular link between the active component of marijuana and Alzheimer's disease pathology. Mol Pharm 3:773–777

Fan P (1995) Cannabinoid agonists inhibit the activation of 5-HT3 receptors in rat nodose ganglion neurons. J Neurophysiol 73 (2):907–910

Fernandes ES, Passos GF, Medeiros R et al (2007) Anti-inflammatory effects of compounds alpha-humulene and (−)-trans-caryophyllene isolated from the essential oil of *Cordia verbenacea*. Eur J Pharmacol 569:228–236

Fischedick JT, Hazekamp A, Erkelens T et al (2010) Metabolic fingerprinting of *Cannabis sativa* L., cannabinoids and terpenoids for chemotaxonomic and drug standardization purposes. Phytochemistry 71:2058–2073

Freundt-Revilla J, Kegler K, Baumgärtner W et al (2017) Spatial distribution of cannabinoid receptor type 1 (CB1) in normal canine central and peripheral nervous system. PLoS One 12:e0181064

Fujita W, Gomes I, Devi LA (2014) Revolution in GPCR signalling: opioid receptor heteromers as novel therapeutic targets: IUPHAR review 10. Br J Pharmacol 171(18):4155–4176

Galindo L, Moreno E, López-Armenta F et al (2018) Cannabis users show enhanced expression of CB_1-$5HT_{2A}$ receptor heteromers in olfactory neuroepithelium cells. Mol Neurobiol:1–15

Gallily R, Yekhtin Z, Hanuš LO (2015) Overcoming the bell-shaped dose-response of cannabidiol by using cannabis extract enriched in cannabidiol. Pharmacol Pharm 6:75–85

Gamble L-J et al (2018) Pharmacokinetics, safety, and clinical efficacy of cannabidiol treatment in osteoarthritic dogs. In: Proceedings of the World Rehabilitation Summit (IAVRPT), ACVSMR track; July 31, Knoxville, TN

Gaoni Y, Mechoulam R (1964) Isolation, structure, and partial synthesis of an active constituent of hashish. J Am Chem Soc 86:1646–1647

Gertsch J (2017) Cannabimimetic phytochemicals in the diet–an evolutionary link to food selection and metabolic stress adaptation? Br J Pharmacol 174:1464–1483

Gertsch J, Schoop R, Kuenzle U et al (2004) Echinacea alkylamides modulate TNF-α gene expression via cannabinoid receptor CB2 and multiple signal transduction pathways. FEBS Lett 577:563–569
Gertsch J, Leonti M, Raduner S et al (2008a) Beta-caryophyllene is a dietary cannabinoid. Proc Natl Acad Sci 105:9099–9104
Gertsch J, Raduner S, Tytgat J et al (2008b) Analgesic and neuropsychological effects of Echinacea N-alkylamides. Planta Med 74 (9):1014–PA302
Gesell FK, Zoerner AA, Brauer C et al (2013) Alterations of endocannabinoids in cerebrospinal fluid of dogs with epileptic seizure disorder. BMC Vet Res 9:262
Glenn H (2017) A stakeholder review of the feasibility of industrial hemp by-products as animal feed ingredients: a report to the Colorado legislature in response to SB17–109
Griffin G, Wray EJ, Tao Q et al (1999) Evaluation of the cannabinoid CB2 receptor-selective antagonist, SR144528: further evidence for cannabinoid CB2 receptor absence in the rat central nervous system. Eur J Pharmacol 377:117–125
Gulluni N, Re T, Aoiacono I et al (2018) Cannabis essential oil: a preliminary study for the evaluation of the brain effects. Evid Based Complement Altern Med 2018:1709182
Guzmán-Gutiérrez SL, Bonilla-Jaime H et al (2015) Linalool and β-pinene exert their antidepressant-like activity through the monoaminergic pathway. Life Sci 128:24–29
Hampson A, Grimaldi M, Axelrod J et al (1998) Cannabidiol and (−) Δ9-tetrahydrocannabinol are neuroprotective antioxidants. Proc Natl Acad Sci USA 95:8268–8273
Hampson AJ, Axelrod J, Grimaldi M (2003) Cannabinoids as antioxidants and neuroprotectants. Google Patents
Han J et al (2012) Acute cannabinoids impair working memory through astroglial CB 1 receptor modulation of hippocampal LTD. Cell 148:1039–1050
Hand M, Thatcher C, Remillard R et al (2010) Small animal clinical nutrition. Mark Morris Institute, Topeka
Hartsel JA, Eades J, Hickory B, Makriyannis A (2016) Nutraceuticals, efficacy, safety and toxicity: *Cannabis sativa* and Hemp. Elsevier, Amsterdam, pp 735–754
Haustein M, Ramer R, Linnebacher M et al (2014) Cannabinoids increase lung cancer cell lysis by lymphokine-activated killer cells via upregulation of ICAM-1. Biochem Pharmacol 92:312–325
Hayakawa K et al (2008) Cannabidiol prevents a post-ischemic injury progressively induced by cerebral ischemia via a high-mobility group box1-inhibiting mechanism. Neuropharmacology 55:1280–1286
Heitland I, Klumpers F, Oosting RS et al (2012) Failure to extinguish fear and genetic variability in the human cannabinoid receptor 1. Transl Psychiatry 2:e162
Henry P (2017) Cannabis chemovar classification: terpenes hyperclasses and targeted genetic markers for accurate discrimination of flavours and effects. Peer J Prepr 5:e3307v3301
Hepburn C, Walsh S, Wainwright C (2011) 17 Cannabidiol as an antiarrhythmic, the role of the CB1 receptors. Heart 97:e8
Hill MN, McLaughlin RJ, Morrish AC et al (2009) Suppression of amygdalar endocannabinoid signaling by stress contributes to activation of the hypothalamic–pituitary–adrenal axis. Neuropsychopharmacology 34:2733
Hill AJ, Williams CM, Whalley BJ et al (2012) Phytocannabinoids as novel therapeutic agents in CNS disorders. Pharmacol Ther 133:79–97
Hillig KW, Mahlberg PG (2004) A chemotaxonomic analysis of cannabinoid variation in *Cannabis* (Cannabaceae). Am J Bot 91:966–975
Hirota R et al (2012) Limonene inhalation reduces allergic airway inflammation in Dermatophagoides farinae-treated mice. Inhal Toxicol 24:373–381
Ho B, Uezono Y, Takada S et al (1999) Coupling of the expressed cannabinoid CB1 and CB2 receptors to phospholipase C and G protein-coupled inwardly rectifying K^+ channels. Receptors Channels 6:363–374
Howlett AC (2002) The cannabinoid receptors. Prostaglandins Other Lipid Mediat 68:619–631
Howlett A, Fleming R (1984) Cannabinoid inhibition of adenylate cyclase. Pharmacology of the response in neuroblastoma cell membranes. Mol Pharmacol 26:532–538
Ito K, Ito M (2011) Sedative effects of vapor inhalation of the essential oil of *Microtoena patchoulii* and its related compounds. J Nat Med 65:336–343
Iwata N, Kitanaka S (2011) New cannabinoid-like chromane and chromene derivatives from Rhododendron anthopogonoides. Chem Pharm Bull 59:1409–1412
Izzo AA, Borrelli F, Capasso R et al (2009) Non-psychotropic plant cannabinoids: new therapeutic opportunities from an ancient herb. Trends Pharmacol Sci 30:515–527
Jiang W, Zhang Y, Xiao L et al (2005) Cannabinoids promote embryonic and adult hippocampus neurogenesis and produce anxiolytic- and antidepressant-like effects. J Clin Invest 115:3104–3116
Kallendrusch S, Kremzow S, Nowicki M et al (2013) The G protein-coupled receptor 55 ligand l-α-lysophosphatidylinositol exerts microglia-dependent neuroprotection after excitotoxic lesion. Glia 61:1822–1831
Kalra EK (2003) Nutraceutical-definition and introduction. AAPS Pharm Sci 5(3):5. http://www.pharmsci.org
Kapoor R, Huang Y-S (2006) https://www.ingentaconnect.com/content/ben/cpb/2006/00000007/00000006/art00016?crawler=true
King A, Lodola A, Carmi C et al (2009) A critical cysteine residue in monoacylglycerol lipase is targeted by a new class of isothiazolinone-based enzyme inhibitors. Br J Pharmacol 157:974–983
Klauke A-L, Racz I, Pradier B et al (2014) The cannabinoid CB2 receptor-selective phytocannabinoid beta-caryophyllene exerts analgesic effects in mouse models of inflammatory and neuropathic pain. Eur Neuropsychopharmacol 24:608–620
Klein TW, Lane B, Newton CA, Friedman H (2000) The cannabinoid system and cytokine network. Proc Soc Exp Biol Med 225:1–8
Koch M, Varela L, Kim JG et al (2015) Hypothalamic POMC neurons promote cannabinoid-induced feeding. Nature 519:45
Kogan L, Hellyer P, Robinson N (2015) Consumers perceptions of animal hemp products. J Am Holist Vet Med Assoc 14:34–35
Kogan L, Hellyer P, Schoenfeld-Tacher R (2018) Dog owner's use and perceptions of cannabis products. J Am Holist Vet Med Assoc 4:34–35
Komiya M, Takeuchi T, Harada E (2006) Lemon oil vapor causes an anti-stress effect via modulating the 5-HT and DA activities in mice. Behav Brain Res 172:240–249
Lafourcade M, Larrieu T, Mato S et al (2011) Nutritional omega-3 deficiency abolishes endocannabinoid-mediated neuronal functions. Nat Neurosci 14:345
Laun AS, Song Z-H (2017) GPR3 and GPR6, novel molecular targets for cannabidiol. Biochem Biophys Res Commun 490:17–21
Le Foll B, Trigo JM, Sharkey KA et al (2013) Cannabis and Δ9-tetrahydrocannabinol (THC) for weight loss? Med Hypotheses 80:564–567
Legault J, Pichette A (2007) Potentiating effect of β-caryophyllene on anticancer activity of α-humulene, isocaryophyllene and paclitaxel. J Pharm Pharmacol 59:1643–1647
Leizer C, Ribnicky D, Poulev A, Dushenkov S, Raskin I (2000) The composition of hemp seed oil and its potential as an important source of nutrition. J Nutraceut Funct Med Foods 2(4):35–53. https://www.tandfonline.com/doi/abs/10.1300/J133v02n04_04
Lewis MA, Russo EB, Smith KM (2018) Pharmacological foundations of cannabis chemovars. Planta Med 84:225–233
Ligresti A, Moriello AS, Starowicz K et al (2006) Antitumor activity of plant cannabinoids with emphasis on the effect of cannabidiol

on human breast carcinoma. J Pharmacol Exp Ther 318: 1375–1387
Lima NG et al (2013) Anxiolytic-like activity and GC–MS analysis of (R)-(+)-limonene fragrance, a natural compound found in foods and plants. Pharmacol Biochem Behav 103:450–454
Lissoni P, Resentini M, Mauri R et al (1986) Effects of tetrahydrocannabinol on melatonin secretion in man. Horm Metabol Res 18:77–78
Lorenzetti BB, Souza GE, Sarti SJ et al (1991) Myrcene mimics the peripheral analgesic activity of lemongrass tea. J Ethnopharmacol 34:43–48
MacCallum CA, Russo EB (2018) Practical considerations in medical cannabis administration and dosing. Eur J Intern Med 49:12–19
Mackie K (2006) Cannabinoid receptors as therapeutic targets. Annu Rev Pharmacol Toxicol 46:101–122
Mackie K (2008) Cannabinoid receptors: where they are and what they do. J Neuroendocrinol 20:10–14
Makriyannis A (2014) 2012 Division of medicinal chemistry award address. Trekking the cannabinoid road: a personal perspective. J Med Chem 57:3891–3911
Marcu JP, Schechter JB (2016) Molecular pharmacology of CB1 and CB2 cannabinoid receptors. In: Neuropathology of Drug Addictions and Substance Misuse. Elsevier, London, pp 713–721
McAllister SD, Christian RT, Horowitz MP et al (2007) Cannabidiol as a novel inhibitor of Id-1 gene expression in aggressive breast cancer cells. Mol Cancer Ther 6:2921–2927
McGrath S (2018) Cannabis clinical trials in dogs—CSU paving the way. In: Proceedings of the AVMA Annual Conference, Denver, CO, July, 2018
McGrath S, Bartner L, Rao S, et al (2018) A report of adverse effects associated with the administration of cannabidiol in healthy dogs. J Am Holistic Vet Med Assoc. Fall 2018
McHugh D, Hu SS, Rimmerman N et al (2010) N-arachidonoyl glycine, an abundant endogenous lipid, potently drives directed cellular migration through GPR18, the putative abnormal cannabidiol receptor. BMC Neurosci 11:44
McPartland J, Marzo VD, Petrocellis LD et al (2001) Cannabinoid receptors are absent in insects. J Comp Neurol 436:423–429
McPartland JM, Agraval J, Glesson D et al (2006) Cannabinoid receptors in invertebrates. J Evol Biol 19:366–373
Mechoulam R, Ben-Shabat S, Hanus L et al (1995) Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors. Biochem Pharmacol 50:83–90
Meola SD, Tearney CC, Haas SA et al (2012) Evaluation of trends in marijuana toxicosis in dogs living in a state with legalized medical marijuana: 125 dogs (2005–2010). J Vet Emerg Crit Care 22:690–696
Mercati F, Dall'Aglio C, Pascucci L et al (2012) Identification of cannabinoid type 1 receptor in dog hair follicles. Acta Histochem 114:68–71
Miyazawa M, Yamafuji C (2005) Inhibition of acetylcholinesterase activity by bicyclic monoterpenoids. J Agric Food Chem 53:1765–1768
Mo X-L, Yang Z, Tao Y-X (2014) Targeting GPR119 for the potential treatment of type 2 diabetes mellitus. Prog Mol Biol Transl Sci 121:95–131
Moon AM, Buckley SA, Mark NM (2018) Successful treatment of cannabinoid hyperemesis syndrome with topical capsaicin. ACG Case Rep J 5:e3. https://doi.org/10.14309/crj.2018.3
Morello G, Imperatore R, Palomba L et al (2016) Orexin-A represses satiety-inducing POMC neurons and contributes to obesity via stimulation of endocannabinoid signaling. Proc Natl Acad Sci USA 113:4759–4764
Morena M, Patel S, Bains JS et al (2016) Neurobiological interactions between stress and the endocannabinoid system. Neuropsychopharmacology 41:80
Moreno-Sanz G (2016) Can you pass the acid test? Critical review and novel therapeutic perspectives of Δ9-tetrahydrocannabinolic acid A. Cannabis Cannabinoid Res 1(1):124–130
Muniyappa R, Sable S, Ouwekerk R et al (2013) Metabolic effects of chronic cannabis smoking. Diabetes Care 36:2415–2422
Nadal X, Del Río C, Casano S et al (2017) Tetrahydrocannabinolic acid is a potent PPARγ agonist with neuroprotective activity. Br J Pharmacol 174:4263–4276
Ndong C, O'donnell D, Ahmad S et al (2011) Cloning and pharmacological characterization of the dog cannabinoid CB2 receptor. Eur J Pharmacol 669:24–31
Niederhoffer N, Szabo B (1999) Effect of the cannabinoid receptor agonist WIN55212-2 on sympathetic cardiovascular regulation. Br J Pharmacol 126(2):457–466
Niederhoffer N, Szabo B (2000) Cannabinoids cause central sympathoexcitation and bradycardia in rabbits. J Pharmacol Exp Ther 294(2):707–713
Nissen L, Zatta A, Stefanini I et al (2010) Characterization and antimicrobial activity of essential oils of industrial hemp varieties (*Cannabis sativa* L.). Fitoterapia 81:413–419
Ottani A, Leone S, Sandrini M et al (2006) The analgesic activity of paracetamol is prevented by the blockade of cannabinoid CB1 receptors. Eur J Pharmacol 531:280–281
Pacioni G et al (2015) Truffles contain endocannabinoid metabolic enzymes and anandamide. Phytochemistry 110:104–110
Parray HA, Yun JW (2016) Cannabidiol promotes browning in 3T3-L1 adipocytes. Mol Cell Biochem 416:131–139
Patel S, Kingsley PJ, Mackie K et al (2009) Repeated homotypic stress elevates 2-arachidonoylglycerol levels and enhances short-term endocannabinoid signaling at inhibitory synapses in basolateral amygdala. Neuropsychopharmacology 34:2699
Pertwee RG (2000) Cannabinoid receptor ligands: clinical and neuropharmacological considerations, relevant to future drug discovery and development. Exp Opin Invest Drugs 9:1553–1571
Pertwee RG (2001) Cannabinoid receptors and pain. Prog Neurobiol 63:569–611
Pertwee RG (2005) Pharmacological actions of cannabinoids. In: Cannabinoids. Springer, Cham, pp 1–51
Pertwee RG (2008) The diverse CB1 and CB2 receptor pharmacology of three plant cannabinoids: Δ9-tetrahydrocannabinol, cannabidiol and Δ9-tetrahydrocannabivarin. Br J Pharmacol 153:199–215
Pertwee Handbook of Cannabis (2014) https://www.biblio.com/book/handbook-cannabis-roger-pertwee-ed/d/1139894567?aid=frg&utm_source=google&utm_medium=product&utm_campaign=feed-details&gclid=EAIaIQobChMIsrjyvqy04QIVXSCtBh0gMwygEAYYASABEgIB1fD_BwE
Pirone A, Lenzi C, Coli A et al (2015) Preferential epithelial expression of type-1 cannabinoid receptor (CB1R) in the developing canine embryo. Springerplus 4:804
Pollan M (2001) The botany of desire: a plant's-eye view of the world. In: How to change your mind: what the new science of psychedelics teaches us about consciousness, dying, addiction, depression, and transcendence. Random house trade paperbacks
Pollastro F, De Petrocellis L, Schiano-Moriello A et al (2017) Amorfrutin-type phytocannabinoids from *Helichrysum umbraculigerum*. Fitoterapia 123:13–17
Prates Ong T, Testoni Cardozo M, de Conti A et al (2012) Chemoprevention of hepatocarcinogenesis with dietary isoprenic derivatives: cellular and molecular aspects. Curr Cancer Drug Targets 12:1173–1190
Raduner S et al (2006) Alkylamides from Echinacea are a new class of cannabinomimetics Cannabinoid type 2 receptor-dependent and-independent immunomodulatory effects. J Biol Chem 281:14192–14206

Radwan MM, ElSohly MA, Slade D et al (2008) Non-cannabinoid constituents from a high potency *Cannabis sativa* variety. Phytochemistry 69(14):2627–2633
Rao V, Menezes A, Viana G (1990) Effect of myrcene on nociception in mice. J Pharm Pharmacol 42:877–878
Rashidi H, Akhtar MT, van der Kooy F et al (2009) Hydroxylation and further oxidation of Δ9-tetrahydrocannabinol by alkane-degrading bacteria. Appl Environ Microbiol 75(22):7135–7141
Reddy AT, Lakshmi SP, Reddy RC (2012) Murine model of allergen induced asthma. J Visual Exp, JoVE
Ribeiro A et al (2015) Cannabidiol improves lung function and inflammation in mice submitted to LPS-induced acute lung injury. Immunopharmacol Immunotoxicol 37:35–41
Riedel G, Fadda P, McKillop-Smith S et al (2009) Synthetic and plant-derived cannabinoid receptor antagonists show hypophagic properties in fasted and non-fasted mice. Br J Pharmacol 156:1154–1166
Rock EM, Parker LA (2013) Effect of low doses of cannabidiolic acid and ondansetron on LiCl-induced conditioned gaping (a model of nausea-induced behaviour) in rats. Br J Pharmacol 169:685–692
Rock E, Kopstick R, Limebeer C et al (2013) Tetrahydrocannabinolic acid reduces nausea-induced conditioned gaping in rats and vomiting in *Suncus murinus*. Br J Pharmacol 170:641–648
Rock EM et al (2014) A comparison of cannabidiolic acid with other treatments for anticipatory nausea using a rat model of contextually elicited conditioned gaping. Psychopharmacology 231:3207–3215
Roehrs T, Roth T (2017) Medication and substance abuse. In: Principles and practice of sleep medicine, 6th edn. Elsevier, Philadelphia, pp 1380–1389.e1384
Rogerio AP, Andrade EL, Leite DF et al (2009) Preventive and therapeutic anti-inflammatory properties of the sesquiterpene α-humulene in experimental airways allergic inflammation. Br J Pharmacol 158:1074–1087
Romano B, Borrelli F, Pagano E et al (2014) Inhibition of colon carcinogenesis by a standardized *Cannabis sativa* extract with high content of cannabidiol. Phytomedicine 21:631–639
Rufino AT, Ribeiro M, Sousa C et al (2015) Evaluation of the anti-inflammatory, anti-catabolic and pro-anabolic effects of E-caryophyllene, myrcene and limonene in a cell model of osteoarthritis. Eur J Pharmacol 750:141–150
Russo EB (2011) Taming THC: potential cannabis synergy and phytocannabinoid-terpenoid entourage effects. Br J Pharmacol 163:1344–1364
Russo EB (2016a) Beyond cannabis: plants and the endocannabinoid system. Trends Pharmacol Sci 37:594–605
Russo EB (2016b) Clinical endocannabinoid deficiency reconsidered: Current research supports the theory in migraine, fibromyalgia, irritable bowel, and other treatment-resistant syndromes. Cannabis Cannabinoid Res 1:154–165
Russo E, Etges T, Stott C, et al (2011) Sativex safety profile is improving over time. International Cannabinoid Research Society, St Charles, pp 1122–1131
Saito VM, Rezende RM, Teixeira AL (2012) Cannabinoid modulation of neuroinflammatory disorders. Curr Neuropharmacol 10:159–166
Saliba SW, Marcotequi AR, Fortwängler E et al (2017) AM404, paracetamol metabolite, prevents prostaglandin synthesis in activated microglia by inhibiting COX activity. J Neuroinflammation 14:246
Satsu H, Matsuda T, Toshimitsu T et al (2004) Regulation of interleukin-8 secretion in human intestinal epithelial Caco-2 cells by α-humulene. Biofactors 21:137–139
Schmitt S, Schaefer UF, Doebler L et al (2009) Cooperative interaction of monoterpenes and phenylpropanoids on the in vitro human skin permeation of complex composed essential oils. Planta Med 75:1381–1385
Scialdone MA (2017) U.S. Patent Application No. 15/613,633
Sellers EM, Schoedel K, Bartlett C et al (2013) A multiple-dose, randomized, double-blind, placebo-controlled, parallel-group QT/QTc study to evaluate the electrophysiologic effects of THC/CBD spray. Clin Pharmacol Drug Dev 2(3):285–294
Silvestri C, Paris D, Martella A et al (2015) Two non-psychoactive cannabinoids reduce intracellular lipid levels and inhibit hepatosteatosis. J Hepatol 62(6):1382–1390
Simopoulos AP (2002) The importance of the ratio of omega-6/omega-3 essential fatty acids. Biomed Pharmacother 56(8):365–379
Smith TH, Blume LC, Straiker A et al (2015) Cannabinoid receptor–interacting protein 1a modulates CB1 receptor signaling and regulation. Mol Pharmacol 87(4):747–765
Smith DR, Stanley C, Foss T et al (2017) Rare genetic variants in the endocannabinoid system genes *CNR1* and *DAGLA* are associated with neurological phenotypes in humans. PLoS One 12:e0187926
Solowij N, Walterfang M, Lubman DI et al (2013) Alteration to hippocampal shape in cannabis users with and without schizophrenia. Schizophrenia Res 143:179–184
Stanley CP, Hind WH, O'sullivan SE (2013) Is the cardiovascular system a therapeutic target for cannabidiol? Br J Clin Pharmacol 75:313–322
Takeda S, Misawa K, Yamamoto I et al (2008) Cannabidiolic acid as a selective cyclooxygenase-2 inhibitory component in cannabis. Drug Metab Dispos 36:1917–1921
Tashkin DP, Shapiro BJ, Frank IM (1974) Acute effects of smoked marijuana and oral Δ9-tetrahydrocannabinol on specific airway conductance in asthmatic subjects. Am Rev Respir Dis 109:420–428
Tate G et al (1989) https://www.researchgate.net/profile/Guillermo_Tate/publication/20604782_Suppression_of_acute_and_chronic_inflammation_by_dietary_gamma_linolenic_acid/links/56e9a36a08aec8bc078113e9/Suppression-of-acute-and-chronic-inflammation-by-dietary-gamma-linolenic-acid.pdf
Tishcler J (2018) Microdosing for the medical market: Why who and how. Paper presented at the Institute for Cannabis Research, Colorado State University, Pueblo, April 27–28 2018
Toyota M, Shimamura T, Ishii H et al (2002) New bibenzyl cannabinoid from the New Zealand liverwort *Radula marginata*. Chem Pharm Bull 50:1390–1392
Trumbly B (1990) Double-blind clinical study of cannabidiol as a secondary anticonvulsant. In: Presented at Marijuana '90 international Conference on Cannabis and Cannabinoids, Kolympari (Crete)
Turner CE, Elsohly MA, Boeren EG (1980) Constituents of *Cannabis sativa* L. XVII. A review of the natural constituents. J Nat Prod 43:169–234
Ulbricht C (2011) Focus: Diabetes. J Diet Suppl 8:239–256
Upton R, Craker I, ElSohly M, et al. (2014) Cannabis inflorescence Cannabis Spp.: standards of identity, analysis, and quality control. American Herbal Pharmacopoeia, Scott's Valley
Vaccani A, Massi P, Colombo A et al (2005) Cannabidiol inhibits human glioma cell migration through a cannabinoid receptor-independent mechanism. Br J Pharmacol 144:1032–1036
Van der Stelt M, Veldhuis W, Bär P et al (2001) Neuroprotection by Δ9-tetrahydrocannabinol, the main active compound in marijuana, against ouabain-induced in vivo excitotoxicity. J Neurosci 21:6475–6479
Vemuri VK, Makriyannis A (2015) Medicinal chemistry of cannabinoids. Clin Pharmacol Ther 97:553–558
Veress T, Szanto J, Leisztner L (1990) Determination of cannabinoid acids by high-performance liquid chromatography of their neutral derivatives formed by thermal decarboxylation: I. Study of the decarboxylation process in open reactors. J Chromatogr A 520:339–347
Verhoeckx KC, Korthout HA, van Meeteren-Kreikamp AP et al (2006) Unheated *Cannabis sativa* extracts and its major compound THC-acid have potential immuno-modulating properties not

mediated by CB1 and CB2 receptor coupled pathways. Int Immunopharmacol 6(4):656–665

Viñals X, Moreno E, Lanfumey L et al (2015) Cognitive impairment induced by delta9-tetrahydrocannabinol occurs through heteromers between cannabinoid CB1 and serotonin 5-HT2A receptors. PLoS Biol 13(7):e1002194

Vogelmann AF, Turner JC, Mahlberg PG (1988) Cannabinoid composition in seedlings compared to adult plants of *Cannabis sativa*. J Nat Prod 51:1075–1079

Wagner JA, Varga K, Kunos G (1998) Cardiovascular actions of cannabinoids and their generation during shock. J Mol Med 76 (12):824–836

Wargent E, Zaibi MS, Silvestri C et al (2013) The cannabinoid Δ 9-tetrahydrocannabivarin (THCV) ameliorates insulin sensitivity in two mouse models of obesity. Nutr Diabetes 3:e68

Weiland BJ, Thayer RE, Depue BE et al (2015) Daily marijuana use is not associated with brain morphometric measures in adolescents or adults. J Neurosci 35:1505–1512

Whyte LS, Ryberg E, Sims NA et al (2009) The putative cannabinoid receptor GPR55 affects osteoclast function in vitro and bone mass *in vivo*. Proc Natl Acad Sci USA 106:16511–16516

Woelkart K, Bauer R (2007) The role of alkamides as an active principle of Echinacea. Planta Med 73:615–623

Xi Z-X, Peng X-Q, Li X et al (2011) Brain cannabinoid CB2 receptors modulate cocaine's actions in mice. Nat Neurosci 14:1160–1166. http://www.nature.com/neuro/journal/v14/n9/abs/nn.2874.html#supplementary-information

Yücel M, Solowij N, Respondek C et al (2008) Regional brain abnormalities associated with long-term heavy cannabis use. Arch Gen Psychiatry 65:694–701

Yücel M, Lorenzetti V, Suo C et al (2016) Hippocampal harms, protection and recovery following regular cannabis use. Transl Psychiatry 6:e710

Zuardi AW, Cosme RA, Graeff FG et al (1993) Effects of ipsapirone and cannabidiol on human experimental anxiety. J Psychopharmacol 7 (1 Suppl):82–88

Essential Oils

Ajay Srivastava, Rajiv Lall, Anita Sinha, and Ramesh C. Gupta

Abstract

The essential oils (EOs) have been used in humans and animals for several millennia, as they represent an important part of folk medicine for their medicinal properties. EOs are a very heterogeneous group of complex mixtures of secondary plant metabolites. The nature of an EO varies from plant to plant, species to species, and within botanical families. By now, more than 3000 varieties of volatile aromatic compounds have been identified. Hundreds of chemical compounds have been identified in the essential oils (EOs) of some plants, with properties such as antioxidative, anti-inflammatory, antibacterial, antiviral, antifungal, antiseptic, antimycotic, antitumor, antispasmodic, immunostimulating, etc. In addition to aromatherapy, they are either ingested or topically applied for conditions such as pain, arthritis, bruises, scratches, scars, flea control, and many others. This chapter describes EOs of plant and non-plant origins, their active constituents, and clinical applications in animal health and disease.

Keywords

Nutraceuticals · Veterinary nutraceuticals · Essential oils · Animal diseases

1 Introduction

Essential oils (EOs) are naturally occurring volatile aromatic compounds that are found in the bark, stems, flowers, seeds, and other parts of many plants. EOs are a very heterogeneous group of complex mixtures of secondary plant metabolites. The composition of EOs can vary depending on a number of factors, such as geographic location, cultivation, time of day, weather, season, and method and duration of distillation. In fact, every step during the production process is a critical determinant of the quality of the EO product.

EOs have been used for at least several millennia for natural healings in humans and animals. EOs also offer calming and invigorating fragrances via aromatherapy. The physical and chemical properties of EOs allow them to quickly move through the air and directly interact with the olfactory sensors in the nose, which is why EOs are commonly used for aromatherapy for dogs. There is growing interest in aromatic and medicinal plants because of their antioxidative and anti-inflammatory properties in both scientific research and industry (Singh et al. 2005; Isbilir and Sagiroglu 2011). EOs can be used for a wide range of emotional wellness of dogs and cats. Depending on a need, they can be used as a single EO or in combination with other EOs. In case of dogs, EOs are commonly used for arthritis, bruises, scars, strains, skin issues (such as eczema, bacterial or fungal infections), flea control, and many other conditions. Additionally, EOs can be used as biopreservatives, reducing or eliminating pathogenic bacteria and increasing the overall quality of animal and vegetable food products (Solórzano-Santos and Miranda-Novales 2012). In dairy cows, EOs such as garlic oil, eugenol, cinnamaldehyde, and capsicum (singly or in combination) have been found to modulate rumen fermentation and responses of immune and lactation (Cardozo et al. 2004; Tekippe et al. 2013; Oh et al. 2015; Oguey and Wall 2016; Stelwagen et al. 2016). This chapter offers a brief account of some commonly used EOs, while other are listed in Table 1.

A. Srivastava · R. Lall · A. Sinha
Vets Plus Inc., Menomonie, WI, USA

R. C. Gupta (✉)
Toxicology Department, Breathitt Veterinary Center, Murray State University, Hopkinsville, KY, USA
e-mail: rgupta@murraystate.edu

R. C. Gupta et al. (eds.), *Nutraceuticals in Veterinary Medicine*, https://doi.org/10.1007/978-3-030-04624-8_11

Table 1 Essential oils (EOs), chemical constituents, and their clinical applications

Essential oil/source	Chemical constituents	Clinical applications	References
Ajenjo oil (*Artemisia echegarayi* Hieron.)	Thujone, camphor	Antibacterial, free radical scavenging	Laciar et al. (2009)
Alpinia galanga Willd. (L.) oil	1,8-Cineole, α-terpineol, α-pinene, β-pinene, terpinene-4-ol, chavicol, (*E*)-β-farnesene, β-sesquiphellandrene, β-bisabolene, eugenol acetate	Antiseptic, expectorant, anesthetic	Raina et al. (2014)
Alpinia officinarum Hance. oil	1,8-Cineole, α-terpineol, α-pinene, β-pinene, terpinene-4-ol, camphor, α-fenchyl acetate	Antiseptic, expectorant, anesthetic	Raina et al. (2014)
Alpinia polyantha D. Fang oil	Camphor, α-pinene, β-pinene, β-agarofuran, globulol, β-cubebene, fenchyl acetate, β-maaliene, aristolone, δ-cadinene, β-caryophyllene, α-muurolene		Huong et al. (2015)
Anthemis aciphylla Boiss.	α-pinene, terpinen-4-ol, isofaurinone	Antimicrobial	Baser et al. (2006)
Arborvitae oil (*Platycladus orientalis* L.)	α-Pinene, α-cedrol, δ-3-carene, limonene, β-caryophyllene, myrcene	Anti-inflammatory, antibacterial, antifungal, antihypertensive, hemostatic, anticancer, anti-gout, insecticidal	Hashemi and Safavi (2012), Puškárová et al. (2017)
Basil oil (*Ocimum basilicum* L.)	Monoterpenes, sesquiterpene hydrocarbons, oxygenated sesquiterpenes	Antioxidative, anti-inflammatory, antiviral, antibacterial, diuretic, antispasmodic, appetizer, nervous system stimulatory, anti-warts, anti-fatigue, muscle relaxant, food preservative	Lemberkovics et al. (1998), Suppakul et al. (2003), Kuorwel et al. (2011), Chenni et al. (2016), Sakkas and Papadopoulou (2017)
Cardamom oil (*Elettaria cardamomum*)	Pinene, methyl eugenol, sabinene, geraniol, myrcene, phellandrene, 1,8-cineole, citronellol, linalool, limonene, *p*-cymene, terpinolene, *trans*-nerolidol	Restores hair loss, skin treatment, antioxidative, anti-inflammatory, analgesic, muscle relaxant, antispasmodic, diuretic	Al-Zuhair et al. (1996)
Chamomile oil/Blue oil (*Matricaria chamomilla* L.)	Terpenoids, Flavonoids, α-bisabolol, sesquiterpene derivatives, chamazulene	Anti-inflammatory, antiseptic, carminative, sedative, spasmolytic	Salmon (1992), McKay and Blumberg (2000), Srivastava et al. (2010), Singh et al. (2011)
Cinnamon/Cassia oil (*Cinnamomum cassia*)	(−)-Cinnamaldehyde, *o*-methoxy-cinnamaldehyde, cinnamyl acetate, cinnamic acid	Antibacterial	Burt (2004), Oussalah et al. (2006)
Citrus maxima Burm., *Citrus sinensis* L.	DL-Limonene	Antioxidative, antifungal	Singh et al. (2010)
Clove oil (*Syzygium aromaticum*)	Eugenol, eugenin, eugenitin, acetyl eugenol, bicornin, β-caryophyllene, vanillin, crategolic acid, campesterol, sesquiterpenes, α-humulene	Antioxidative, anti-inflammatory, antibacterial, antifungal, anti-gingivitis	Pinto and Santos (2017), Nagababu and Lakshmaiah (1992), Viuda-Martos et al. (2007a, b), Rodrigues et al. (2009), Białoń et al. (2017), Puškárová et al. (2017)
Coriander oil (*Coriandrum sativum*)	Tannins, terpenoids, alkaloids, phenolics, flavonoids, sterols, glycosides	Antioxidative, anti-inflammatory, analgesic, antibacterial, antifungal, anthelmintic, anxiolytic, antidepressant, sedative, hypnotic, anticonvulsant, anti-Alzheimer's, neuroprotective, hypolipidemic, antidiabetic, anticancer, hepatoprotective	Cantore et al. (2004), Al-Snafi (2016)
Cudrania tricuspidata oil	Cudraxanthone L, cudratricusxanthone A, cudratricusxanthone E, macluraxanthone B	Antibacterial, hepatoprotective	Tian et al. (2005), Bajpai et al. (2013)

(continued)

Table 1 (continued)

Essential oil/source	Chemical constituents	Clinical applications	References
Cumin oil (*Cuminum cyminum* L.)	β-Pinene, γ-pinene, *p*-cymene, γ-terpinene, cuminic aldehyde, cuminal	Antimicrobial, antifungal	Viuda-Martos et al. (2007b), Wanner et al. (2010)
Dill seed oil (*Anethum graveolens* L.)	α-Phellandrene, β-phellandrene, dill ether, *p*-cymene, limonene, anethofuran, carvone, carveol, isoeugenol, dill apiole	Antioxidative, antibacterial, antifungal, sensory importance	Porter et al. (1983), Brunke et al. (1991), Vera and Chane-Ming (1998), Hosseinzadeh et al. (2002), Singh et al. (2005), Isbilir and Sagiroglu (2011), Rana and Blazquez (2015)
Eucalyptus oil (*Eucalyptus citriodora*, *E. tereticornis*, *E. globulus*)	Borneol, 1,8-Cineole, globulol, *p*-cymene, limonene, α-pinene, cryptone, α-terpineol	Antibacterial, anti-gingivitis, anti-gum bleeding, anti-plaque, anti-inflammatory, central and peripheral analgesic	Silva et al. (2003), Sebei et al. (2015), Singh et al. (2016)
Fennel oil (*Foeniculum vulgare*)	*Trans*-anethole, (*E*)-anethole	Antioxidative, anti-inflammatory, analgesic, antimicrobial, laxative, carminative, facial cleanser	Yamini et al. (2002), Choi and Hwang (2004), Cetin et al. (2010), Picon et al. (2010), Mohamad et al. (2011), Qiu et al. (2012)
Frankincense/ Olibanum/Levona oil (*Boswellia Sacra*)	Monoterpenes, sesquiterpenes, monoterpenols, sesquiterpenols, phellandrene, ketones, limonene, α-thujone, α-pinene, β-pinene	Perfume, cosmetic, aromatherapy, antidiabetic, cytochrome P450 inhibitor	Frank and Unger (2006), Woolley et al. (2012)
Garlic oil (*Allium sativum* L.)	Diallyl trisulfide, diallyl disulfide, methyl allyl trisulfide, thiosulfinates, allicin	Antibacterial, antifungal, antiviral, antiparasitic	Rao et al. (1999), Kyung (2012), Djiri et al. (2014)
Geranium oil (*Pelargonium graveolens* L.)	Geraniol, citronellol, linalool, α-pinene, myrcene, linalool, γ-terpinene, neral	Antioxidative, aromatherapy, muscle relaxant, skin care, anti-dysentery, anti-hemorrhoids, anti-inflammatory, antidiabetic, anti-diarrhea, anti-gastric ulcers, anticancer	Sharopov et al. (2014)
Ginger oil (*Zingiber officinale* Roscoe)	Zingiberene, e-citral, z-citral, camphene, ocimene, ar-curcumene, β-bisabolene, β-sesquiphellandrene	Antioxidative, free radical scavenging, hydrogen peroxide scavenging, antibacterial, antifungal	Stoyanova et al. (2006), Bellik et al. (2013), Raina et al. (2013)
Helichrysum oil (*Helichrysum italicum*)	α-cedrene, α-curcumene, geranyl acetate, limonene, nerol, neryl acetate, α-pinene	Antioxidative, anti-inflammatory, antibacterial, antiviral, antifungal	Sala et al. (2002), Nostro et al. (2003), Djihane et al. (2017)
Hemp oil/CBD oil (*Cannabis sativa* L.)	Cannabidiol (CBD), THC, β-caryophyllene, limonene, β-myrcene	Relief of pain, anti-arthritis, anti-atopic dermatitis, antidiabetic, anti-epileptic seizures, neuroprotection, antioxidative, anti-inflammatory	–
Juniper oil (*Juniperus communis* L., *J. oxycedrus*)	α-Pinene, sabinene, β-myrcene, *p*-cymene, geranial, α-caryophyllene, β-caryophyllene, germacrene D, δ-cadinene, *trans*-calamenene, D-limonene, terpinene-4-ol, terpinolene, 3-carene, β-phellandrene, β-thujone, α-humulene	Antibacterial, antifungal	Filipwicz et al. (2003), Białoń et al. (2017), Glisic et al. (2007)
Lantana xenica Mold. oil	(*E*)-caryophyllene, γ-cadinene, α-pinene, ocimene, germacrene D	Antimicrobial	Juliani Jr et al. (2002)
Lantana achyranthifolia Desf. oil	Carvacrol, 1,8-cineole, isocaryophyllene, α-bisabolol, β-bisabolene	Antibacterial	Hernández et al. (2005)
Lavender oil (*Lavandula latifolia*, *L. angustifolia*)	1,3,7-Octatriene, 3,7-dimethyl, α-pinene, limonene, 1,8-cineole, *cis*-ocimene, *trans*-ocimene, camphor, linalool, caryophyllene, terpinen-4-ol, lavandulyl acetate, eucalyptol, camphor	Antiseptic, analgesic, anticonvulsant, antidepressant, antirheumatic, antioxidative, anti-inflammatory, antiviral, antibacterial, carminative, cholagogue, deodorant, diuretic, emmenagogue, hypotensive, sedative, sudorific	Shellie et al. (2002), Hui et al. (2010), Robu et al. (2011), Adaszynska-Skwirzynska and Szczerbinska (2017)

(continued)

Table 1 (continued)

Essential oil/source	Chemical constituents	Clinical applications	References
Lemon citrus oil (*Citrus limon*)	Limonene, β-pinene, α-pinene, sabinene, myrcene, limonene, 1,8-cineole, linalool, α-terpineol, nerol, neradiol, geranial, farnesol	Antioxidative, antimicrobial, antifungal	Hsouna et al. (2017)
Lemon grass oil (*Cymbopogon citratus*)	Myrcene, citronellal, geranyl acetate, neral, nerol, geraniol, limonene, citral	Anti-stress, anti-mental fatigue, glandular secretion stimulatory, antimicrobial, insect-repellent	Abdulazeez (2016),Lawal (2017)
Marjoram oil	α-Pinene, β-pinene, limonene	Antibacterial, antiviral, antifungal, antidiabetic, antidepression, pain killer	Białoń et al. (2017)
Metasequoia glyptostroboides Mikiex oil	α-Pinene, β-caryophyllene, α-thujone, bornylene, totarol, δ-3-carene, 2-β-pinene, α-humulene	Antifungal	Bajpai et al. (2007), Bajpai and Kang (2010)
Myracrodruon urundeuva oil	α-Pinene, β-pinene, *trans*-caryophyllene, limonene	Antibacterial, cytotoxic	Rebouças de Araujo et al. (2017)
Myrrh oil (*Commiphora myrrha*)	Furanoeudesma-1, 3-diene, curzarene, sterols, steroids	Antibacterial, antiviral, antifungal, astringent, analgesic, antispasmodic, amoebocidal, anti-inflammatory, hypoglycemic	Dolara et al. (2000), Zhu et al. (2003), Tipton et al. (2006)
Myrtle oil (*Myrtus communis* var. *italica* L.)	α-pinene, 1,8-cineole, flavonoids, hydrolysable tannins (gallotannins), catechin	Antioxidative	Aidi et al. (2010)
Nigella sativa oil	Thymoquinone	Antioxidative, anti-inflammatory, anticancer, anti-proliferative	Burits and Bucar (2000), Gupta et al. (2016)
Nutmeg seed oil (*Myristica fragrans* Houtt.)	Sabinene, α-pinene, α-thujone, α-myrcene, α-terpinene, α-terpineol, 4-terpineol, myristicin, safrol, citronellol	Locomotor inhibitory, anxiogenic, anti-chronic inflammatory pain	Muchtaridi et al. (2010), Zhang et al. (2016)
Olive oil (extra virgin)	Phenolic compounds	Antioxidative, anti-inflammatory, antimicrobial	Cicerale et al. (2012)
Omani luban oil (*Boswellia sacra* Flueck)	Limonene, myrcene, α-pinene	Antibacterial	Al-Saidi et al. (2012)
Oregano oil (*Origanum vulgare* L.)	α-Thujone, α-pinene, sabinene, β-pinene, β-myrcene, α-phellandrene, α-terpinene, *p*-cymene, limonene, 1,8-cineole, linalool, γ-terpinene, thymol, carvacrol, caryophyllene, etc.	Antioxidative, antibacterial, antiviral, antifungal, cytotoxic, food preservative	Lambert et al. (2001), Figiel et al. (2010), Bolechowski et al. (2011), Kuorwel et al. (2011), Sakkas and Papadopoulou (2017), De Falco et al. (2013), Siroli et al. (2014), Stanojević et al. (2016), Białoń et al. (2017), Puškárová et al. (2017)
Palmarosa oil (*Cymbopogon martinii* var. *motia* Burk.)	(*E*)-β-ocimene, linalool, geraniol, geranyl acetate, (*E,Z*)-farnesol, β-caryophyllene	Antioxidative, anti-inflammatory, antibacterial, antiviral, antifungal, fever, arthritis	Puškárová et al. (2017)
Palo Santo oil (*Bursera graveolens*)	α-Terpinene	Antioxidative, antimicrobial	Mendez et al. (2017)
Peppermint oil (*Mentha piperita* L.)	Menthol, menthone, menthyl acetate, 1,8-cineole, limonene, β-pinene, flavonoids, β-caryophyllene	Antioxidative, anti-inflammatory, analgesic, cytoprotective, antiulcer, chemopreventive, anti-gingivitis	Blumenthal et al. (1998), Kumar et al. (2009), Taheri et al. (2011)
Perilla oil (*Perilla frutescens* L.)	(−)-perillaldehyde, (−)-perillylalcohol, (+)-limonene, α-pinene, luteolin, perillic acid, carvane, *trans*-shisool, perillaketone	Olfactory stimulation, prefrontal cortex inhibitory, cytostatic, antiallergic, antidepressant, antibacterial, antifungal, anti-inflammatory	Solórzano-Santos and Miranda-Novales (2012), Igarashi and Miyazaki (2013), Igarashi et al. (2014)
Piper cernuum oil	α-Pinene, β-pinene, bicyclogermacrene, β-caryophyllene, spathulenol, germacrene D	Antimicrobial	Constantin et al. (2001)

(continued)

Table 1 (continued)

Essential oil/source	Chemical constituents	Clinical applications	References
Piper regnellii oil	Linalool, myrcene, bicyclogermacrene, β-caryophyllene	Antimicrobial	Constantin et al. (2001)
Plectranthus spp. (*P. cylindraceus*, *P. asirensis*, *P. barbatus*) oil	Maaliol, β-caryophyllene, α-pinene, borneol, spathulenol	Antioxidative, Antimicrobial, anticancer, larvicidal	Govindarajan et al. (2016), Mothana et al. (2018)
Protium oil *heptaphyllum* Aubl.	α-Pinene, *p*-mentha-1,4(8)-diene, α-phellandrene, α- and β-amyrin	Gastroprotective, anxiolytic, antidepressant, antioxidative	Aragão et al. (2006), Araujo et al. (2011)
Rose oil (*Rosa damascene* Mill.)	Citronellol, geraniol, nonadecane, nerol, heneicosane, linalool	Flavor, fragrance	Almasirad et al. (2011), Verma et al. (2011)
Rosemary oil (*Rosmarinus officinalis*)	1,8-Cineol, camphor, α-pinene, β-pinene	Antioxidative, anti-gastric ulcers, antimicrobial	Bozin et al. (2007), Viuda-Martos et al. (2007b), Zaouali et al. (2010), Takayama et al. (2016)
Sage oil (*Salvia officinalis*, *Salvia hispanica*)	Aesculetin, camphor, 1,8-cineole, camphene, α-humulene, α-thujene, α-thujone, β-thujone, α-terpineol, α-terpenes, α-pinene, linalool, limonene, salviol, thujanol, terpinolene	Antibacterial, antifungal, antioxidative, antiseptic, antispasmodic, cholagogue, choleretic, febrifuge, emmenagogue, anti-dermatitis, anti-dementic	Perry et al. (2003), Delamare et al. (2007), Viuda-Martos et al. (2007b), Pierozan et al. (2009), Porte et al. (2013)
Sandalwood oil (*Santalum spicatum*, *S. acuminatum*, *S. album*, *S. latifolium*)	Santalols, α-bergamotol, nuciferol, γ-elemene, *cis*-lanceol, α-cedrol	Anti-inflammatory, aromatherapy	Howes et al. (2004), Kusuma and Mahfud (2016)
Savory oil (*Satureja montana* L.)	α-Thujone, α-pinene, myrcene, *p*-cymene, sabinene, α-terpinene, camphor, borneol, carvacrol, thymol, caryophyllene oxide	Antibacterial	Milos et al. (2001), Oussalah et al. (2006)
Senecio atacamensis Phil. oil	α-terpinene, α-phellandrene, *p*-cymene	Antimicrobial	Benites et al. (2011)
Sideritis hirsuta L. oil	α-Phellandrene, β-phellandrene, α-pinene, (*Z*)-β-guaiene, Sabinene, 1,8-cineole	Anti-inflammatory, antirheumatic, antibacterial, antimicrobial	Palá-Paul et al. (2006)
Sidris italica Miller. oil	Sesquiterpenes, phenyl propane derivative	Antibacterial, antioxidative	Lemberkovics et al. (1998), Basile et al. (2006)
Spanish oregano oil (*Corydothymus capitatus*)	–	Antibacterial	Oussalah et al. (2006)
Spearmint oil (*Mentha spicata* L.)	Carvone, 1,8-cineole, β-pinene, β-caryophyllene, *trans*-dihydrocarvone	Antioxidative, antimicrobial	Hussain et al. (2011), Şarer et al. (2011)
Spruce oil (*Picea abies* L.)	α-Pinene, camphene, limonene, myrcene, bomyl acetate,δ-cadinene, muurolene, cadinol, muurolol, manool	Antimicrobial	Radulescu et al. (2011)
Summer savory oil (*Satureja hortensis* L.)	γ-Terpinene, carvacrol, α-thujone	Antioxidative, antibacterial, antifungal, antispasmodic	Sahin et al. (2003), Khalid (2016)
Tea tree oil (*Melaleuca alternifolia*)	Terpinen-4-ol, γ-terpinene, 1,8-cineol, α-cymene, δ-cadinene, globulol, linalool, limonene	Antimicrobial	Carson et al. (2006)
Thyme oil (*Thymus vulgaris*, *T. zygis*, *T. hyemalis*)	Thymol, carvacrol, terpinene-4-ol, α-pinene, γ-terpinene, *cis*-sabinene hydrate, caryophyllene, *p*-cymene, myrcene, borneol, linalool	Antibacterial, antiseptic bandages, mouth rinses, antifungal agent in toenails, anxiolytic, antioxidative, anti-inflammatory, carminative, antispasmodic, food preservative	Pierce (1999), Ramsewak et al. (2003), Burt (2004), Tawfik et al. (2006), Viuda-Martos et al. (2007a, b), Rota et al. (2006), Sakkas and Papadopoulou (2017), Kuorwel et al. (2011), Fachini-Queiroz et al. (2012), Cerda et al. (2013), Borugă et al. (2014), Komaki et al. (2016), Al-Asmari et al. (2017), Puškárová et al. (2017)

(continued)

Table 1 (continued)

Essential oil/source	Chemical constituents	Clinical applications	References
Thymus longicaulis subsp. *Chaubardii* oil	Limonene, thymol, geraniol, geranyl acetate, linalool, α-terpinyl acetate	Antimicrobial	Tzakou et al. (1998)
Valerian oil (*Valeriana officinalis* L.)	Valerenic acid, β-sitosterol, ursolic acid, caryophyllene, valerane	Anti-insomnia, anxiolytic, anti-epilepsy, antirheumatic, aphrodisiac, antispasmodic, anthelmintic, diuretic, diaphoretic, emmenagogue	Jiang et al. (2007), Nandhini et al. (2018)
Vetiver oil (*Vetiveria zizanioides*)	Khusimol, germacrene D, α- and β-vetivone, cedrane, bisabolane, eudesmane, eremophilane, zizaane, valerenol, β-caryophyllene	Antibacterial, antifungal, antioxidative, anti-inflammatory, insecticidal, herbicidal, calming dogs afraid of loud noises	Khesorn et al. (2010), Chahal et al. (2015)
Ylang Ylang oil (*Cananga odorata*)	Monoterpenes, Sesquiterpenes, phenylpropanoids	Stomach ailments, asthma, gout, rheumatism, antibacterial, antifungal, amoebocidal, cytotoxic, antibiofilm, anti-inflammatory, anti-vector, antidiabetic, antifertility, insect-repellent	Tan et al. (2015)

2 Essential Oils

2.1 Thyme Oil

There are more than 150 species of Thymus that are found in North America, Asia, and Africa. *Thymus vulgaris* is an aromatic perennial evergreen herbal plant with great medicinal value. Thyme oil contains vital ingredients for human and animal health. Oil of thyme contains 12–61% thymol. The structural formulas of thymol and carvacrol are shown in Fig. 1.

Thyme oil may also contain carvacrol (0.4–20.6%), 1,8-cineol (0.2–14.2%), *p*-cymene (9.1–22.2%), linalool (2.2–4.8%), borneol (0.6–7.5%), α-pinene (0.9–6.6%), and camphor (0.6–7.3%). Thymol and carvacrol are the main phenolic components that are primarily responsible for thyme's antioxidative activity (Yanishlieva et al. 1999; Rota et al. 2006; Komaki et al. 2016; Al-Asmari et al. 2017).

Thymol possesses an antiseptic property, and therefore it is used in bandages and several commercially available mouthwashes, including Listerine (Pierce 1999). Ramsewak et al. (2003) reported that thyme oil can be used as an antifungal agent for infected toenails. Its use has also been indicated for coughs and bronchitis. Recently, Komaki et al. (2016) demonstrated that the extract of *Thymus vulgaris* exerts an anxiolytic effect in rats.

In recent years, thyme oil as a nutraceutical has become more popular than ever before due to its antioxidant and anti-inflammatory properties (Amiri 2012; Cerda et al. 2013). Additionally, thyme is known to exert antispasmodic, antivirotic, antifungal, carminative, sedative, diaphoretic, antihypertensive, anticancer, and anti-acne effects (Viuda-Martos et al. 2007a, b; Fachini-Queiroz et al. 2012; Ahmad et al. 2014).

2.2 Rosemary Oil

Rosemary essential oil (EO) is obtained from the leaves and flowers of the *Rosmarinus officinalis* plant, which is native to the Mediterranean region. The ancient Greeks, Romans, Egyptians, and Hebrews considered rosemary oil a sacred substance as it improved memory, soothed digestive problems, boosted the immune system, and relieved aches and pains. Phytochemical analysis of rosemary oil revealed

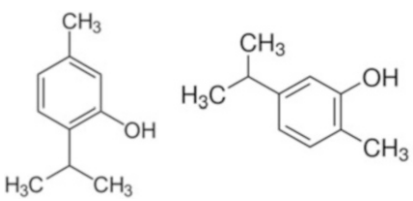


Fig. 1 Structural formula of thymol (left) and carvacrol (right)

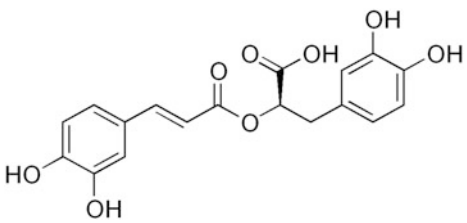


Fig. 2 Structural formula of rosmarinic acid

the presence of rosmarinic acid (Fig. 2), 1,8-cineol, camphor, carnosol, carnosic acid, ursolic acid, and caffeic acid (Zaouali et al. 2010; Takayama et al. 2016). Using GC/MS, Takayama et al. (2016) identified three major monoterpenes: cineol (28.5%), camphor (27.7%), and α-pinene (21.3%) in *R. officinalis* essential oil.

For thousands of years, rosemary EO has been used in many health conditions, including alopecia, cognition dysfunction, and as an antinociception, liver cleansing, cortisol lowering, and anticancer agent. In GI abnormalities, the gastric epithelium is often attacked by physical, chemical, or microbiological agents acting in the gastric lumen. Rosemary EO protected the gastric mucosa via its antioxidative property (Rozza and Pellizzon 2013; Dawidowicz and Olszowy 2014) by modulating the activities of enzymes (superoxide dismutase and glutathione peroxidase) and increasing or maintaining the levels of glutathione (Takayama et al. 2016). In several other experimental models of GI inflammatory conditions, rosemary EO has been found effective (Dias et al. 2000; Minaiyan et al. 2011).

Zaouali et al. (2010) reported antioxidative and antimicrobial activities of *R. officinalis* L. varieties (var. *typicus* and var. *troglodytorum*). The bactericidal and antioxidative activities were higher with EO from var. *troglodytorum* than those from var. *typicus*.

2.3 Oregano Oil

Oregano (*Origanum vulgare*) is native to the Mediterranean countries, such as Italy and Spain, and is used as a spicy herb. The EO of oregano contains more than 25 compounds, including α-thujone, α-pinene, sabinene, β-pinene, β-myrcene, α-phellandrene, α-terpinene, *p*-cymene, limonene, 1,8-cineol, γ-terpinene, linalool, thymol, carvacrol, caryophyllene, etc. (Figiel et al. 2010; Bolechowski et al. 2011). Oregano EO is known to exert strong antioxidant, antimicrobial, cytotoxic, and antifungal activities (Viuda-Martos et al. 2007a, b; Wojdylo et al. 2008; Siroli et al. 2014). By having these properties, the EO of oregano can be used in many ailments of humans and animals, as well as a food preservative.

2.4 Basil Oil

The EOs of basil (*Ocimum basilicum* L.) have been used extensively for a long time in food products, dental and oral products, and perfumery. Chenni et al. (2016) identified 65 chemical compounds in EOs extracted from Egyptian basil leaves. The main components were linalool (43.5–48.4%), methyl chavicol (13.3–14.3%), and 1,8-cineol (6.8–7.3%). These authors suggested that the solvent-free microwave extraction (SFME) method may be a better alternative to the conventional hydro-distillation (HD) method for the extraction of EO from basil leaves. This is because the SFME method may provide a richer source of natural antioxidants as well as strong antimicrobial agents for food preservation. Basil EOs and their major chemical constituents have been reported to exhibit antimicrobial activity against a wide range of Gram-negative and Gram-positive bacteria, fungi, and yeast (Suppakul et al. 2003).

2.5 Chamomile Oil

Chamomile is one of the medicinal herbs native to Southern and Eastern Europe. Currently, it is grown in many European countries, Brazil, India, and many other parts of the world. On the international market, there is a growing demand for chamomile oil. German chamomile (*Matricaria chamomilla*), commonly referred to as "Blue oil," and Roman chamomile (*Chamaemelum nobile*) are more popular than other chamomiles. Phytochemical analysis of chamomile has revealed more than 120 secondary metabolites, 28 terpenoids, and 36 flavonoids (McKay and Blumberg 2000; Singh et al. 2011). The principle components of the essential oil extracted from the German chamomile flowers are the terpenoids α-bisabolol and its oxide azulenes, including chamazulene and acetylene derivatives. Both chamazulene and bisabolol are very unstable and are best preserved in an alcoholic tincture. The essential oil of Roman chamomile contains less chamazulene and is mainly constituted of esters of angelic acid and tiglic acid. It also contains farnesene and α-pinene. In chamomile, α-bisabolol, bisabolol oxides A and B, chamazulene or azulene, farnesene, spiroether sesquiterpene lactones, glycosides, hydroxycoumarines, flavonoids (apigenin, luteolin, patuletin, and quercetin), coumarins (herniarin and umbelliferone), terpenoids, and mucilage are considered to be the major bioactive ingredients (Singh et al. 2011). Depending upon the chemical constituents and qualitative and quantitative composition, there are four basic types of chamomile essential oils (EOs):

Type A: Dominant component is bisabolol oxide A
Type B: Dominant component is bisabolol oxide B
Type C: Dominant component is (−)-α-bisabolol
Type D: (−)-α-bisabolol and bisabolol oxide A and B present in 1:1 ratio

The biological and pharmacological properties of chamomile oil include antioxidative, anti-inflammatory, antiseptic, carminative, sedative, and spasmolytic activities, and as a result its preparations have been used for many human ailments such as hay fever, skin conditions (eczema, chicken pox, and psoriasis), muscle spasms, neuralgia, sciatica,

rheumatic pain, menstrual disorders, insomnia, ulcers, wounds, GI disorders, and hemorrhoids (Salmon 1992; Srivastava et al. 2010; Singh et al. 2011). In veterinary medicine, the use of chamomile is yet to be explored.

Srivastava et al. (2010) reported an anti-inflammatory effect due to chamomile constituents, such as bisabolol, chamazulene, apigenin, and luteolin, although the exact mechanism is yet to be elucidated. In experimental studies, coumarins, bisabolol, and flavonoids have been shown to exert smooth muscle relaxant and antispasmodic effects (Gardiner 2007; Srivastava et al. 2010). In humans, Merfort et al. (1994) demonstrated that chamomile flavonoids and essential oils penetrate below the skin surface into the deeper skin layers, thereby exerting an anti-inflammatory activity. Chamomile oil produced an anti-inflammatory effect by inhibiting LPS-induced prostaglandin E_2 release and accumulation of cyclooxygenase-2 (COX-2) enzyme activity without affecting the constitutive from COX-1 (Srivastava et al. 2009).

Topical applications of chamomile have been shown to be moderately effective in the treatment of atopic eczema and other skin diseases (Nissen et al. 1988; Graf 2000). Results revealed that it was about 60% as effective as 0.25% hydrocortisone cream (Albring et al. 1983). In a partially double-blind randomized study, Roman chamomile-based cream Kamillosan® showed a slight superiority over 0.5% hydrocortisone (Patzelt-Wenczler and Ponce-Pöschl 2000). More investigations need to be done in this area of research.

Chamomile has been used in many GI conditions, including GI spasms, GI irritation, stomach upset, flatulence, and ulcers (Kroll and Cordes 2006), and GI inflammatory conditions such as esophageal reflux, diverticular disease, etc. Chamomile has specifically been found useful in dispelling gas, soothing the stomach, and relaxing the muscles that move food through the intestines (reviewed in Srivastava et al. 2010). Reports suggested that chamomile ointment may improve hemorrhoids by reducing the inflammation (Misra and Parshad 2000). Studies also proved that chamomile inhibits *Helicobacter pylori*, the bacteria that can contribute to stomach ulcers. Chamomile reduces smooth muscle spasms associated with various GI inflammatory disorders.

Chamomile has been indicated in the treatment of generalized anxiety disorder. One of chamomile's bioactive compounds apigenin has been shown to reduce latency in the onset of picrotoxin-induced convulsions and reduction in locomotor activity but did not demonstrate any anxiolytic, myorelaxant, or anticonvulsant activities (Avallone et al. 2000).

Chamomile has been found very effective in managing hyperglycemia and diabetes. It ameliorates hyperglycemia and diabetic complications, independent of insulin secretion, by suppressing blood sugar levels, increasing liver glycogen storage, inhibiting sorbitol in the erythrocytes, and protecting pancreatic beta cells from oxidative stress (Cemek et al. 2008; Srivastava et al. 2010).

Chamomile has been reported to be statistically efficacious in producing wound drying, speedy epithelialization, and wound healing (Glowania et al. 1987; Nayak et al. 2007). In aromatherapy, chamomile massage has also been shown to exert positive effects on anxiety (Hadfield 2001). Additionally, chamomile can be used in many other conditions such as cardiovascular, mucositis, osteoporosis, sleep disorder, sore throat, vaginitis, immunodeficiency, and cancer (Srivastava et al. 2010; Singh et al. 2011).

Although chamomile is listed on the FDA's GRAS (generally recognized as safe) list, it may produce allergic reaction probably due to its contamination with "dog chamomile," a highly allergenic plant of similar appearance (Srivastava et al. 2010).

2.6 Cinnamon Oil

Cinnamon essential oil (EO) is obtained from the outer bark or leaves of *Cinnamomum zeylanicum* and *Cinnamomum vervun* (both names refer to the same tree). The main chemical constituents of cinnamon bark and leaf EOs, in varying amounts, are cinnamaldehyde, cinnamyl acetate, eugenol, and eugenol acetate. The EO of the bark of *C. altissimum* Kosterm consists of linalool (36.0%), methyl eugenol (12.8%), limonene (8.3%), α-terpineol (7.8%), terpinen-4-ol (6.4%), γ-terpinene (3.5%), α-terpinene (2.3%), and 1,8-cineol (2.3%) (Abdelwahab et al. 2017).

Cinnamaldehyde exhibits antibacterial and antifungal activities, in addition to imparting an aroma scent. Cinnamyl acetate is usually used to repel and prevent insect infestation, in addition to its common use as a fixative in manufactured perfumes. Eugenol exerts anti-inflammatory, antiseptic, and analgesic properties, soothes ulcers and gastric pain, reduces chances of developing sores, eliminates bacteria, and prevents the growth of many fungi. Eugenol acetate is a well-known antioxidant.

Cinnamon EO is known to diminish the feelings of depression, faintness, and exhaustion. It stimulates the libido and is often regarded as a natural aphrodisiac. It also stimulates the GI and immune systems and relieves muscle and joint pain associated with rheumatism. Cinnamon EO has calming and tonic effects on the mind and can improve cognitive function and memory. In essence, cinnamon EO has many medicinal properties such as antioxidative, anti-inflammatory, antibacterial, antifungal, antiviral, astringent, antispasmodic, immunostimulating, carminative, emmenagogue, antirheumatic, etc.

2.7 Clove Oil

Clove essential oil or oil of clove is obtained from the buds and stems of clove trees (*Syzygium aromaticum*) which grow in tropical regions. Xu et al. (2016) identified 22 chemical compounds in the EO of clove with 76.23% eugenol. Clove EO has been found very effective against many strains of bacteria, including *E. coli*, *Salmonella*, *Helicobacter pylori*, *Staphylococcus aureus*, *Streptococcus pneumonia*, and many others. Its other properties include antioxidative, anti-inflammatory, antibacterial, antiviral, antifungal, antitumoral, anticoagulant, and anticancer (cytotoxicity and apoptosis toward cancerous cells).

2.8 Dill Seed Oil

Anethum graveolens L., commonly known as dill, is native to the Mediterranean countries and Southeastern Europe. In an earlier study, Porter et al. (1983) found four major constituents (phellandrene, limonene, anethofuran, and carvone) in dill oil. Limonene and phellandrene were maximally present in stems and leaves. Anethofuran was a major component of the oil from umbels at flowering but declined during seed maturation. Within the umbels, the carvone was present in the seeds only and not the pedicels (Porter et al. 1983). Using GC/MS, at least 36 chemicals (including α-phellandrene 56.5%, dill ether 20.8%, limonene 10.9%, *p*-cymene 3.8%) have been identified in the EO of *A. graveolens*, totaling 92% (Vera and Chane-Ming 1998; Singh et al. 2005; Rana and Blazquez 2015). Carvone is commonly regarded as the component having the strongest influence on the character of dill oils.

Phytoconstituents of dill oil and its extract have free radical scavenging, hydrogen peroxide scavenging, antioxidative, antibacterial, and antifungal properties (Singh et al. 2005; Isbilir and Sagiroglu 2011). Dill is used as a diuretic and also to solve certain gastrointestinal (GI) problems, such as flatulence, intestinal spasms, and various digestive problems (Hosseinzadeh et al. 2002).

2.9 *Eucalyptus* Oil

Eucalyptus EO is obtained from *Eucalyptus citriodora*, *E. camaldulensis*, *E. urophylla*, *E. lehmani*, *E. leucoxylon*, *E. cinerea*, and many other species. The value of *Eucalyptus* oil for medicinal purposes can vary depending on several factors, such as species of *Eucalyptus* and its chemical constituents (Sebei et al. 2015). The EOs from *Eucalyptus* leaves have been found to contain chemicals from three classes: (1) oxygenated monoterpenes (1,8-cineole, *trans*-pinocarveol, and α-terpineol), (2) oxygenated sesquiterpenes (borneol, spathulenol, viridiflorol, and globulol), and (3) monoterpene hydrocarbons (α-pinene, *p*-cymene, and limonene). The major constituents in *E. camaldulensis* leaf EO are α-pinene (22.52%), *p*-cymene (21.69%), α-phellandrene (20.08%), 1,8-cineole (9.48%), c-terpinene (9.36%), and limonene (4.56%) (Cheng et al. 2008). In a recent study, Sebei et al. (2015) reported that the EOs of *Eucalyptus* leaves contain two major phytoconstituents, 1,8-cineole (49.07–83.59%) and α-pinene (1.27–26.35%).

Eucalyptus EO has been reported to exert antiradical and antioxidative (Ben Hassine et al. 2012), anti-inflammatory (Silva et al. 2003), antipyretic (Silva et al. 2003), antibacterial (Cimanga et al. 2006; Sebei et al. 2015), and antifungal (Su et al. 2006) properties. In humans, *Eucalyptus* oil is widely used for many health conditions, including asthma, bronchitis, plaques and gingivitis, head lice, and toenail fungi.

2.10 Citrus Lemon Oil

Citrus, including lemon (*Citrus limon*), plants constitute one of the main valuable sources of EOs used in foods and for medicinal purposes (Hsouna et al. 2017). Phytochemical analysis of citrus EO revealed the presence of many chemicals with an abundance of limonene (39.74%) and β-pinene (25.44%). In a number of studies, citrus EO has been investigated for antimicrobial activities against a wide range of food-associated microorganisms (bacteria, molds, and yeasts) (Hsouna et al. 2017). Hsouna et al. (2017) suggested that citrus EO has great potential as a natural antioxidant and antimicrobial agent and can be applied in food systems and the nutraceutical industry.

2.11 Lavender Oil

Lavender EO is obtained from the flowers of certain species of lavender (*Lavandula latifolia*, *L. angustifolia*, *L. intermedia*). The phytoconstituents present in lavender oil may be more than 100 and vary from species to species. The EO primarily consists of linalool and linalyl acetate, with moderate amounts of lavandulyl acetate, terpinene-4-ol, and lavandulol. Camphor and 1,8-cineole are present in small amounts. Smigielski et al. (2009) reported 78 chemical constituents in the EO of dried flowers of *Lavandula angustifolia* (cultivated in Poland), including linalool (30.6%), linalyl acetate (14.2%), geraniol (5.3%), β-caryophyllene (4.7%), and lavandulyl acetate (4.4%). In a similar study, Robu et al. (2011) found linalool (20.60–35.99%), linalyl acetate (12.58–19.65%), lavandulyl acetate (3.74–10.48%), t-p3-ocimene (1.26–9.23%), α-terpineol (3.67–6.73%), nerol (0.81–3.32%), neryl acetate (0.95–3.64%), and β-caryophyllene (0.93–2.43%). Recently, Adaszynska-Skwirzynska and Szczerbinska (2017)

investigated the antimicrobial activity of lavender EO and its influence on the production performance of broiler chickens. These investigators found 26 chemical compounds in lavender EO with 87.79% oxygenated monoterpenes, 8.17% monoterpene hydrocarbons, and 2.93% sesquiterpene hydrocarbons. Linalool acetate (46.25%) and linalool (35.17%) were the main compounds. Results revealed that the lavender EO exhibited antimicrobial activity and significantly enhanced chicken production performance. In another study, Hui et al. (2010) reported antioxidative activity and inhibitory activity of lavender EO against rhinitis-related bacteria (*Staphylococcus aureus*, *Micrococcus ascoformans*, *Proteus vulgaris*, and *Escherichia coli*). Other uses of lavender EO include antiseptic, analgesic, anticonvulsant, antidepressant, antirheumatic, antioxidative, anti-inflammatory, antiviral, antibacterial, carminative, cholagogue, deodorant, diuretic, emmenagogue, hypotensive, sedative, and sudorific.

2.12 Sage Oil

Sage EO is obtained from *Salvia officinalis*, a perennial shrub native to the Mediterranean region. Porte et al. (2013) identified 47 chemicals in the EO of sage, including α-thujone (40.9%), camphor (26.12%), α-pinene (5.85%), and β-thujone (5.62%). By having antimicrobial and antioxidative properties, sage EO is commonly used on meat, sausage, poultry stuffing, fish, soups, canned foods, and other food stuffs (reviewed in Porte et al. 2013).

2.13 Peppermint Oil

Peppermint (*Mentha piperita* L.) is a hybrid of water mint and spearmint and is native to Europe. The EO is extracted from the leaves and flowers of the plant. Its oil consists of menthol, menthone, menthyl acetate, 1,8-cineole, limonene, β-pinene, β-caryophyllene, and flavonoids. Peppermint EO is used for more than 35 purposes, including neurological pain, digestive issues, oral health, infectious diseases, immune deficiency, muscle and arthritic pain, skin care, burn, allergies, and lice and flea control.

2.14 Hemp/CBD/Cannabis Oil

Cannabis/hemp/cannabidiol (CBD) oil has been used in medicine for millennia. The phytochemistry of hemp oil is complex as it contains CBD, THC, β-caryophyllene, limonene, β-myrcene, humulene, α-pinene, linalool, terpinolene, etc. Due to behavioral side effects from the abuse of cannabis products, the use of medical marijuana continues to be an emotionally and politically charged issue. CBD oil has shown remarkable health benefits in many conditions including pain, seizures, osteoarthritis, atopic dermatitis, obesity, diabetes, COPD, hypertension, cancer, etc. Readers are referred to a chapter on cannabis in this book for detailed phytochemistry, pharmacology, uses, and health risks.

2.15 Palmarosa Oil

Palmarosa EO is extracted from the *Cymbopogon martinii* var. motia plant. The plant is indigenous to India. Rao et al. (1999) identified 17 phytoconstituents accounting for 95.6–97.1% of the oil. Major phytoconstituents were (*E*)-β-ocimene (1.2–4.3%), linalool (0.8–2.0%), geraniol (70.1–85.3%), geranyl acetate (4.3–14.8%), and (*E,Z*)-farnesol (1.6–3.4%). Whole herb oil was found to be richer in linalool, β-caryophyllene, and *(E,Z)*-farnesol. While leaf lamina and leaf sheath oils were found to be rich in geraniol, inflorescence oil was rich in (*E*)-β-ocimene and geranyl acetate. In addition its rose-like aroma, palmarosa EO has antioxidative (Puškárová et al. 2017), anti-inflammatory, bactericidal, antiviral, and antifungal activities and is commonly used for fever, infectious diseases, wounds, arthritis and rheumatism, digestive disorders, and damaged and dry skin as well as stress and nerve-related pain.

2.16 Cardamom Oil

Cardamom EO is obtained from *Elettaria cardamomum*. The oil consists of many phytochemicals, including pinene, methyl eugenol, sabinene, geraniol, myrcene, phellandrene, 1,8-cineole, citronellol, linalool, limonene, *p*-cymene, terpinolene, and *trans*-nerolidol. In addition to aromatherapy, cardamom EO exhibits antioxidative, anti-inflammatory, analgesic, and antispasmodic properties. The oil is commonly used for skin treatment, coughs, nausea, and heartburn (Al-Zuhair et al. 1996). It is also used as a muscle relaxant, diuretic, and an antibacterial agent.

2.17 Coriander Oil

Coriander EO is extracted from *Coriandrum sativum*. Phytochemical analysis revealed the presence of many bioactive chemicals, including alkaloids, terpenoids, tannins, phenolics, flavonoids, sterols, and glycosides (Al-Snafi 2016). Al-Snafi (2016) and Cantore et al. (2004) reported that coriander EO has many biological and pharmacological properties and is thereby used as an antioxidative, anti-inflammatory, analgesic, antibacterial, antifungal, anthelmintic, anxiolytic, antidepressant, sedative, hypnotic, anticonvulsant, anti-Alzheimer's, neuroprotective, hypolipidemic, antidiabetic, anticancer, and hepatoprotective agent.

2.18 Geranium Oil

Geranium EO is extracted from *Pelargonium graveolens* L. The oil contains many phytochemical compounds, including geraniol, citronellol, linalool, α-pinene, myrcene, γ-terpinene, and neral. Pharmacologically, geranium oil is very important because it has antioxidative and anti-inflammatory activities. Geranium EO has been indicated in aromatherapy, muscle relaxation, skin care, dysentery, hemorrhoids, inflammatory conditions, diabetes, diarrhea, gastric ulcers, and cancer.

2.19 Lemon Grass Oil

Lemon grass EO is obtained from *Cymbopogon citratus*, which is native to warm and tropical regions, such as India, Southeast Asia, and Oceania. Phytochemicals present in lemon grass EO are heterogeneous in nature including limonene, citronellal, myrcene, geranyl acetate, neral, nerol, geraniol, and citral. Additionally, the oil is rich in vitamins (vitamin A, B1, B2, B3, B5, B6, C, and folate) and essential minerals (calcium, copper, iron, manganese, magnesium, phosphorus, and zinc). The oil exerts antioxidative and anti-inflammatory activities. Lemon grass EO has a wide application in aromatherapy, health, and diseases. It reduces fever, stress, mental fatigue, and pain associated with nerves, muscles, and the GI tract (gas irritation and gastric ulcers). The oil also has glandular secretion stimulatory, diuretic, immune stimulatory, antibacterial, antifungal, skin healing (antiseptic and astringent), and insect-repellent properties (Abdulazeez 2016; Lawal 2017).

2.20 *Sideritis hirsuta* Oil

The EO of *Sideritis hirsuta* L. is commonly used in the Mediterranean region. Palá-Paul et al. (2006) identified more than 70 chemicals in the EO, predominantly β-phellandrene (23.8%), α-phellandrene (9.2%), α-pinene (8.2%), and (*Z*)-β-guaiene (8.1%). The oil exerts anti-inflammatory, antirheumatic, antimicrobial, and antibacterial properties. The EO of *Sideritis hirsuta* L. appears to have great potential as a nutraceutical in veterinary medicine.

2.21 Tea Tree Oil

The EO of tea tree is extracted from fresh leaves and wood of the tree *Melaleuca alternifolia*. Chemical constituents present in the oil include terpinen-4-ol, γ-terpinene, 1,8-cineol, limonene, linalool, α-cymene, δ-cadinene, and globulol. The applications of tea tree oil are diverse encompassing from cleaning to skin care to massage to prevention and treatment of diseases. In the 1920s, Arthur Penfold, a chemist in Australia, stated that tea tree oil has 11–13 times more antimicrobial activity than phenol. Due to high terpinene-4-ol content, the oil exerts antibacterial and antifungal activities. Its use in oral health and periodontal diseases is well established (discussed in detail in Chap. on nutraceuticals in periodontal health and diseases in dogs and cats). Other uses of tea tree oil include in the treatment of acne, cuts, scrapes, wounds, scars, eczema, psoriasis, itch, warts, toenail fungus, athlete's foot, ringworm, and cancer. Additionally, it can be used as a powerful insecticide.

2.22 Myrrh Oil

Myrrh EO is obtained from the tree trunk bark and branches of *Commiphora myrrha*, which is native to Middle Eastern countries. Its use has been described throughout history (Roman literature, Bible, Koran) for various purposes, including rituals, ceremonies, mummification, and in health and diseases (Ayurvedic, Unani, and Chinese medicine). Myrrh oil contains many chemicals including sesquiterpenes (furanoeudesma-1,3-diene and curzarene), sterols, and steroids (Zhu et al. 2003). Its use has been well documented for oral hygiene and periodontal diseases. Phytoconstituents in myrrh oil exert antibacterial, antiviral, antifungal, amoebocidal, astringent, antispasmodic, hypoglycemic, and anti-inflammatory properties (Dolara et al. 2000; Tipton et al. 2006).

2.23 Ozonated Olive Oil

Studies have shown that when ozone gas is passed through olive oil, the ozone reacts with polyunsaturated fatty acids (PUFA). This ozonated olive oil thus contains a highly reactive and oxidizing agent ozonide which displays a strong antimicrobial effect. Vets Plus Inc. (Menomonie, WI, USA) has developed a patent pending ozonated olive oil formulation Ozoderm, which is a proprietary blend of omega fatty acids and curcumin in ozonated olive oil. In clinical studies (unpublished), Ozoderm has been found to be very effective against a variety of skin conditions.

2.24 Emu Oil

Emu oil is obtained in Australia from the fat of an emu. The oil is very rich in antioxidants and polyunsaturated fatty acids (including omega-3 fatty acids). By having antioxidative and anti-inflammatory properties, emu oil is commonly used to treat skin conditions (such as acne, eczema, psoriasis, rashes, and rosacea), for hair health, and

healing wounds. Emu oil is believed to improve back pain, chronic pain, headache, diabetes, gum disease, osteoarthritis, and rheumatoid arthritis.

3 Essential Oils as Modifiers of Rumen Fermentation, Immune System, and Lactation

Busquet et al. (2006) reported that increased rumen fermentation is indicated by an increase in propionate and a decrease in methane, acetate, and ammonia nitrogen, without reducing total volatile fatty acids (VFAs). VFAs are natural hydrogen sinks that help maintain the equilibrium of hydrogen and keep the fermentation process active. Retention of energy from glucose is the highest in propionate (109%), intermediate in butyrate (78%), and the lowest in acetate (62.5%). Recently, some EOs have been reported as modifiers of rumen fermentation (Lillehoj et al. 2018). In in vitro studies, garlic oil is reported to reduce the proportions of acetate and branched-chain VFAs and increase the proportions of propionate and butyrate (Busquet et al. 2005, 2006). Its fermentation profile is consistent with changes observed when methane inhibitors are supplied to ruminants (Lillehoj et al. 2018). The anti-methanogenic effect of garlic and its active components is due to direct inhibition of Archaea microorganisms in the rumen through the inhibition of hydroxymethylglutaryl coenzyme A (HMG-CoA) reductase, a specific pathway essential for the membrane stability of Archaea. Garlic oil has a strong inhibitory action on VFA production with variable effect, and the margin of safety in the doses between adequate and toxic levels is narrow.

In other studies, cinnamaldehyde and eugenol are reported to reduce the molar proportion of acetate and increase the molar proportions of propionate and butyrate (Cardozo et al. 2004; Busquet et al. 2005). Cinnamaldehyde caused (1) inhibition of methanogenesis, (2) reduction of ammonia nitrogen, and (3) increase in free amino acids, suggesting that deamination of amino acids was inhibited in the rumen. In an in vitro rumen simulation system, cinnamaldehyde has been reported to reduce *Prevotella* spp. (bacteria involved in deamination) (Ferme et al. 2004). Busquet et al. (2005) demonstrated that eugenol inhibits the breakdown of large peptides to small peptides and amino acids. Recently, Lillehoj et al. (2018) suggested that the combination of cinnamaldehyde and eugenol may work in synergy to inhibit peptidolysis and deamination and then improve the overall supply of amino acids and small peptides to rumen flora and the host.

Tekippe et al. (2013) reported that feeding cinnamaldehyde alone or in combination with eugenol results in increase in milk production (1.7–2.7%). In some other studies, a combination of cinnamaldehyde, eugenol, and capsicum, when fed to dairy cattle, increased energy-corrected milk production from 3.2% to 5.2% (Bravo et al. 2009; Wall et al. 2014; Oguey and Wall 2016). Feeding rumen-protected capsicum has been shown to improve milk production by modulating immune function (Oh et al. 2015; Stelwagen et al. 2016).

4 Safety of Essential Oils

Do not add concentrated EOs to dog or cat food and drinking water. Also, avoid using EOs with puppies under 10 weeks of age. Use hydrosols, instead. As needed, dilute EOs and then apply topically or administer via food or water.

5 Concluding Remarks and Future Directions

Essential oils (EO) are extracted from plant and non-plant origins. They may contain up to 100 plus chemicals, which can be of heterogeneous classes. A good number of these chemicals possess antioxidative, anti-inflammatory, immunostimulatory, and antimicrobial properties. Some of the oils also have analgesic, antispasmodic, antiparasitic, antifungal, skin healing, and many other medicinal properties. In dairy cows, some EOs (such as eugenol, cinnamaldehyde, and capsicum) can stimulate immune system and improve milk production. These oils are of immense value to promote human and animal health and prevent and treat diseases. In the future, new oils and novel uses of existing oils will be discovered for the prevention and treatment of animal diseases.

Acknowledgment The authors would like to thank Ms. Robin B. Doss for her technical assistance in preparation of this chapter.

References

Abdelwahab SI, Mariod AA, Taha MME et al (2017) Chemical composition and antioxidant properties of the essential oil of *Cinnamomum altissimum* Kosterm. (*Lauraceae*). Arab J Chem 10:131–135

Abdulazeez MA (2016) In: Preedy VR (ed) Essential oils in food preservation, flavor and safety. Elsevier, Amsterdam, pp 509–516

Adaszynska-Skwirzynska M, Szczerbinska D (2017) The antimicrobial activity of lavender essential oil (*Lavandula angustifolia*) and its influence on the production performance of broiler chickens. J Anim Physiol Anim Nutr 102:1020–1025

Ahmad A, van Vuuren S, Viljoen A (2014) Unravelling the complex antimicrobial interactions of essential oils—the case of *Thymus vulgaris* (Thyme). Molecules 19:2896–2910

Aidi WW, Mhamdi B, Sriti J et al (2010) Antioxidant activities of the essential oils and methanol extracts from myrtle (*Myrtus communis* var. *italica* L.) leaf, stem and flower. Food Chem Toxicol 48:1362–1370

Al-Asmari AK, Athar MT, Al-Faraidy AA et al (2017) Chemical composition of essential oil of *Thymus vulgaris* collected from Saudi Arabian market. Asian Pac J Trop Biomed 7(2):147–150

Albring M, Albrecht H, Alcorn G et al (1983) The measuring of the anti-inflammatory effect of a compound on the skin of volunteers. Methods Find Exp Clin Pharmacol 5:75–77

Almasirad A, Amanzadeh Y, Taheri A et al (2011) Composition of a historical rose oil sample (*Rosa damascena* Mill., *Rosaceae*). J Essent Oil Res 19(2):110–112

Al-Saidi S, Rameshkumar KB, Hisham A et al (2012) Composition and antibacterial activity of the essential oils of four commercial grades of Omani luban, the oleo-gum resin of *Boswellia sacra* FLUECK. Chem Biodivers 9(3):615–624

Al-Snafi AE (2016) A review on chemical constituents and pharmacological activities of *Coriandrum sativum*. IOSR J Pharm 6(7):17–42

Al-Zuhair H, Al-Sayed B, Ameen HA et al (1996) Pharmacological studies of cardamom oil in animals. Pharmacol Res 34:79–82

Amiri H (2012) Essential oils composition and antioxidant properties of three *Thymus species*. Evid Based Complement Alternat Med 2012:728065

Aragão GF, Carneiro LMV, Junior APF et al (2006) A possible mechanism for anxiolytic and antidepressant effect of alpha- and beta-amyrin from *Protium heptaphyllum* (Aubl.) March. Pharmacol Biochem Behav 85:827–834

Araujo DAO, Takayama C, de Faria FM et al (2011) Gastroprotective effects of essential oil from *Protium heptaphyllum* on experimental gastric ulcer models in rats. Rev Bras Farm 21:721–729

Avallone R, Zanoli P, Puia G et al (2000) Pharmacological profile of apigenin, a flavonoid isolated from *Matricaria chamomilla*. Biochem Pharmacol 59:1387–1394

Bajpai VK, Kang SC (2010) Antifungal activity of leaf essential oil and extracts of *Metasequoia glyptostroboides* Mikiex Hu. J Am Oil Chem Soc 87:327–336

Bajpai VK, Rahman A, Kang SC (2007) Chemical composition and anti-fungal properties of the essential oil and crude extracts of *Metasequoia glyptostroboides* Mikiex Hu. Ind Crop Prod 26 (1):28–35

Bajpai VK, Sharma A, Back KH (2013) Antibacterial mode of action of *Cudrania tricuspidata* fruit essential oil, affecting membrane permeability and surface characteristics of food-borne pathogens. Food Control 32:582–590

Baser KH, Demirci B, Iscan G et al (2006) The essential oil constituents and antimicrobial activity of *Anthemis aciphylla* BOISS. Var. discoidea BOISS. Chem Pharm Bull 54:222–225

Basile A, Senatore F, Gargano R et al (2006) Antibacterial and antioxidant activities in *Sidris italica* (Miller) Greuter et Burdet essential oils. J Ethnopharmacol 107:240–248

Bellik Y, Benabdesselam F, Ayad A et al (2013) Antioxidant activity of the essential oil and oleoresin of *Zingiber officinale* Roscoe as affected by chemical environment. Int J Food Prop 16:1304–1313

Ben Hassine D, Abderrabba M, Yvon Y et al (2012) Chemical composition and in vitro evaluation of the antioxidant and antimicrobial activities of *Eucalyptus gillii* essential oil and extracts. Molecules 17 (8):9540–9558

Benites J, Bravo F, Rojas M et al (2011) Composition and microbiological screening of the essential oil from the leaves and stems of *Senecio atacamensis*. Phil from Chile. J Chil Chem Soc 56 (2):712–714

Białoń M, Krzyśko-Łupicka T, Pik A et al (2017) Chemical composition of herbal macerates and corresponding commercial essential oils and their effect on Bacteria *Escherichia coli*. Molecules 22:1887

Blumenthal M, Busse WR, Goldberg A et al (1998) The complete German commission E monographs: therapeutic guide to herbal medicines. Austin, American Botanical Council and Boston, Integr Med Commun, pp 180–182

Bolechowski A, Moral R, Bustamante MA et al (2011) Composition of oregano essential oil (*Origanum vulgare*) as affected by the use of winery-distillery composts. J Essent Oil Res 23:32–38

Borugă O, Jianu C, Mişcă C et al (2014) *Thymus vulgaris* essential oil: chemical composition and antimicrobial activity. J Med Life 7 (3):56–60

Bozin B, Mimica-Dukic N, Samojlik I et al (2007) Antimicrobial and antioxidant properties of rosemary and sage (*Rosmarinus officinalis* L. and *Salvia officinalis* L., *Lamiaceae*) essential oils. J Agric Food Chem 55:7879–7885

Bravo D, Pyatt N, Doane PH et al (2009) Meta analysis of growing ruminants fed a mixture of eugenol, cinnamaldehyde and capsicum oleoresin. J Dairy Sci 92:374

Brunke E-J, Hammerschmidt F-J, Koester F-H et al (1991) Constituents of dill (*Anethum graveolens* L.) with sensory importance. J Essent Oil Res 3(4):257–267

Burits M, Bucar F (2000) Antioxidant activity of *Nigella sativa* essential oil. Phytother Res 14:323–328

Burt S (2004) Essential oils: their antibacterial properties and potential applications in foods-a review. Int J Food Microbiol 94:223–253

Busquet M, Calsamiglia S, Ferret A et al (2005) Effects of cinnamaldehyde and garlic oil on rumen microbial fermentation in a dual flow continuous culture. J Dairy Sci 88:2508–2516

Busquet M, Calsamiglia S, Ferret A et al (2006) Plant extract affect in vitro rumen microbial fermentation. J Dairy Sci 89:761–771

Cantore LP, Iacobellis SN, Marco DA et al (2004) Antibacterial activity of *Coriandrum sativum* L. and *Foeniculum vulgare* Miller var. (Miller) essential oil. J Agric Food Chem 52(26):7862–7866

Cardozo PW, Calsamiglia S, Ferret A et al (2004) Effects of natural plant extracts on ruminal protein degradation and fermentation profiles in continuous culture. J Anim Sci 83:3230–3236

Carson CF, Hammer KA, Riley TV (2006) *Melaleuca alternifolia* (Tea Tree) oil: a review of antimicrobial and other medicinal properties. Clin Microbiol Rev 19(1):50–62

Cemek M, Kaga S, Simsek N et al (2008) Antihyperglycemic and antioxidative potential of *Matricaria chamomilla* L. in streptozotocin-induced diabetic rats. J Nat Med 62:284–293

Cerda A, Martínez ME, Soto C et al (2013) The enhancement of antioxidant compounds extracted from *Thymus vulgaris* using enzymes and the effect of extracting solvent. Food Chem 139:138–143

Cetin B, Ozer H, Cakir A et al (2010) Antimicrobial activities of essential oil and hexane extract of Florence fennel [*Foeniculum vulgare* var. *azoricum* (Mill.) Thell.] against foodborne microorganisms. J Med Food 13(1):196–204

Chahal KK, Bhardwaj U, Kaushal S et al (2015) Chemical composition and biological properties of *Chrysopogon zizanioides* (L.) Roberty syn. *Vetiveria zizanioides* (L.) Nash-a review. Indian J Nat Prod Resour 6(4):251–260

Cheng SS, Huang CG, Chen YJ et al (2008) Chemical compositions and larvicidal activities of leaf essential oils from two *Eucalyptus* species. Bioresour Technol 99(9):3617–3622

Chenni M, El Abed D, Rakotomanomana N et al (2016) Comparative study of essential oils extracted from Egyptian basil leaves (*Ocimum basilicum* L.) using hydro-distillation and solvent-free microwave extraction. Molecules 21(1):E113. https://doi.org/10.3390/molecules21010113

Choi E-M, Hwang J-K (2004) Antiinflammatory, analgesic and antioxidant activities of the fruit of *Foeniculum vulgare*. Fitoterapia 75:557–565

Cicerale S, Lucas LJ, Keast RSJ (2012) Antimicrobial, antioxidant and anti-inflammatory phenolic activities in extra virgin olive oil. Curr Opin Biotechnol 23(2):129–135

Cimanga K, Kambu K, Tona L et al (2006) Correlation between chemical composition and antibacterial activity of essential oils of some

aromatic medicinal plants growing in the Democratic Republic of Congo. J Ethnopharmacol 79(2):213–220
Constantin M, Sartorelli P, Limburger R et al (2001) Essential oils from *Piper cernuum* and *Piper regnellii*: antimicrobial activities and analysis by GC/MS and ^{13}C-NMR. Planta Med 67:771–773
Dawidowicz AL, Olszowy M (2014) Does antioxidant properties of the main component of essential oil reflect its antioxidant properties? The comparison of antioxidant properties of essential oils and their main components. Nat Prod Res 28:1952–1963
De Falco E, Mancini E, Roscigno G et al (2013) Chemical composition and biological activity of essential oils of *Origanum vulgare* L. subsp. *vulgare* L. under different growth conditions. Molecules 18:14948–14960
Delamare APL, Moschen-Pistorello IT, Artico L et al (2007) Antibacterial activity of the essential oil of *Salvia officinalis* L. and *Salvia triloba* L. cultivated in South Brazil. Food Chem 100:603–608
Dias PC, Foglio MA, Possenti A et al (2000) Antiulcerogenic activity of crude hydroalcoholic extract of *Rosmarinus officinalis* L. J Ethnopharmacol 69:57–62
Djihane B, Wafa N, Elkhamssa S et al (2017) Chemical constituents of *Helichrysum italicum* (Roth) G. Don essential oil and their antimicrobial activity against Gram-positive and Gram-negative bacteria, filamentous fungi and *Candida albicans*. Saudi Pharm J 25:780–787
Djiri S, Casabianca H, Hanchi B et al (2014) Composition of garlic essential oil (*Allium sativum* L.) as influenced by drying method. J Essent Oil Res 26(2):91–96
Dolara P, Corte B, Ghelardini C et al (2000) Local anesthetic, antibacterial and antifungal properties of sesquiterpenes from myrrh. Planta Med 66(4):356–358
Fachini-Queiroz FC, Kummer R, Estevao-Silva CF et al (2012) Effects of thymol and carvacrol, constituents of *Thymus vulgaris* L. essential oil, on the inflammatory response. Evid Based Complement Alternat Med 2012:657026
Ferme D, Banjac M, Calsamiglia S et al (2004) The effects of plant extracts on microbial community structure in a rumen-simulating continuous-culture system as revealed by molecular profiling. Folia Microbiol (Praha) 49:151–155
Figiel A, Szumny A, Gutiérrez-Ortíz A et al (2010) Composition of oregano essential oil (*Origanum vulgare*) as affected by drying method. J Food Eng 98(2):240–247
Filipwicz N, Kaminski M, Kurlenda J et al (2003) Antibacterial and antifungal activity of juniper berry oil and its selected components. Phytother Res 17:227–231
Frank A, Unger M (2006) Analysis of frankincense from various *Boswellia* species with inhibitory activity on human drug metabolizing cytochrome P450 enzymes using liquid chromatography mass spectrometry after automated on-line extraction. J Chromatogr A 1112(1–2):255–262
Gardiner P (2007) Complementary, holistic, and integrative medicine: chamomile. Pediatr Rev 28(4):16–18
Glisic SB, Milojevic SZ, Dimimitrijevic SL et al (2007) Antimicrobial activity of the essential oil and different fractions of *Juniperus communis* L., and comparison with some commercial antibiotics. J Serb Chem Soc 2:311–320
Glowania HJ, Raulin C, Swoboda M (1987) Effect of chamomile on wound healing-a clinical double-blind study. Z Hautkr 62:1267–1271
Govindarajan M, Rajeswary M, Hoti SL et al (2016) Eugenol, α-pinene and β-caryophyllene from *Plectranthus barbatus* essential oil as eco-friendly larvicides against malaria, dengue and Japanese encephalitis mosquito vectors. Parasitol Res 15(2):807–815
Graf J (2000) Herbal anti-inflammatory agents for skin diseases. Skin Therapy Lett 5:3–5
Gupta B, Ghosh KK, Gupta RC (2016) Thymoquinone. In: Gupta RC (ed) Nutraceuticals: efficacy, safety and toxicity. Academic Press/Elsevier, Amsterdam, pp 541–550
Hadfield N (2001) The role of aromatherapy massage in reducing anxiety in patients with malignant brain tumors. Int J Palliat Nurs 7:279–285
Hashemi SM, Safavi SA (2012) Chemical constituents and toxicity of essential oils of oriental arborvitae, *Platycladus orientalis* L. Franco, against three stored-product beetles. Chilean J Agric Res 72 (2):188–194
Hernández T, Canales M, Avila JG et al (2005) Comparative and antibacterial activity of essential oil of *Lantana achyranthifolia* Desf. (*Verbenaceae*). J Ethnopharmacol 96(3):551–554
Hosseinzadeh H, Karimi GR, Ameri M (2002) Effects of *Anethum graveolens* L. seed extracts on experimental gastric irritation models in mice. BMC Pharmacol 2:21–27
Howes MJR, Simmonds MSJ, Kite GC (2004) Evaluation of the quality of sandalwood essential oils by gas chromatography-mass spectrometry. J Chromatogr 1028(2):307–312
Hsouna AB, Halima NB, Smaoui S et al (2017) Citrus lemon essential oil: chemical composition, antioxidant and antimicrobial activities with its preservative effect against *Listeria monocytogenes* inoculated in minced beef meat. Lipids Health Dis 16:146
Hui L, He L, Huan L et al (2010) Chemical composition of *Lavender* essential oil and its antioxidant activity and inhibition against rhinitis-related bacteria. Afr J Microbiol Res 4(4):309–313
Huong T, Thang TD, Ogunwande IA (2015) Chemical constituents of essential oils from the leaves, stems, roots and fruits of *Alpinia polyantha*. Nat Prod Commun 10(2):367–368
Hussain AI, Anwar F, Shahid M et al (2011) Chemical composition, and antioxidant and antimicrobial activities of essential oil of spearmint (*Mentha spicata* L.) from Pakistan. J Essent Oil Res 22(1):78–84
Igarashi M, Miyazaki Y (2013) A review on bioactivities of *Perilla*: progress in research on the functions of *Perilla* as medicine and food. Evid Based Complement Alternat Med 2013:925342
Igarashi M, Song C, Ikei H et al (2014) Effects of olfactory stimulation with *Perilla* essential oil on prefrontal cortex activity. J Altern Complement Med 20(7). https://doi.org/10.1089/acm.214.0100
Isbilir SS, Sagiroglu A (2011) Antioxidant potential of different dill (*Anethum graveolens* L.) leaf extract. Int J Food Prop 14:894–902
Jiang X, Zhang JC, Liu YW et al (2007) Studies on chemical constituents of *Valeriana officinalis*. Zhong Yao Cai 30 (11):1391–1393
Juliani HR Jr, Biurrun F, Koroch AR et al (2002) Chemical constituents and antimicrobial activity of the essential oil of *Lantana xenica*. Planta Med 68(8):762–764
Khalid KA (2016) Essential oil constituents of summer savory plants propagated and adapted under Egyptian climate. J Appl Sci 16 (2):54–57
Khesorn N, Manasnant B, Banyong K et al (2010) Antimicrobial activity of alkaloid from roots of *Vetiveria zizanioides* (L.) Nash ex small. Thai Pharm Health Sci J 5(2):99–102
Komaki A, Hoseini F, Shahidi S et al (2016) Study of the effect of extract of *Thymus vulgaris* on anxiety in male rats. J Tradit Complement Med 6:257–261
Kroll U, Cordes C (2006) Pharmaceutical prerequisites for a multi-target therapy. Phytomedicine 5:12–19
Kumar P, Ansari SH, Ali J (2009) Herbal remedies for the treatment of periodontal disease-a patent review. Recent Pat Drug Deliv Formul 3:221–228
Kuorwel KK, Cran MJ, Sonneveld K et al (2011) Essential oils and their principal constituents as antimicrobial agents for synthetic packaging films. J Food Sci 76(9):R164–R177
Kusuma HS, Mahfud M (2016) Chemical composition of essential oil of Indonesia sandalwood extracted by microwave-assisted

hydrodistillation. AIP Conf Proc 1755(1):50001. https://doi.org/10.1063/1.4958484
Kyung KH (2012) Antimicrobial properties of *Allium* species. Curr Opin Biotechnol 23(2):142–147
Laciar A, Ruiz ML, Flores RC et al (2009) Antibacterial and antioxidant activities of the essential oil of *Artemisia echegarayi* Hieron. (*Asteraceae*). Rev Argent Microbiol 41:226–231
Lambert RJW, Skandamis PN, Coote PJ et al (2001) A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol. J Appl Microbiol 91:453–462
Lawal OA (2017) Medicinal spices and vegetables from Africa. Academic Press, London, pp 397–423
Lemberkovics E, Kéry A, Marczal G et al (1998) Phytochemical evaluation of essential oils, medicinal plants and their preparations. Acta Pharm Hung 68:141–149
Lillehoj H, Liu Y, Calsamiglia S et al (2018) Phytochemicals as antibiotic alternatives to promote growth and enhance host health. Vet Res 49:76
McKay DL, Blumberg JB (2000) A review of the bioactivity and health benefits of chamomile tea (*Matricaria ricutita* L.). Phytother Res 20:519–530
Mendez AHS, Cornejo CGF, Coral MFC et al (2017) Chemical composition, antimicrobial and antioxidant activities of the essential oil of *Bursera graveolens* (*Burseraceae*) from Peru. Indian J Pharm Educ Res 51(3):S429–S435
Merfort I, Heilmann J, Hagedorn-Leweke U et al (1994) In vivo skin penetration studies of chamomile flavones. Pharmazie 49:509–511
Milos M, Radonic A, Bezic N et al (2001) Localities and seasonal variations in the chemical compositions of essential oils of *Satureja montana* L. and *S. cuneifolia* Ten. Flavour Fragr J 16:157–160
Minaiyan M, Ghannadi AR, Afsharipour M et al (2011) Effects of extract and essential oil of *Rosmarinus officinalis* L. on TNBS-induced colitis in rats. Res Pharm Sci 6:13–21
Misra MC, Parshad R (2000) Randomized clinical trial of micronized flavonoids in the early control of bleeding from acute internal hemorrhoids. Br J Surg 87:868–872
Mohamad RH, El-Bastawesy AM, Abdel-Monem MG et al (2011) Antioxidant and anticarcinogenic effects of methanolic extract and volatile oil of fennel seeds (*Foeniculum vulgare*). J Med Food 14 (9):986–1001
Mothana RA, Khaled JM, Noman OM et al (2018) Phytochemical analysis and evaluation of the cytotoxic, antimicrobial and antioxidant activities of essential oils from three *Plectranthus* species grown in Saudi Arabia. BMC Complement Altern Med 18:237
Muchtaridi, Subarnas A, Apriyantono A et al (2010) Identification of compounds in the essential oil of nutmeg seeds (*Myristica fragrans* Houtt.) that inhibit locomotor activity in mice. Int J Mol Sci 11:4771–4781
Nagababu E, Lakshmaiah M (1992) Inhibitory effect of eugenol on non-enzymatic lipid peroxidation in rat liver mitochondria. Biochem Pharmacol 43:2393–2400
Nandhini S, Narayanan KB, Ilango K (2018) *Valeriana officinalis*: a review of its traditional uses, phytochemistry and pharmacology. Asian J Pharm Clin Res 11(1):36–41
Nayak BS, Raju SS, Rao AV (2007) Wound healing activity of *Matricaria ricutita* L. extract. J Wound Care 16:298–302
Nissen HP, Blitz H, Kreyel HW (1988) Prolifometrie, eine methode zur beurteilung der therapeutischen wirsamkeit kon Kamillosan®-Salbe. Z Hautkr 63:84–90
Nostro A, Cannatelli MA, Marino A et al (2003) Evaluation of antiherpesvirus-1 and genotoxic activities of *Helichrysum italicum* extract. New Microbiol 26:125–128
Oguey C, Wall EH (2016) 1570A blend of cinnamaldehyde, eugenol, and capsicum oleoresin improves milking performance in lactating daily cows. J Anim Sci 94:763
Oh J, Giallongo F, Frederick T et al (2015) Effects of dietary Capsicum oleoresin on productivity and immune responses in lactating dairy cows. J Dairy Sci 98:6327–6339
Oussalah M, Caillet S, Lacroix M (2006) Mechanism of action of Spanish oregano, Chinese cinnamon, and savory essential oils against cell membranes and walls of *Escherichia coli* 0157:H7 and *Listeria monocytogenes*. J Food Prot 69(5):1046–1055
Palá-Paul J, Pérez-Alonso J, Velasco-Negueruela A et al (2006) Essential oil composition of *Sideritis hirsuta* L. from Guadalajara province, Spain. Flavour Fragr J 21:410–415
Patzelt-Wenczler R, Ponce-Pöschl E (2000) Proof of efficacy Kamillosan® cream in atopic eczema. Eur J Med Res 5:171–175
Perry NS, Bollen C, Perry EK et al (2003) Salvia for dementia therapy: review of pharmacological activity and pilot tolerability clinical trial. Pharmacol Biochem Behav 75:651–659
Picon PD, Picon RV, Costa AF et al (2010) Randomized clinical trial of a phytotherapic compound containing *Pimpinella anisum*, *Foeniculum vulgare*, *Sambucus nigra*, and *Cassia angustifolia* for chronic constipation. BMC Complement Altern Med 10:17
Pierce A (1999) American pharmaceutical association practical guide to natural medicines. Stonesong Press, New York, pp 338–340
Pierozan MK, Pauletti GF, Rota L et al (2009) Chemical characterization and antimicrobial activity of essential oils of *Salvia* L. species. Cienc Technol Aliment 29:764–770
Pinto P, Santos CN (2017) Worldwide (poly)phenol intake: assessment methods and identified gaps. Eur J Nutr 56:1393–1408
Porte A, Godoy RLO, Maia-Porte LH (2013) Chemical composition of sage (*Salvia officinalis* L.) essential oil from the Rio de Janeiro State (Brazil). Rev Bras Plantas Med 15(3):438–441
Porter NG, Shaw ML, Shaw GJ et al (1983) Content and composition of dill herb oil in the whole plant and the different plant parts during crop development. N Z J Agric Res 26(1):119–127
Puškárová A, Bučková M, Kraková L et al (2017) The antibacterial and antifungal activity of six essential oils and their cyto/genotoxicity to human HEL 12469 cells. Sci Rep 7:8211
Qiu J, Li H, Su H et al (2012) Chemical composition of fennel essential oil and its impact on *Staphylococcus aureus* exotoxin production. World J Microbiol Biotechnol 28(4):1399–1405
Radulescu V, Saviuc C, Chifiriuc C et al (2011) Chemical composition and antimicrobial activity of essential oil from Shoots Spruce (*Picea abies* L.). Rev Chim (Bucharest, Rom) 62(1):69–74
Raina VK, Kumar A, Aggarwal KK (2013) Essential oil composition of Ginger (*Zingiber officinale* Roscoe) rhizomes from different place in India. J Essent Oil Bear Plants 89(2):187–191
Raina AP, Verma SK, Abraham Z (2014) Volatile constituents of essential oils isolated from *Alpinia galanga* Willd. (L.) and *A. officinarum* Hance rhizomes from North East India. J Essent Oil Res 26(1):24–28
Ramsewak RS, Nair MG, Stommel M et al (2003) In vitro antagonistic activity of monoterpenes and their mixtures against 'toe nail fungus' pathogens. Phytother Res 17(4):376–379
Rana VS, Blazquez MA (2015) Chemical composition of the essential oil of *Anethum graveolens* aerial parts. J Essent Oil Bear Plants 17 (6):1219–1223
Rao PGP, Rao LJ, Raghavan B (1999) Chemical composition of essential oils of garlic (*Allium sativum* L.). J Spices Aromat Crops 8 (1):41–47
Rebouças de Araujo JD, Coriolano de Aquino N, Véras de Aguiar Guerra AC et al (2017) Chemical composition and evaluation of the antibacterial and cytotoxic activities of the essential oil from the leaves of *Myracrodruon urundeuva*. BMC Complement Alternat Med 17(1):419
Robu S, Aprotosoaie AC, Spac A et al (2011) Studies regarding chemical composition of lavender volatile oils. Rev Med Chir Soc Med Nat Iasi 115(2):584–589

Rodrigues TG, Fernandes A Jr, Sousa JP et al (2009) In vitro and in vivo effects of clove on pro-inflammatory cytokines production by macrophages. Nat Prod Res 23(4):319–326

Rota MC, Herrera A, Martinez RM et al (2006) Antimicrobial activity and chemical composition of *Thymus vulgaris*, *Thymus zygis*, and *Thymus hyemalis* essential oils. Food Control 19:681–687

Rozza AL, Pellizzon CH (2013) Essential oils from medicinal and aromatic plants: a review of the gastroprotective and ulcer-healing activities. Fundam Clin Pharmacol 27:51–63

Sahin F, Karaman I, Gulluce M et al (2003) Evaluation of antimicrobial activities of *Satureja hortensis* L. J Ethnopharmacol 87:61–65

Sakkas H, Papadopoulou C (2017) Antimicrobial activity of basil, oregano, and thyme essential oils. J Microbiol Biotechnol 27 (3):429–438

Sala A, Recio M, Giner RM et al (2002) Anti-inflammatory and antioxidant properties of *Helichrysum italicum*. J Pharm Pharmacol 54:365–371

Salmon I (1992) Chamomile a medicinal plant. J Herbs Spices Med Plants 10:1–4

Şarer E, Toprak Y, Otlu B et al (2011) Composition and antimicrobial activity of the essential oil from *Mentha spicata* L. subsp. spicata. J Essent Oil Res 23(1):105–108

Sebei K, Sakouhi F, Herchi W et al (2015) Chemical composition and antibacterial activities of seven *Eucalyptus* species essential oils leaves. Biol Res 48(1):7

Sharopov FS, Zhang H, Setzer WN (2014) Composition of geranium (*Pelargonium graveolens*) essential oil from Tajikistan. Am J Essent Oils Nat Prod 2(2):13–16

Shellie R, Mondello L, Marriott P et al (2002) Characterization of lavender essential oils by gas chromatography-mass spectrometry with correlation of linear retention indices and comparison with comprehensive two-dimensional gas chromatography. J Chromatogr A 970(1–2):225–234

Silva J, Abebe W, Sousa SM et al (2003) Analgesic and anti-inflammatory effects of essential oils of *Eucalyptus*. J Ethnopharmacol 89(2–3):277–283

Singh G, Maurya S, de Lamposana MP et al (2005) Chemical constituents, antimicrobial investigation, and antioxidative potential of *Anethum graveolens* L. essential oil and acetone extract. Part 52. J Food Sci 70(4):208–215

Singh P, Shukla R, Prakash B et al (2010) Chemical profile, antifungal, antiaflatoxigenic and antioxidant activity of *Citrus maxima* Burm. and *Citrus sinensis* (L.) Obseck essential oils and their cyclic monoterpene, DL-limonene. Food Chem Toxicol 48:1734–1740

Singh O, Khanam Z, Misra N et al (2011) Chamomile (*Matricaria chamomilla* L.): an overview. Pharmacogn Rev 5(9):82–95

Singh J, Singh R, Gambhir RS et al (2016) Local drug delivery system in treatment of periodontitis: a review. J Periodontol Med Clin Pract 3 (3):153–160

Siroli L, Patrignani F, Montanari C et al (2014) Characterization of oregano (*Origanum vulgare*) essential oil and definition of its antimicrobial activity against *Listeria monocytogenes* and *Escherichia coli* in vitro system and on foodstuff surfaces. Afr J Microbiol Res 8 (29):2746–2753

Smigielski K, Raj A, Krosowiak K et al (2009) Chemical composition of the essential oil of *Lavandula angustifolia* cultivated in Poland. J Essent Oil Bear Plants 12(3):338–347

Solórzano-Santos F, Miranda-Novales MG (2012) Essential oils from aromatic herbs as antimicrobial agents. Curr Opin Biotechnol 23 (2):136–141

Srivastava JK, Pandey M, Gupta S (2009) Chamomile, a novel and selective Cox-2 inhibitor with anti-inflammatory activity. Life Sci 85:663–669

Srivastava JK, Shankar E, Gupta S (2010) Chamomile: a herbal medicine of the past with bright future. Mol Med Rep 3(6):895–901

Stanojević LP, Stanojević JS, Cvetković DJ et al (2016) Antioxidant activity of oregano essential oil (*Origanum vulgare* L.). Biol Nyssana 7(2):131–139

Stelwagen K, Wall EH, Bravo DM (2016) Effect of rumen-protected capsicum on milk production in early lactating cows in a pasture-based system. J Anim Sci 94:675

Stoyanova A, Konakchiev A, Damyanova S et al (2006) Composition and antimicrobial activity of ginger essential oil from Vietnam. J Essent Oil Bear Plants 84(4):93–98

Su YC, Ho CL, Wang EI et al (2006) Antifungal activities and chemical compositions of essential oils from leaves of four eucalyptus. Taiwan J For Sci 21:49–61

Suppakul P, Miltz J, Sonneveld K et al (2003) Antimicrobial properties of basil and its possible application in food packaging. J Agric Food Chem 51(11):3197–3207

Taheri JB, Azimi S, Rafieian N et al (2011) Herbs in dentistry. Int Dent J 61:287–296

Takayama C, de-Faria FM, de Almeida CA et al (2016) Chemical composition of *Rosmarinus officinalis* essential oil and antioxidant action against gastric damage induced by absolute ethanol in the rat. Asian Pac J Trop Biomed 6(8):677–681

Tan LTH, Lee LH, Yin WF et al (2015) Traditional uses, phytochemistry, and bioactivities of *Cananga odorata* (Ylang Ylang). Evid Based Complement Alternat Med 2015:896314

Tawfik SS, Abbady MI, Ahmed M et al (2006) Therapeutic efficacy attained with thyme essential oil supplementation throughout γ-irradiated rats. Egypt J Rad Sci Appl 19(1):1–22

Tekippe JA, Tacoma R, Hristov AN et al (2013) Effect of essential oils on ruminal fermentation and lactation performance of dairy cows. J Dairy Sci 96:7892–7903

Tian YH, Kim HC et al (2005) Hepatoprotective constituents of *Cudrania tricuspidata*. Arch Pharm Res 28:44–48

Tipton DA, Hamman NR, Dabbous MKH (2006) Effect of myrrh oil on IL-1beta stimulation of NF-kappaB activation and PGE_2 production in human gingival fibroblasts and epithelial cells. Toxicol In Vitro 20 (2):248–255

Tzakou O, Verykokidou E, Roussis V et al (1998) Chemical composition and antibacterial properties of *Thymus longicaulis* subsp. *Chaubardii* oils: three chemotypes in the same population. J Essent Oil Res 10:97–99

Vera RR, Chane-Ming J (1998) Chemical composition of essential oil of dill (*Anethum graveolens* L.) growing in Reunion island. J Essent Oil Res 10(5):539–542

Verma RS, Padalia RC, Chauhan A et al (2011) Volatile constituents of essential oil and rose water of damask rose (*Rosa damascena* Mill.) cultivars from North Indian hills. Nat Prod Res 25(17):1577–1584

Viuda-Martos M, Ruíz-Navajas Y, Fernández-López J et al (2007a) Antifungal activities of thyme, clove and oregano essential oils. J Food Saf 27:91–101

Viuda-Martos M, Ruíz-Navajas Y, Fernández-López J et al (2007b) Chemical composition of the essential oils obtained from some spices widely used in Mediterranean region. Acta Chim Slov 54:921–926

Wall EH, Doane PH, Donkin SS et al (2014) The effects of supplementation with a blend of cinnamaldehyde and eugenol on feed intake and milk production of dairy cows. J Dairy Sci 97:5709–5717

Wanner J, Bail S, Jirovetz L et al (2010) Chemical composition and antimicrobial activity of cumin oil (*Cuminum cyminum*, *Apiaceae*). Nat Prod Commun 5(9):1355–1358

Wojdylo A, Figiel A, Oszmianski J (2008) Dehydration techniques effect on polyphenols content, antioxidant activity and color of oregano herb. J Clin Biochem Nutr 43:1–4

Woolley CL, Suhail MM, Smith BL et al (2012) Chemical differentiation of *Boswellia sacra* and *Boswellia carterii* essential oils by gas chromatography and chiral gas chromatography-mass spectrometry. J Chromatogr A 1261:158–163

Xu J-G, Liu T, Hu Q-P et al (2016) Chemical composition, antibacterial properties and mechanism of action of essential oil from clove buds against *Staphylococcus aureus*. Molecules 21:1194

Yamini Y, Sefidkon F, Pourmortazavi SM (2002) Comparison of essential oil composition of Iranian fennel (*Foeniculum vulgare*) obtained by supercritical carbon dioxide extraction and hydrodistillation methods. Flavour Fragr J 17(5):345–348

Yanishlieva NV, Marinova EM, Gordon MH et al (1999) Antioxidant activity and mechanism of action of thymol and carvacrol in two lipid systems. Food Chem 64:59–66

Zaouali Y, Bouzaine T, Boussaid M (2010) Essential oils composition in two *Rosmarinus officinalis* L. varieties and incidence for antimicrobial and antioxidant activities. Food Chem Toxicol 48:3144–3152

Zhang WK, Tao SS, Li TT et al (2016) Nutmeg oil alleviates chronic inflammatory pain through inhibition of COX-2 expression and substance P release in vivo. Food Nutr 60:30849

Zhu N, Sheng S, Sang S et al (2003) Isolation and characterization of several aromatic sesquiterpenes from *Commiphora myrrha*. Flavour Fragr J 18:282–285

Omega Fatty Acids

Szabina A. Stice

Abstract

Nutraceuticals are bioactive compounds that may be used to prevent and treat diseases and to promote health. They include vitamins, minerals, and other bioactive chemical compounds, such as omega fatty acids. This chapter discusses the potential benefits of omega fatty acids to animals, including their use in treating osteoarthritis, atopy, inflammatory bowel disease and other inflammatory conditions, renal and cardiovascular diseases, lipid and metabolic disorders, and cancer. Additionally, the effects of omega fatty acids on neurological development, behavior, and cognitive function are summarized along with recommended dosing levels and potential adverse effects such as immune function impairment, platelet dysfunction, and altered glucose and lipid metabolism.

Keywords

Omega fatty acids · Omega-3 fatty acids · Omega-6 fatty acids · Polyunsaturated fatty acids

1 Introduction

Nutraceuticals may have profoundly beneficial effects on the well-being and health of animals and humans, including both the prevention and treatment of disease. They may also help reduce or eliminate the need for conventional medications and may confer some beneficial effects that are not available from pharmaceuticals. Nutraceuticals include vitamins, minerals, and other bioactive chemical compounds, or mixtures of compounds, such as fish oil. Fish and marine-life oils, important sources of omega-3 fatty acids, along with purified polyunsaturated fatty acids, are examples of widely available nutraceuticals.

Omega-3 fatty acids have beneficial effects in animals with osteoarthritis, atopy, inflammatory bowel disease, or other inflammatory conditions. They are important for neurologic and retinal development and can improve skin and coat. A low-fat diet combined with omega-3 fatty acid supplementation can be effective in managing canine hypercholesterolemia and hypertriglyceridemia. In dogs, supplementation with fish oil seems to help preserve renal function, as well as to prevent arrhythmias, treat ventricular premature contractions, minimize the loss of heart muscle in congestive heart failure, and exhibit beneficial effects in early chronic valvular diseases. Omega-3 fatty acids have been investigated in both humans and animals for cancer prevention, support, or therapy, both as lone agents or, more often, combined with chemotherapeutic agents, to potentially enhance their effects. Fish oil treatment reduces cachexia (wasting syndrome) and improves food intake in dogs with anorexia (Fig. 1).

While the use of polyunsaturated fatty acids for the management and treatment of numerous conditions in animals is mostly considered safe, it is not completely without some adverse effects. For example, while supplementation with omega-3 fatty acids appears to be renoprotective, long-term supplementation with omega-6 fatty acids has enhanced renal injury in dogs. In addition, concurrent administration of the NSAID carprofen or the antiplatelet medication clopidogrel and omega-3 fatty acids may negatively impact hemostasis.

2 Omega Fatty Acids

A fatty acid is a carboxylic acid with a long aliphatic chain that is either saturated or unsaturated. In the case of unsaturated fatty acids, the position of the double bond in the aliphatic chain can be indicated in two ways. When employing the ω-n notation, the position of the double bond is counted from the methyl ($–CH_3$) end of the fatty acid. For

The opinions expressed in this chapter are the author's personal opinions, and they do not necessarily reflect that of FDA, DHHS, or the Federal Government.

S. A. Stice (✉)
Rockville, MD, USA
e-mail: szabina.stice@fda.hhs.gov

R. C. Gupta et al. (eds.), *Nutraceuticals in Veterinary Medicine*, https://doi.org/10.1007/978-3-030-04624-8_12

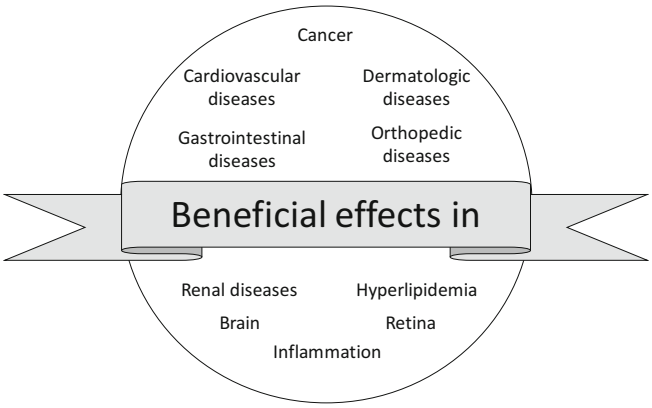


Fig. 1 Potential beneficial effects of omega fatty acids in animals

instance, in the case of an 18-carbon fatty acid, a double bond between C-15 and C-16 counted from the carboxylic acid (–COOH) end may be reported as ω-3 (or omega-3). The naming of the unsaturated fatty acids (i.e., if they are ω-3 fatty acids, ω-6 fatty acids, or ω-9 fatty acids) is based on the location of the first double bond determined by the ω-n notation. In this chapter, the term "omega-3 fatty acids" refers to compounds that are polyunsaturated fatty acids containing a double bond at the ω-3 position. The International Union of Pure and Applied Chemistry (IUPAC) naming of unsaturated fatty acids starts from the –COOH end (Fig. 2).

Fatty acids are important structural components of cells, precursors for other chemicals that are needed for many physiological processes, and provide fuel for animals. For example, eicosapentaenoic acid (EPA) is a precursor of many important chemicals in the body, such as prostaglandin-3, thromboxane-3, and leukotriene-5 eicosanoids; hence, it contributes to platelet aggregation and the regulation of immune responses, along with other functions. Docosahexaenoic acid (DHA) is an important component of the retina, skin, and brain and may lower markers of inflammation. Arachidonic acid (AA) is present in the phospholipids of cell membranes and is abundant in the liver, brain, and muscles. Additionally, AA is involved in cellular signaling and is a key inflammatory intermediate.

Essential fatty acids (EFAs) are fatty acids that humans and other animals cannot synthesize. Therefore, they must obtain EFAs from the diet for optimal health. Diets deficient in EFAs may cause essential fatty acid deficiency (EFAD), characterized by scaly dermatitis, alopecia, thrombocytopenia, hyperlipidemia, altered platelet aggregation, and poor wound healing (Morley 2016; Mogensen 2017). Treatment of fatty acid deficiency requires the dietary administration of, or supplementation with, EFAs. Important fatty acids include, but are not limited to, the ω-3 fatty acids α-linolenic acid (ALA), EPA, and DHA and the ω-6 fatty acids linoleic acid (LA), γ-linolenic acid (GLA), and AA. In humans, LA and ALA are EFAs, while DHA, EPA, GLA, and AA may be synthesized from EFAs or obtained from the diet. Some mammals either have a limited capacity, or completely lack the ability, to convert LA to AA, and for these animals, AA is an essential fatty acid. Due to the inefficient rate of conversion of ALA to EPA, diets containing ALA as the only source of omega-3 fatty acids may not be as effective as those containing EPA and DHA. Natural sources of fatty acids include seeds (ALA, LA, GLA); nuts (ALA, LA, GLA); vegetable oils (ALA, LA, GLA); oily fish, fish oil, or marine-life oil (EPA, DHA); algae (EPA, DHA); meat (AA); and eggs (AA).

3 Omega Fatty Acids as Nutraceuticals in Veterinary Medicine

3.1 Development of the Brain and Retina

DHA is an important component of the brain and the retina and can be synthesized from ALA or obtained from the diet. Kittens and puppies need high concentrations of DHA during gestation and as neonates to support the healthy development of their brain and retinas (Bauer 2006, 2007). Insufficient amounts of maternal DHA during gestation and lactation may lead to suboptimal neurological and retinal development in offspring; therefore, adequate maternal intake of ALA and DHA must be ensured. After weaning, dietary supplementation of DHA from fish oil is recommended to improve overall retinal and nervous system development in puppies and kittens. This is preferred to feeding them foods containing ALA, as neonatal dogs appear to preferentially synthesize DHA from ALA only for a short time while they are suckling, and because the conversion of precursors may be insufficient to meet kittens' DHA needs.

3.2 Anti-inflammatory Effects

3.2.1 Modification of Inflammatory and Immune Responses

Omega-3 fatty acids were shown to modify inflammatory and immune responses. A fish oil-supplemented diet consisting of 1.75 g EPA/kg and 2.2 g DHA/kg diet (ω-6:ω-3 ratio of 3.4:1) appears to suppress, not promote, lymphocyte proliferation in healthy dogs (LeBlanc et al. 2007). The exact same diet was associated with significant reductions in serum prostaglandin E_2 (PGE_2, a potent inflammatory mediator) and concentrations and activities of interleukin-1 and interleukin-6 (IL-1 and IL-6, regulators of immune and inflammatory responses) in dogs (LeBlanc et al. 2008).

The effect of dietary omega-3 fatty acids on inflammation and immune response was also evaluated in cats (Park et al. 2011). For 12 weeks, cats were fed either control, fish oil (high in EPA and DHA)-supplemented, or flaxseed oil (high in ALA)-supplemented diets with ω-6:ω-3 ratios of 20:1, 5:1, and 5:1, respectively. Skin inflammatory response to histamine

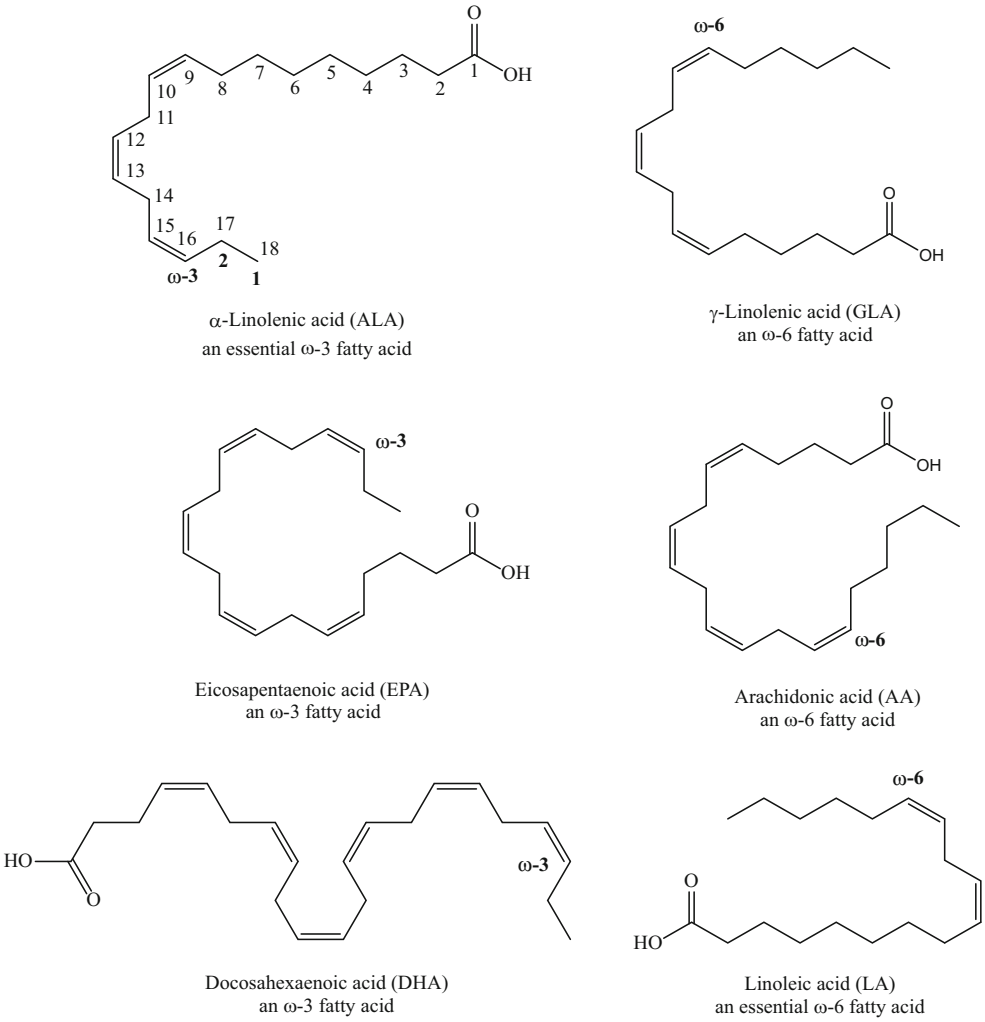


Fig. 2 Numbering of carbon atoms in fatty acids and naming of omega fatty acids

significantly decreased in the fish and flaxseed oil groups. In these groups, lowered B, total T and T_h subpopulations, and leukocyte proliferative response to pokeweed mitogen were noted. Cats fed fish oil had significantly higher leukotriene LTB_5, but cats fed flaxseed oil did not. These results indicate that even though both fish and flaxseed oils can reduce feline skin inflammatory responses, flaxseed oil is less immunosuppressive than fish oil.

The effects of omega-3 fatty acid supplementation on selected immune responses were also evaluated in normal horses by a delayed-type hypersensitivity skin test to keyhole limpet hemocyanin (KLH) and by measuring antibody titers to KLH along with other measurements (Hall et al. 2004). Supplementation with fish oil inhibited the lipopolysaccharide (endotoxin)-induced increase in PGE_2 production of bronchoalveolar lavage fluid (BALF) cells. The authors concluded that fish oil could have a beneficial role in the treatment of equine recurrent airway obstruction or other equine inflammatory diseases, as fish oil did not increase PGE_2 production. Indeed, in horses with recurrent airway obstruction or inflammatory airway disease, omega-3 fatty acid supplementation was later shown to improve clinical signs, lung function, and BALF (decreased neutrophils) when compared to placebo (Nogradi et al. 2015).

The anti-inflammatory and immunosuppressive effects of omega-3 fatty acids are potentially beneficial to animals with chronic inflammatory diseases. Some of these inflammatory diseases and the reported beneficial effects of treatments with omega fatty acids are discussed below.

3.2.2 Therapeutic Use in Inflammatory Bowel Disease

Inflammatory bowel disease (IBD) is common in cats (Trepanier 2009) and has also been reported in dogs. IBD is usually treated by modifying the diet first, and if that fails, by administering immunosuppressive therapies. Unfortunately, these treatments do not always help or do not help enough; hence, adjunctive treatments are often sought. One of these potential adjunctive therapies is omega-3 fatty acid supplementation, because omega-3 fatty acids have anti-

inflammatory properties. They have been shown to be beneficial in the treatment of IBD in humans.

In rats, cod liver oil showed positive effects in treating inflammatory colitis; i.e., cod liver oil supplementation significantly diminished the severity of the inflammatory lesions of the bowel and their progression to chronicity (Guarner et al. 1992). In a mouse model of colitis, dietary administration of omega-3 fatty acids reduced clinical colitis and colonic immunopathology by multiple mechanisms (Whiting et al. 2005). A second study in mice showed that dietary omega-3 fatty acids reduced the severity and manifestation of colitis, in addition to suppressing the production of tumor necrosis factor-α that appears to play a pivotal role in the pathogenesis of chronic IBD (Chapkin et al. 2007).

In dogs with food-responsive diarrhea (FRD) or IBD, omega-3 fatty acids significantly decreased the canine IBD activity index (CIBDAI) in both the FRD and IBD groups, demonstrating that omega-3 fatty acids may be beneficial for the treatment of canine chronic enteropathies (Ontsouka et al. 2012). While marked suppression of intestinal inflammatory activity was reported in dogs with FRD, dogs with IBD required a combination of dietary omega-3 fatty acids and immunosuppressive drugs to reduce their CIBDAI values to levels similar to those of dogs in the FRD group receiving omega-3 fatty acids. Unfortunately, efficacy studies in cats are lacking; thus, dosing in cats is empirical and extrapolated from dosages used to treat IBD in humans. Careful titration of the dose is recommended.

3.2.3 Treatment of Pruritus and Atopic Dermatitis

Pruritus, or itch, is a skin sensation that provokes the desire to scratch. Atopic dermatitis (AD), a type of skin inflammation, is characterized by chronic itch, along with red, swollen, and cracked skin or skin lesions. Allergen-specific immunotherapy, corticosteroids, antihistamines, and cyclosporine have been used to control or reduce itching. These treatments may have undesired side effects, take a long time to produce improvement (immunotherapy), or are not effective in all patients. Some cases require a combination of medications to adequately control symptoms. Hence, there is a need for a safer alternative or one that may reduce the number and/or the dosage of medications given to an animal. The effects of fish oil supplementation were examined in multiple studies to determine if omega fatty acid supplementation may be a safer alternative for the treatment of pruritus and atopic dermatitis or to help reduce the doses of medications used.

In a single-blinded, self-controlled study, atopic dogs were fed a diet with an ω-6 to ω-3 fatty acid ratio of 5.5 to 1 (Scott et al. 1997). The itching was satisfactorily controlled within 1–3 weeks in almost half of the animals. After the fatty acid supplementation was stopped, the itching returned soon after but was controlled again when the diet was reinstated. In a second single-blinded, randomized trial, the effects of a similar dietary ω-6 to ω-3 fatty acid ratio (i.e., 5:1) on canine perennial atopic dermatitis were evaluated (Bensignor et al. 2008). The test diet contained 1.6% ω-6 and 0.32% ω-3 EFAs. While both the canine atopic dermatitis extent and severity index scores and the pruritus scores significantly declined on the test diet, proving its efficacy, the score reductions were less than 50%, and most cases required adjunctive therapy to fully resolve symptoms.

In a double-blinded, placebo-controlled, randomized trial, dogs with nonseasonal atopic dermatitis were supplemented with either flax oil (containing ALA and LA), a commercial fatty acid supplement (containing EPA and DHA), or mineral oil as placebo for 10 weeks (Mueller et al. 2004). Average global (supplementation plus dietary) intakes of omega-3 fatty acids were 84, 327, and 141 mg/kg body weight (bw) in the placebo, flax oil, and commercial capsule groups, respectively. Average global intakes of omega-6 fatty acids were 509, 468, and 426 mg/kg bw in the placebo, flax oil, and commercial capsule groups, respectively. In each treatment group, approximately half of the dogs improved by more than 50% and 10–20% achieved complete remission. In a double-blind, crossover study, dogs with idiopathic pruritus, confirmed atopy, and/or flea allergy were randomly placed on either 1 omega-3 fatty acid capsule containing 180 mg EPA and 120mg DHA or a control capsule containing 570 mg LA and 50 mg GLA per 4.55 kg (10 lbs) of body weight for 6 weeks (Logas and Kunkle 1994). This was followed by a 3-week period of no supplementation. After the washout period, the dogs were crossed over to the other supplement for an additional 6 weeks. Dogs on 180 mg EPA and 120 mg DHA supplementation from marine oil displayed significant improvements in pruritus, alopecia, and coat character over time, providing evidence that high-dose marine oil is an effective anti-inflammatory treatment of canine allergic skin disease and may provide a safe alternative to glucocorticoids for short-term symptom relief.

The steroid-sparing effect of omega fatty acid supplementation was demonstrated in studies by Bond and Lloyd (1994) and Saevik et al. (2004). Bond and Lloyd (1994) studied perennially affected atopic dogs, whom they gave a concentrated fatty acid supplement containing GLA and EPA for 12 weeks to examine if it would reduce their prednisolone (steroid) requirements. Seventy-three percent of the dogs required reduced doses of prednisolone for satisfactory control of their atopy. The steroid-sparing effect of essential fatty acid supplementation in canine atopic dermatitis was also indicated in the 12-week randomized, double-blind, placebo-controlled clinical trial by Saevik et al. (2004). In this study, dogs received either a combination of borage seed oil and fish oil, or a placebo, in addition to prednisolone tablets. Investigators noted a 64-daytime lag before finding a statistically significant difference between the placebo and control groups; nonetheless the results indicated that the treatment had a steroid-sparing effect. Unfortunately, based

on the results of this study, they could not suggest an optimal dose for a steroid-sparing effect of fatty acid supplementation for the treatment of canine atopic dermatitis.

A review of the literature by Saevik et al. (2004) concluded that different studies showed different lengths of time needed for the fatty acid treatment to show benefits. For example, in the study by Scott et al. (1997), results were seen just after 1–3 weeks, while in the studies by Bond and Lloyd (1994) and Saevik et al. (2004), the period was much longer; hence, the length of time required to achieve maximum benefit is still being debated.

The timing of the start of fatty acid supplementation may also be important in achieving optimal benefits in treating pruritus and atopic dermatitis. In a study by Abba et al. (2005), the effects of omega fatty acid supplementation were evaluated in nonseasonal atopic dogs during a 2-month trial. While animals were divided into two groups, dogs with early stages of atopy and dogs with chronic atopy (immunotherapy nonresponsive), all dogs received 17 mg EPA/kg, 5 mg DHA/kg, and 35 mg GLA/kg supplementation that provided an ω-6 to ω-3 fatty acid ratio of 5.5 to 1 globally. Based on the results, early stages of atopy seem to be more responsive to fatty acid supplementation than chronic atopy; hence, the study authors recommended that omega fatty acid administration be started early in treatment.

In addition to the enteral administration of polyunsaturated fatty acids, a topical preparation of polyunsaturated fatty acids and essential oils was shown to be beneficial in reducing the clinical signs of canine atopic dermatitis. In a randomized, double-blinded, placebo-controlled clinical trial, dogs were treated once a week for 8 weeks with placebo or a spot-on formulation containing 6 mg ALA/mL, 30 mg LA/mL, neem oil, rosemary extract, lavender oil, clove oil, tea tree oil, oregano extract, peppermint extract, cedar bark extract, and vitamin E (Blaskovic et al. 2014). Weekly, dogs weighing less than 10 kg, 10–20 kg, or 20–40 kg were administered 0.6, 1.2, or 2.4 mL, respectively. While significant improvement of symptoms was reported in the treatment group compared to the placebo group, most dogs did not attain complete remission. According to Blaskovic et al. (2014), the efficacy of this topical treatment with polyunsaturated fatty acids and essential oils appears to be comparable to oral treatment with polyunsaturated fatty acids, but neither oral nor dermal treatment with polyunsaturated fatty acids is as efficacious as treatment with glucocorticoids or cyclosporine. Nonetheless, omega fatty acids are safe alternatives to other anti-inflammatory therapies and may have steroid-sparing effects when used as adjunctive therapy.

3.3 Treatment of Osteoarthritis

Osteoarthritis (OA) results from the degeneration of joint cartilage. It causes joint pain, swelling, stiffness, and restricted movement of the joint. It is a prevalent cause of lameness in equine species and is also common in dogs and older cats (Vandeweerd et al. 2012; Corbee et al. 2013). In cats, dogs, and horses, nonsteroidal anti-inflammatory drugs (NSAIDs) are the basic treatment for OA. Corticosteroids, polysulfated glycosaminoglycans, or hyaluronic acid (a nonsulfated glycosaminoglycan) injections are also employed in horses and dogs (Vandeweerd et al. 2012). Various nutraceuticals for the management of OA are also available.

Based on a review of scientific literature, Vandeweerd et al. (2012) concluded that omega-3 fatty acids significantly alleviate the clinical signs of OA in dogs, unlike other nutraceuticals, whose evidence of efficacy is poor. Dogs fed diets containing a total of 0.8% (baseline therapeutic food), 2.0%, and 2.9% EPA and DHA on a dry matter (DM) basis were evaluated for the symptomatic relief of OA (Fritsch et al. 2010a). Animals on the diet containing 2.9% EPA and DHA, but not those on the 2.0% diet, displayed significant improvement in clinical signs of lameness and weight bearing, overall arthritic condition, and progression of OA, compared to those on the baseline diet (0.8%) at day 90 of the study. In a second dietary study, dogs were fed diets containing either approximately 0.11% omega-3 fatty acids and 2.78% omega-6 fatty acids (very high ω-6:ω-3, control diet) or 3.47% omega-3 fatty acids and 2.46% omega-6 fatty acids (low ω-6:ω-3, test food) (Roush et al. 2010a). Owners of dogs that were provided the test food reported some improvements in their dogs' clinical signs of OA, such as the ability to walk after 12 and 24 weeks on the test food. To evaluate the effects of food supplemented with omega-3 fatty acids on weight bearing in dogs with OA, investigators randomly assigned dogs to consume either typical commercial food (controls) or test food containing 3.5% omega-3 fatty acids (Roush et al. 2010b). Supplementation with omega-3 fatty acids resulted in significant improvement in weight bearing and lameness. When the effects of diets containing either 1.47% omega-3 and 1.86% omega-6 fatty acids (ω-6:ω-3 ratio of 1.3) or 0.18% omega-3 and 2.43% omega-6 fatty acids (ω-6:ω-3 ratio of 13.6) were compared in dogs with OA over a 13-week period, dogs on the high omega-3 fatty acid diet displayed improved locomotor ability and performance of daily living activities (Moreau et al. 2013). Moreover, the results of a randomized controlled study suggested that dietary supplementation at the 3.5% level with omega-3 fatty acids in dogs with chronic OA receiving carprofen (a NSAID) to manage pain may allow for the reduction of carprofen dosage (Fritsch et al. 2010b). In summary, multiple studies have shown that omega-3 fatty acids can significantly alleviate the clinical signs of OA in dogs.

The efficacy of omega-3 fatty acids in the management of OA was also evaluated in cats and horses. In a randomized, controlled, blinded clinical study, cats with degenerative joint

disease fed a diet high in EPA and DHA and supplemented with green-lipped mussel extract and glucosamine/chondroitin sulfate significantly improved in objective measures of mobility over a 9-week period (Lascelles et al. 2010). The test diet contained 2.97 g of total omega-3 fatty acids/1000 Kcal and 8.03 g of total omega-6 fatty acids/1000 Kcal. The effects of omega-3 fatty acid supplementation on owners' perception of behavior and locomotion in cats with OA were investigated in a randomized, double-blinded, placebo-controlled study (Corbee et al. 2013). Fish oil containing 15 mg eicosatetraenoic acid (ETA)/mL, 500 mg EPA/mL, and 100 mg DHA/mL (test supplement) or corn oil containing 0 mg EPA and DHA/mL (control supplement) were provided to cats at a dosage of 1 mL/5 kg bw/day over a 10-week period. Cats on the test supplement had higher activity levels, more walking up and down stairs, less gait stiffness, more interaction with their owners, and higher jumps compared with cats on the control supplement.

Arthritic horses that consumed approximately 89.2 g of omega-6 fatty acids and 17.8 g omega-3 fatty acids (ω-6:ω-3 ratio of 5:1) for 90 days experienced a greater decrease in synovial fluid white blood cell concentration and plasma prostaglandin E_2, as well as a trend toward lower normalized plasma fibrinogen concentrations compared to arthritic horses in the control group consuming approximately 114 g of omega-6 fatty acids and 10.2 g omega-3 fatty acids (ω-6:ω-3 ratio of 11:1) (Manhart et al. 2009). While more studies are needed, the above results indicate that omega-3 fatty acids may be beneficial in the treatment of feline and equine OA as well.

3.4 Renoprotective Effects

To examine the utility of EPA as a renoprotective agent, the effects of fish oil supplementation were investigated in an ischemic model of acute renal failure in dogs (Neumayer et al. 1992). Randomly selected dogs received either vehicle capsules (controls) or 55 mg EPA and 40 mg DHA/kg/day (fish oil group) throughout the study. After 3 weeks on the diet, each dog's right kidney was removed. Three weeks after the right-sided nephrectomy, the renal artery of the left kidney was occluded for 2 h to initiate ischemia. Unlike in control animals, no significant effect on renal function or urine volume was reported in dogs pretreated with fish oil. In uninephric dogs, pretreatment with fish oil seemed to protect the animals from ischemic renal failure.

Marked reduction of renal mass in dogs leads to progressive loss of renal function, and as such, this model has been used to study progressive renal injury (Brown et al. 1998, 2000). The long-term responses of dogs with reduced renal mass to dietary manipulation of polyunsaturated fatty acids were studied to investigate the chronic course of induced renal disease (Brown et al. 1998). Partially nephrectomized dogs were randomly assigned to three supplement groups 2 months post-nephrectomy: omega-3 fatty acids (fish oil), omega-6 fatty acids (safflower oil), and saturated fatty acids (beef tallow). Over a 20-month period, significantly reduced survival in the safflower oil-supplemented group was observed compared to the fish oil group. The fish oil group also had significantly lower proteinuria, which may indicate damage to kidneys, than in the safflower oil and beef tallow groups and higher mean urinary clearance of exogenously administered creatinine, a measure for approximating glomerular filtration rate and used to assess the excretory function of the kidneys. Histologic severity of glomerular injury was significantly higher, and renal interstitial cellular infiltrate was more severe in the safflower oil and beef tallow groups compared to the fish oil group. The incidence of glomerulosclerosis, the hardening of the glomeruli responsible for filtration, was significantly lower in the fish oil group compared to the safflower oil and beef tallow groups. Renal tubulointerstitial fibrosis, a progressive detrimental connective tissue deposition on the kidney parenchyma, was significantly more severe in the safflower oil group than in the fish oil group. In summary, while supplementation with omega-3 fatty acids was suggested to be renoprotective, long-term supplementation with omega-6 fatty acids enhanced renal injury in dogs.

Unlike the studies by Neumayer et al. (1992) and Brown et al. (1998), the study by Brown et al. (2000) studied the same model of kidney disease and diets but before the onset of decreased kidney function (Brown et al. 2000). That is, they investigated the effects of omega-3 and omega-6 fatty acid supplementation in dogs with early chronic renal insufficiency. The partially nephrectomized dogs were randomly assigned to three supplement groups 8 weeks post-nephrectomy: omega-3 fatty acids (fish oil), omega-6 fatty acids (safflower oil), and saturated fatty acids (beef tallow) (Brown et al. 2000). In addition, the animals were supplemented with 5 IU of vitamin E/kg/day. The supplemented diets were administered for 10–13 weeks, during which time the kidney injury was minimal and not different among groups. Omega-6 fatty acid supplementation resulted in worsening of glomerular hypertension and hypertrophy. Significant lowering of serum cholesterol concentration and concurrent maintenance or reduction of the ratio of HDL cholesterol to total cholesterol was observed with omega-3 fatty acid supplementation.

In conclusion, there is plenty of evidence supporting the use of omega-3 fatty acids in slowing the progression of chronic kidney disease in dogs. Omega-3 fatty acid supplementation is recommended in dogs and cats with chronic kidney disease in addition to renin-angiotensin antagonism, calcium channel antagonism, and antihypertensive and antiproteinuric therapy (Brown 2008). Dietary

supplementation with specific antioxidants, such as vitamin E, is also important in helping to slow the progression of chronic kidney disease. Unfortunately, the optimum amount of omega-3 fatty acid and the ratio of ω-3 to ω-6 fatty acid for supplementation have not been established conclusively. Nonetheless, the dietary inclusion of 0.4–2.5% DM omega-3 fatty acids has been recommended for dogs with chronic kidney disease (Forrester et al. 2010). In a review of the therapeutic use of fish oils in companion animals, Bauer (2011) recommends that a diet with 0.4% DM [equivalent to a dose of approximately 130–140 mg EPA and DHA/(kg bw)$^{0.75}$ or ~790 mg EPA and DHA for a 10-kg dog] may be helpful, and the highest dose recommended by Forrester et al. (2010) should be used with caution until more data is obtained.

3.5 Beneficial Effects in Hyperlipidemia

Lipid disorders are common in dogs (Johnson 2005). Hyperlipidemia refers to abnormally elevated levels of any or all lipids or lipoproteins in blood. They can be classified by the types of lipids that are elevated and as familial (primary) or acquired (secondary). Several of these conditions have been recognized in specific dog breeds. For example, hypercholesterolemia (high cholesterol) has been associated with briards, an ancient breed of large herding dog originally from France. Primary idiopathic hypertriglyceridemia, the most common type of primary hyperlipidemia in dogs living in the USA, is recognized in miniature Schnauzers (Xenoulis and Steiner 2010).

Canine secondary lipid disorders (SLDs) can be caused by high-fat diets, diabetes, hyperadrenocorticism, hypothyroidism, acute pancreatitis, protein-losing nephropathy, and cholestasis. They are far more common than primary lipid disorders. High plasma cholesterol levels predispose dogs to narrowing of the arteries due to plaque buildup. In addition to atherosclerosis, complications of hyperlipidemia in dogs can include pancreatitis, seizures, and liver and ocular diseases. Treating SLDs involves correcting or managing their causes.

A double-blind, randomized, placebo-controlled study investigating the effect of a fish oil-supplemented diet and doxorubicin on hemograms and biochemical profiles of dogs with lymphoma and hemangiosarcoma reported that dogs receiving an omega-3-supplemented diet had significantly lower serum cholesterol levels than dogs fed the control diet (Walton et al. 2000). In the studies by Brown et al. (2000) and Neumayer et al. (1992), mentioned in the section titled "Renoprotective Effects," supplementation with omega-3 fatty acids lessened hypercholesterolemia in canine renal insufficiency and significantly lowered cholesterol and triglyceride values in canine ischemic renal failure, respectively.

Hypertriglyceridemia was also reported to be effectively controlled with reduced intake of dietary fat and supplementation with one omega-3 fatty acid-rich fish oil capsule/4.55 kg bw/day (1 g/4.55 kg bw/day) (Bauer 1995). This dose equates to approximately 120 mg of EPA and DHA/kg bw$^{0.75}$ (Bauer 2011). In one study, fish oil supplementation (1.75 g/kg of dietary EPA and 2.2 g/kg of dietary DHA with an ω-6:ω-3 fatty acid ratio of 3.4:1) safely decreased triglycerides in dogs, further evidencing fish oil supplementation's possible benefit in the treatment of canine hyperlipidemia (LeBlanc et al. 2005). In addition to lipid-decreasing drugs such as gemfibrozil, supplementation with omega-3 fatty acids, fish oil, or marine-life oil, in conjunction with a low-fat diet, is a viable lipid-reducing strategy in dogs.

3.6 Beneficial Effects in Cardiovascular Diseases

Omega-3 fatty acids have been shown to be beneficial in human cardiovascular diseases. Atherosclerosis is commonly reported in captive birds (Petzinger et al. 2010). Based on the review of the scientific literature, Petzinger et al. (2010) concluded that omega-3 fatty acid supplementation of captive birds may reduce the prevalence of atherosclerosis and help maintain their health. In addition, studies evidencing the beneficial effects of omega-3 fatty acids in canine cardiovascular diseases have been reviewed by Freeman (2010) and Bauer (2011). The findings of these reviews support the antiarrhythmic properties of omega-3 fatty acids and their potential usefulness in treating ventricular premature contractions in dogs. Fish oil supplementation providing 780 mg EPA and 497 mg DHA/animal/day significantly reduced the number of ventricular premature contractions in boxer dogs with arrhythmogenic right ventricular cardiomyopathy (Smith et al. 2007). In a dog model of cardiac sudden death, intravenous administration of either 860 mg EPA, DHA, or ALA each was protective against fatal ventricular arrhythmias, indicating that purified omega-3 fatty acids can prevent ischemia-induced ventricular fibrillation (Billman et al. 1999). Omega-3 fatty acids were also found to benefit dogs with early chronic valvular diseases and to significantly reduce the size of myocardial infarct in dogs with occlusion-perfusion myocardial ischemia. In dogs with stable chronic heart failure secondary to idiopathic dilated cardiomyopathy, fish oil treatment (providing approximately 27 mg EPA/kg bw/day and 18 mg DHA/kg bw/day) reduced cachexia (wasting syndrome) and improved food intake in dogs with anorexia (Freeman et al. 2006). Recommended doses of omega-3 fatty acids for dogs with anorexia and cachexia are 40 mg EPA/kg and 25 mg DHA/kg (Freeman and Rush 2006).

That said, the optimal doses of omega fatty acids for different cardiac diseases, for different stages of the same

disease, or for different species, are not yet clearly known; hence, further research is needed. Until more information is available, Freeman (2010) recommends 40 mg EPA/kg and 25 mg DHA/kg with a ratio of EPA:DHA of approximately 1.5:5 for both cats and dogs with cardiac disease. These amounts can be added directly to the diet or administered orally using fish oil capsules. The use of flax and flaxseed oil is not recommended, as the omega-3 fatty acid in these products is ALA and the conversion of ALA to EPA and DHA is inefficient in dogs and nearly nonexistent in cats.

3.7 Therapeutic Role Against Cancer

In vitro cell culture studies, in vivo studies in rodents with various cancers, clinical trials in humans with cancer, and clinical trials in dogs with cancer have indicated that omega-3 fatty acids may prevent the development of carcinogen-induced tumors, alter tumor growth and metastasis, and sensitize tumor cells to anticancer drugs (Hardman et al. 1997; Ogilvie et al. 2000; Roudebush et al. 2004; Gleissman et al. 2010; Nabavi et al. 2015). In a double-blind randomized study, 12 dogs with malignancies of the nasal cavity received either a diet supplemented with menhaden fish oil and arginine (used to improve wound healing and immune responses, experimental diet) or an otherwise identical diet supplemented with corn oil instead of fish oil (control diet) (Anderson et al. 1997). The animals received 4 weeks of radiation therapy. Diets were administered 1 week before radiation therapy, during therapy, and for at least 6 weeks after therapy. Omega-3 fatty acids normalized elevated blood lactic acid levels and decreased inflammatory mediators. Furthermore, histopathological examination of the skin and mucosa revealed that dogs on the experimental diet had less thinning and ulceration.

In a double-blind, randomized, placebo-controlled study, dogs with lymphoma received either a diet supplemented with fish oil and arginine or a control diet supplemented with soybean oil (Ogilvie et al. 2000). These diets were provided before and after the dogs attained remission, along with up to five doses of doxorubicin (a chemotherapeutic agent used to treat lymphoma and other cancers). Dogs fed fish oil had significantly higher mean serum levels of DHA and EPA compared to controls, which in turn helped normalize elevated blood lactic acid in a dose-dependent manner. For dogs with stage III lymphoma who were fed fish oil, higher serum levels of DHA were significantly associated with longer disease-free interval and survival time. As the diet was supplemented with both arginine and omega-3 fatty acids, distinguishing the effects of each was not possible.

Major pathways responsible for the beneficial effects of omega-3 fatty acids in cancer therapy are (a) alteration of membrane-associated signal transduction, (b) increase of lipid peroxidation that results in irreversible cell damage enhancing sensitivity to anticancer drugs and inducing apoptosis, and (c) modulating gene expression in numerous signaling pathways (Nabavi et al. 2015). For a more thorough review on the antineoplastic effects of omega-3 fatty acids and their potential modes of action in animals and humans, see Nabavi et al. (2015), Gleissman et al. (2010), Calviello et al. (2009), and Roudebush et al. (2004). The findings of these reviews indicate that omega-3 fatty acids clearly have a therapeutic role against certain types of cancers and can improve the tolerability and efficacy of chemotherapy and radiation therapy. Cats with cancer, however, should be given lower levels of omega-3 fatty acids or fish oil than are recommended for dogs, as some cat studies indicated a potential for bleeding problems (Roudebush et al. 2004).

3.8 Treatment of Aggression and Depression

Clinical studies in humans suggest that that supplementation with omega-3 fatty acids may reduce aggressive, impulsive, and depressive behaviors (Hibbeln et al. 2006). Male rat pups subjected to an omega-3 fatty acid-deficient diet for 15 weeks exhibited significantly increased depression and aggression compared to pups on an adequate omega-3 fatty acid diet (DeMar et al. 2006). While supplementation with omega-3 fatty acids seems to reduce aggressive behavior and depression, the offspring of mice exposed to high levels of omega-6 fatty acids during gestation through the maternal diet (2.3 g LA/day) were more aggressive than the offspring of mice on the control diet (0.8 g LA/day), indicating that in utero exposure to a high omega-6 fatty acid diet increases aggression (Raygada et al. 1998).

Aggression is a common problem in dogs. Because omega-3 fatty acid supplementation had led to reduced aggression in humans and rodents, Re et al. (2008) studied the relationship between omega-3 fatty acid plasma level and aggressive behavior in dogs. Aggressive dogs were found to have lower DHA plasma levels and higher ω-6 to ω-3 ratio compared to normal dogs, suggesting that low omega-3 fatty acid levels may adversely affect behavior. Whether omega-3 supplementation is useful in reducing aggressive behavior in dogs needs further investigation.

3.9 Potential Use for the Prevention of Preterm Labor

In humans, marine omega-3 fatty acids reduce the rate of preterm birth and increase birthweight when administered in pregnancy (Salvig and Lamont 2011). In rats, average gestational age at birth was positively related to a high maternal dietary intake of omega-3 fatty acids (Olsen et al. 1990). In

sheep, when preterm labor was induced by betamethasone, intravenous administration of omega-3 fatty acids delayed the onset of labor and the time of delivery (Baguma-Nibasheka et al. 1999). In two of the six animals in the treatment group, labor was completely stopped. These observations indicate that omega-3 fatty acids may be beneficial for the prevention of preterm labor in animals, but further investigations are needed before they become widely used for this purpose.

4 Potential Adverse Effects of Omega Fatty Acids in Dogs and Cats

Based on the review of the scientific literature, Nabavi et al. (2015) concluded that high doses of omega-3 fatty acids may be associated with immune function impairment, platelet dysfunction, and altered glucose and lipid metabolism. According to Freeman's (2010) review, most studies have not identified the reported hemostatic alterations due to anti-aggregatory effects on platelets, vitamin E deficiency and lipid peroxidation, and mild diarrhea to be of clinical relevance. Slow transition of the animal to the high omega fatty acid-supplemented diet, decreasing the dosage of omega fatty acids, and other dietary modifications may help with the management of diarrhea. To avoid or decrease lipid peroxidation and to prevent vitamin E deficiency, either the omega fatty acid-enriched diets can be supplemented with vitamin E, or vitamin E can be added to omega fatty acid supplements (Lenox and Bauer 2013). In addition to the abovementioned potential direct adverse effects from omega fatty acids, there is a potential for omega fatty acid-drug interactions. Concurrent administration of the NSAID carprofen or the antiplatelet medication clopidogrel and omega-3 fatty acids may negatively impact hemostasis (Lenox and Bauer 2013). Consequently, it is important to consider the individual animal before starting omega-3 fatty acid treatment. For instance, animals with low platelet counts may not be candidates for omega-3 fatty acid supplementation. All animals supplemented with high doses of omega-3 fatty acids should be monitored regularly for possible adverse effects.

5 Concluding Remarks and Future Directions

Nutraceuticals, when correctly used as supplements, offer health benefits and play a role in the safe management of numerous human and veterinary diseases. Evidence regarding the beneficial effects of omega fatty acids in both human and veterinary diseases has been accumulating. Nonetheless, more research is needed to firmly establish the amount of and the type of omega fatty acids, as well as the ω-6:ω-3 fatty acid ratio required for the treatment of various diseases in different species. Once these studies become available, refinements in the current recommendations or the establishment of recommended doses for clinical veterinary applications can be implemented.

References

Abba C, Mussa PP, Vercelli A et al (2005) Essential fatty acids supplementation in different-stage atopic dogs fed on a controlled diet. J Anim Physiol Anim Nutr 89(3–6):203–207

Anderson CR, Ogilvie GK, Fettman MJ et al (1997) Effect of fish oil and arginine on acute effects of radiation injury in dogs with nasal tumors: a double blind randomized study. Proc Vet Cancer Soc Am Coll Vet Radiol, Chicago:33–34

Baguma-Nibasheka MJ, Brenna T, Nathanielsz PW (1999) Delay of preterm delivery in sheep by omega-3 long-chain polyunsaturates. Biol Reprod 60(3):698–701

Bauer JE (1995) Evaluation and dietary considerations in idiopathic hyperlipidemia in dogs. J Am Vet Med Assoc 206(11):1684–1688

Bauer JE (2006) Metabolic basis for the essential nature of fatty acids and the unique dietary fatty acid requirements of cats. J Am Vet Med Assoc 229(11):1729–1732

Bauer JE (2007) Responses of dogs to dietary omega-3 fatty acids. J Am Vet Med Assoc 231(11):1657–1661

Bauer JE (2011) Therapeutic use of fish oils in companion animals. J Am Vet Med Assoc 239(11):1441–1451

Bensignor E, Morgan DM, Nuttall T (2008) Efficacy of an essential fatty acid-enriched diet in managing canine atopic dermatitis: a randomized, single-blinded, cross-over study. Vet Dermatol 19(3):156–162

Billman GE, Kang JX, Leaf A (1999) Prevention of sudden cardiac death by dietary pure ω-3 polyunsaturated fatty acids in dogs. Circulation 99(18):2452–2457

Blaskovic M, Rosenkrantz W, Neuber A et al (2014) The effect of a spot-on formulation containing polyunsaturated fatty acids and essential oils on dogs with atopic dermatitis. Vet J 199(1):39–43

Bond R, Lloyd DH (1994) Combined treatment with concentrated essential fatty acids and prednisolone in the management of canine atopy. Vet Rec 134(2):30–32

Brown SA (2008) Oxidative stress and chronic kidney disease. Vet Clin Small Anim Pract 38(1):157–166

Brown SA, Brown CA, Crowell WA et al (1998) Beneficial effects of chronic administration of dietary ω-3 polyunsaturated fatty acids in dogs with renal insufficiency. J Lab Clin Med 131(5):447–455

Brown SA, Brown CA, Crowell WA et al (2000) Effects of dietary polyunsaturated fatty acid supplementation in early renal insufficiency in dogs. J Lab Clin Med 135(3):275–286

Calviello G, Serini S, Piccioni E et al (2009) Antineoplastic effects of n-3 polyunsaturated fatty acids in combination with drugs and radiotherapy: preventive and therapeutic strategies. Nutr Cancer 61 (3):287–301

Chapkin RS, Davidson LA, Ly L et al (2007) Immunomodulatory effects of (n-3) fatty acids: putative link to inflammation and colon cancer. J Nutr 137(1):200S–204S

Corbee RJ, Barnier MMC, Van De Lest CHA et al (2013) The effect of dietary long-chain omega-3 fatty acid supplementation on owner's perception of behaviour and locomotion in cats with naturally occurring osteoarthritis. J Anim Physiol Anim Nutr 97(5):846–853

DeMar JC, Ma K, Bell JM et al (2006) One generation of n-3 polyunsaturated fatty acid deprivation increases depression and aggression test scores in rats. J Lipid Res 47(1):172–180

Forrester SD, Adams LG, Allen TA (2010) Chronic kidney disease. In: Small animal clinical nutrition, 5th edn. Mark Morris Institute, Topeka, KS, pp 765–810

Freeman LM (2010) Beneficial effects of omega-3 fatty acids in cardiovascular disease. J Small Anim Pract 51(9):462–470

Freeman LM, Rush JE (2006) Cardiovascular diseases: nutritional modulation. In: Encyclopedia of canine clinical nutrition. Aniwa SAS-Royal Canin, Aimargues, pp 316–341

Freeman LM, Rush JE, Markwell PJ (2006) Effects of dietary modification in dogs with early chronic valvular disease. J Vet Intern Med 20 (5):1116–1126

Fritsch D, Allen TA, Dodd CE et al (2010a) Dose-titration effects of fish oil in osteoarthritic dogs. J Vet Intern Med 24(5):1020–1026

Fritsch DA, Allen TA, Dodd CE et al (2010b) A multicenter study of the effect of dietary supplementation with fish oil omega-3 fatty acids on carprofen dosage in dogs with osteoarthritis. J Am Vet Med Assoc 236(5):535–539

Gleissman H, Johnsen JI, Kogner P (2010) Omega-3 fatty acids in cancer, the protectors of good and the killers of evil? Exp Cell Res 316(8):1365–1373

Guarner F, Vilaseca J, Malagelada JR (1992) Dietary manipulation in experimental inflammatory bowel disease. Agents Actions 36(1): C10–C14

Hall JA, Saun RJ, Tornquist SJ et al (2004) Effect of type of dietary polyunsaturated fatty acid supplement (corn oil or fish oil) on immune responses in healthy horses. J Vet Intern Med 18 (6):880–886

Hardman WE, Barnes CJ, Knight CW et al (1997) Effects of iron supplementation and ET-18-OCH3 on MDA-MB 231 breast carcinomas in nude mice consuming a fish oil diet. Br J Cancer 76 (3):347

Hibbeln JR, Ferguson TA, Blasbalg TL (2006) Omega-3 fatty acid deficiencies in neurodevelopment, aggression and autonomic dysregulation: opportunities for intervention. Int Rev Psychiatry 18 (2):107–118

Johnson MC (2005) Hyperlipidemia disorders in dogs. Compendium 27:361–370

Lascelles BDX, DePuy V, Thomson A, Hansen B, Marcellin-Little DJ, Biourge V, Bauer JE (2010) Evaluation of a therapeutic diet for feline degenerative joint disease. J Vet Intern Med 24(3): 487–495

LeBlanc CJ, Bauer JE, Hosgood G, Mauldin GE (2005) Effect of dietary fish oil and vitamin E supplementation on hematologic and serum biochemical analytes and oxidative status in young dogs. Vet Ther 6(4):325

LeBlanc CJ, Dietrich MA, Horohov DW et al (2007) Effects of dietary fish oil and vitamin E supplementation on canine lymphocyte proliferation evaluated using a flow cytometric technique. Vet Immunol Immunopathol 119(3–4):180–188

LeBlanc CJ, Horohov DW, Bauer JE et al (2008) Effects of dietary supplementation with fish oil on in vivo production of inflammatory mediators in clinically normal dogs. Am J Vet Res 69(4):486–493

Lenox CE, Bauer JE (2013) Potential adverse effects of omega-3 fatty acids in dogs and cats. J Vet Intern Med 27(2):217–226

Logas D, Kunkle GA (1994) Double-blinded crossover study with marine oil supplementation containing high-dose icosapentaenoic acid for the treatment of canine pruritic skin disease. Vet Dermatol 5(3):99–104

Manhart DR, Scott BD, Gibbs PG et al (2009) Markers of inflammation in arthritic horses fed omega-3 fatty acids. Prof Anim Sci 25 (2):155–160

Mogensen KM (2017) Essential fatty acid deficiency. Pract Gastroenterol:37

Moreau M, Troncy E, Del Castillo JRE et al (2013) Effects of feeding a high omega-3 fatty acids diet in dogs with naturally occurring osteoarthritis. J Anim Physiol Anim Nutr 97(5):830–837

Morley JE (2016, October) Essential fatty acid deficiency. https://www.merckmanuals.com/professional/nutritional-disorders/undernutrition/essential-fatty-acid-deficiency. Accessed 8 June 2018

Mueller RS, Fieseler KV, Fettman MJ, Zabel S, Rosychuk RAW, Ogilvie GK, Greenwalt TL (2004) Effect of omega-3 fatty acids on canine atopic dermatitis. J Small Anim Pract 45(6):293–297

Nabavi SF, Bilotto S, Russo GL, Orhan IE, Habtemariam S, Daglia M, Devi KP, Loizzo MR, Tundis R, Nabavi SM (2015) Omega-3 polyunsaturated fatty acids and cancer: lessons learned from clinical trials. Cancer Metastasis Rev 34(3):359–380

Neumayer HH, Heinrich M, Schmissas M et al (1992) Amelioration of ischemic acute renal failure by dietary fish oil administration in conscious dogs. J Am Soc Nephrol 3(6):1312–1320

Nogradi N, Couetil LL, Messick J et al (2015) Omega-3 fatty acid supplementation provides an additional benefit to a low-dust diet in the management of horses with chronic lower airway inflammatory disease. J Vet Intern Med 29(1):299–306

Ogilvie GK, Fettman MJ, Mallinckrodt CH et al (2000) Effect of fish oil, arginine, and doxorubicin chemotherapy on remission and survival time for dogs with lymphoma. Cancer 88(8):1916–1928

Olsen SF, Hansen HS, Jensen B (1990) Fish oil versus *Arachis* oil food supplementation in relation to pregnancy duration in rats. Prostaglandins Leukot Essent Fatty Acids 40(4):255–260

Ontsouka EC, Burgener IA, Luckschander-Zeller N et al (2012) Fish-meal diet enriched with omega-3 PUFA and treatment of canine chronic enteropathies. Eur J Lipid Sci Technol 114(4):412–422

Park HJ, Park JS, Hayek MG, Reinhart GA, Chew BP (2011) Dietary fish oil and flaxseed oil suppress inflammation and immunity in cats. Vet Immunol Immunopathol 141(3–4):301–306

Petzinger C et al (2010) Dietary modification of omega-3 fatty acids for birds with atherosclerosis. J Am Vet Med Assoc 236(5): 523–528

Raygada M, Cho E, Hilakivi-Clarke L (1998) High maternal intake of polyunsaturated fatty acids during pregnancy in mice alters offsprings' aggressive behavior, immobility in the swim test, locomotor activity and brain protein kinase C activity. J Nutr 128 (12):2505–2511

Re S, Zanoletti M, Emanuele E (2008) Aggressive dogs are characterized by low omega-3 polyunsaturated fatty acid status. Vet Res Commun 32(3):225–230

Roudebush P, Davenport DJ, Novotny BJ (2004) The use of nutraceuticals in cancer therapy. Vet Clin Small Anim Pract 34 (1):249–269

Roush JK, Dodd CE, Fritsch DA et al (2010a) Multicenter veterinary practice assessment of the effects of omega-3 fatty acids on osteoarthritis in dogs. J Am Vet Med Assoc 236(1):59–66

Roush JK, Cross AR, Renberg WC et al (2010b) Evaluation of the effects of dietary supplementation with fish oil omega-3 fatty acids on weight bearing in dogs with osteoarthritis. J Am Vet Med Assoc 236(1):67–73

Saevik BK, Bergvall K, Holm BR et al (2004) A randomized, controlled study to evaluate the steroid sparing effect of essential fatty acid supplementation in the treatment of canine atopic dermatitis. Vet Dermatol 15(3):137–145

Salvig JD, Lamont RF (2011) Evidence regarding an effect of marine n-3 fatty acids on preterm birth: a systematic review and meta-analysis. Acta Obstet Gynecol Scand 90(8):825–838

Scott DW, Miller WH Jr, Reinhart GA et al (1997) Effect of an omega-3/omega-6 fatty acid-containing commercial lamb and rice diet on pruritus in atopic dogs: results of a single-blinded study. Can J Vet Res 61(2):145

Smith CE, Freeman LM, Rush JE et al (2007) Omega-3 fatty acids in Boxer dogs with arrhythmogenic right ventricular cardiomyopathy. J Vet Intern Med 21(2):265–273

Trepanier L (2009) Idiopathic inflammatory bowel disease in cats: Rational treatment selection. J Feline Med Surg 11(1):32–38

Vandeweerd JM, Coisnon C, Clegg P et al (2012) Systematic review of efficacy of nutraceuticals to alleviate clinical signs of osteoarthritis. J Vet Intern Med 26(3):448–456

Walton JA, Ogilvie GK, Fettman MJ et al (2000) Effect of fish oil supplemented diet and doxorubicin on hemograms and biochemical profiles from dogs with lymphoma and hemangiosarcoma: a double blind, randomized, placebo controlled study. Proc Vet Cancer Soc Conf:101

Whiting CV, Bland PW, Tarlton JF (2005) Dietary n-3 polyunsaturated fatty acids reduce disease and colonic proinflammatory cytokines in a mouse model of colitis. Inflamm Bowel Dis 11(4): 340–349

Xenoulis PG, Steiner JM (2010) Lipid metabolism and hyperlipidemia in dogs. Vet J 183(1):12–21

Polyphenols and Flavonoids

Satish Kumar Garg, Amit Shukla, and Soumen Choudhury

Abstract

Majority of the plants used in traditional system of medicine are rich in polyphenols and flavonoids which not only regulate the growth of plants but also are rich source of phytochemicals employed in human and animal health. Polyphenols and flavonoids have been advocated as nutraceuticals in human medicine to treat certain modern lifestyle diseases including cancer. These phytochemicals seem to possess great potential for their use in animal health and production system and to replace certain synthetic chemicals which are deleterious on account of their residual effects on human and animal health. Some of the common polyphenols and flavonoids possessing desirable pharmacological activities and the potential for use in livestock and poultry sectors are kaempferol, quercetin, genistein, rutin, catechin, etc. Similarly, some of the plants rich in polyphenols and flavonoids can be used as green fodder and as feed supplements to formulate certain functional foods for augmenting productivity of animals and poultry.

Keywords

Polyphenols · Flavonoids · Anthocyanin · Flavanones · Isoflavones · Flavonols · Nutraceuticals · Human health · Animal production

1 Introduction

Use of plants and vegetables in treatment of human and animal diseases has been advocated since time immemorial. The famous quote of Hippocrates "Let food be thy medicine and medicine be thy food" expressed almost 2000 years ago also adds credence to the fact that before the advent of allopathic drugs, plants were the major sources of drugs in traditional systems of medicine and used by traditional healers in human and veterinary medicine worldwide including India. Ancient literature of therapeutics in Ayurveda also described the role of plants in treatment of diseases. Advancements in science and understanding of the pharmacological activities, mechanism(s) of action at cellular and molecular levels, toxicity potential and possible therapeutic uses of the active principles of plants and their secondary metabolites opened the vistas for discovery and synthesis of various semi-synthetic and synthetic drugs having their origin from plants, e.g. aspirin, quinine, atropine, cardiac glycosides, reserpine, morphine, senna, etc. Therefore, several modern-day drugs have their roots in the ancient medicinal literature.

Understanding of the human health and diseases is becoming more and more complex with advancements in science, especially at molecular level, due to complex interactions between different factors including environmental pollutants, food habits and lifestyle. During the last two decades, incidence of several diseases like hypertension, diabetes, obesity, renal failure and even various types of cancers has increased several folds, and these are becoming more and more challenging both in developed and developing countries. Lifestyle and food habits have been identified to be the precipitating factors for those diseases which were not very rampant few decades ago. Nutraceutical sector has seen unprecedented growth during the last decade, and now these are considered as complementary and supplementary to modern human medicine as these are expected to play an important role in holistic health management in humans. Nonetheless, their importance in veterinary medicine in health management and augmenting animal productivity cannot be undermined.

The term "nutraceutical" was coined from "nutrition" and "pharmaceutical" combo by Stephen DeFelice, founder and Chairman of the Foundation for Innovation in Medicine (FIM), Cranford, New Jersey in 1989 (Maddi et al. 2007).

S. K. Garg (✉) · A. Shukla · S. Choudhury
Department of Pharmacology and Toxicology, College of Veterinary Science and Animal Husbandry, U.P. Pandit Deen Dayal Upadhyaya Veterinary University (DUVASU), Mathura, Uttar Pradesh, India

R. C. Gupta et al. (eds.), *Nutraceuticals in Veterinary Medicine*, https://doi.org/10.1007/978-3-030-04624-8_13

According to De Felice, nutraceutical can be defined as "a food (or a part of food) that provides medical or health benefits, including prevention and/or treatment of a disease". On the other hand, Health Canada has defined nutraceutical as "a product prepared from foods, but sold in the form of pills, or powder (potions) or in other medicinal forms, not usually associated with foods" (Wildman 2001; Bull 2000). According to the report of Business Communications Company Research (2007), world nutraceutical market has grown to $74.7 billion from $46.7 billion in 2002. The USA, UK and Japan are amongst the leading market countries of nutraceuticals. Nutraceuticals provide functional and dietary supplements and impart medicinal effects in individuals. Therefore, functional and dietary food has been stemmed as a key to human health in modern-day lifestyle.

2 Phytochemicals (PCs)

Plants being very rich in several active principles and constituting the active "pharmacophore moieties" possess diverse pharmacological activities and therapeutic potential. Alkaloids, glycosides, tannins, saponins, gum and resins, etc. are the common active principles present in plants (Figs. 1 and 2). There has been tremendous increase in studies pertaining to the discovery of secondary metabolites in plants and their pharmacological activities. Chemical moieties present in plants have been optimistically considered for their adaptogenic properties not only for plant itself but also to improve human and animal health (Valdes et al. 2015).

Basic and applied research in chemistry and biological life science has revealed the role of different primary metabolites in basic physiological functions in plants such as respiration, growth and development, storage and reproduction (Montero 2016). Discovery of plant metabolite-based drugs started with isolation of morphine from opium by Friedrich Wilhelm Sertürner in 1800s. Kossel (1891) was credited for defining the secondary metabolites of plants in comparison to the primary ones for the very first time, and Czapek (1925) was the second person who described the new role of phytochemicals and coined the term "end products" for these compounds.

Fig. 1 Images of flora containing polyphenols and flavonoids. (**a**) Black currant fruit. (**b**) Pecans. (**c**) Clove flower. (**d**) Cocoa. (**e**) Tea leaves. (**f**) Grapes. (Source: Google web page)

Fig. 2 Images of polyphenol- and flavonoid-rich flora for animal use. (**a**) Sea buckthorn. (**b**) *Azadirachta* leaves. (**c**) *Moringa oleifera* leaves (Source: Google web page)

Phytochemicals are pervasive category of diverse and biologically active secondary metabolites of plants, having diverse pharmacological activities, and form an essential part of the feed and fodder for animals and diet of human beings. Phytochemicals have the advantages of accessibility, specificity of responses and comparatively lower toxicity. But the major disadvantages are low bioavailability and rapid elimination due to rapid biotransformation and excretion. Food and feed of humans and animals, respectively, contain complex mixtures of PCs along with numerous antinutritional factors which can affect the concentrations of these PCs in body.

2.1 Polyphenols

Polyphenols are secondary plant metabolites which are present in vegetables, fruits, coffee beans, tea leaves, chocolates, cereals, legumes, beverages etc. More than 8000 polyphenols have been identified in different plants, and these mainly act as antioxidants. Phenolic compounds are also important constituents and affect the quality of fruits which include taste, colour and nutritional properties. On an average, about 300 mg polyphenol is present in 100 mg of fresh fruits such as grapes, apple, pear, cherries etc., and the relief from stress after consumption of coffee and tea is attributed to polyphenols. Several

meta-analysis data and epidemiological findings have revealed the prophylactic and therapeutic roles of polyphenols and flavonoids against chronic diseases such as cardiovascular, cerebrovascular and neurodegenerative diseases, tumour, diabetes mellitus, etc. in human medicine.

2.1.1 Classification of Polyphenols

On the basis of chemical configuration, polyphenols have been classified into several types, namely, phenolic acids (hydroxybenzoic acids and hydroxycinnamic acids), flavonoids (flavonols, flavones, flavanols, flavanones, anthocyanidins, chalcones, catechins, isoflavones and proanthocyanidins), stilbenes (resveratrol) and lignans (matairesinol) (Manach et al. 2004, 2005; Bohn 2014).

Phenolic acids: These are predominantly present in blueberries, coffee, tea, cinnamon, plum, apple and cherries and subdivided into two subgroups termed as hydroxybenzoic acids and hydroxycinnamic acids.

Flavonoids: These are commonly found in fruits, vegetables, legumes, red wine and green tea.

Stilbenes: These are derived from red wine and peanuts, and resveratrol is the most popular entity of this group.

Lignans: These are commonly found in seeds like that of flax, linseed, legumes, cereals, grains and fruits, algae and certain vegetables.

2.1.2 Phenolic Acids

Hydroxybenzoic acid and hydroxycinnamic acid are the two distinguished classes of phenolic acids. Hydroxybenzoic acids are composed of hydrolysable tannins such as gallotannins and ellagitannins and are generally present in red fruits such as raspberries, blackberries and strawberries (Clifford and Scalbert 2000). The percentage of hydroxybenzoic acid content in edible plants is generally very low. There are certain exceptions to it e.g. black radish and onion contain concentration in tens of milligrams per kilogram weight (Shahidi and Naczk 1995). Gallic acid is commonly found in tea leaves, and its concentration is around 4.5 g/kg fresh weight (Tomas-Barberan and Clifford 2000). Plants containing hydroxybenzoic acid are less commonly used in human food; therefore, nutraceutical aspect of these compounds has not been explored well.

Hydroxycinnamic acids are more common form of phenolic acids as compared to hydroxybenzoic acids and are chiefly consisted of chemicals like p-coumaric, ferulic, sinapic, caffeic acids etc. These acids in plant are commonly distributed in bound forms which are the glycosylated products or esters of shikimic acid, quinic acid and tartaric acid. Processing of the food material either by freezing or by fermentation only yields the free form of hydroxycinnamic acid. Chlorogenic acid (CGA) is an important and valuable polyphenol and an inexpensive source of dietary phenol (Clifford 1999; Garg 2016) and is composed of caffeic, ferulic, coumaric, quinic and 3,4 dimethoxycinnamic acid (Belay and Gholap 2009; Tajik et al. 2017) and found in very high concentrations in green coffee beans (Garg 2016). Outer parts of ripe fruits are rich in hydroxycinnamic acids. About 0.5–2 g hydroxycinnamic acids/kg fresh weight is present in the fruits of blueberries, kiwis, plums, cherries, apples etc. Cereal grains are rich in ferulic acid, and wheat grains contain around 0.8–2 g/kg of ferulic acid on dry weight basis which constitutes up to 90% of the total polyphenols (Lempereur et al. 1997; Hatcher and Kruger 1997).

2.1.3 Flavonoids

Flavonoids chemically constitute the class of low-molecular-weight phenolic compounds that are widely distributed amongst the kingdom *Plantae*. Many of them serve as flower pigment commonly encountered in angiosperms. Flavonoids are an important class of secondary metabolite phytochemicals having the polyphenolic structure; are widely present in fruits, vegetables, tea, cocoa and wine; and therefore are termed as "dietary flavonoids" as these constitute an important component of the normal human diet.

Flavonoids are further classified into different subgroups depending on the basis of stereochemistry and chemical structure as the site of attachment of B ring on C ring and degree of oxidation of the C ring and unsaturation (Fig. 1).

Flavonoids where B ring is linked in position 3 of the C ring are called isoflavones. But if B ring is linked in position 4 of the C ring, these are classified as neoflavonoids (Fig. 3). Those in which the B ring is linked in position 2 of the C ring can be further divided into several subgroups on the basis of oxidation and unsaturation of C ring. These subgroups are flavones, flavonols, flavanones, flavanonols, catechins, anthocyanins and chalcones. Examples of the different subtypes have been illustrated in Fig. 4.

Flavonols

Flavonols are the most ubiquitous flavonoids present in dietary foods, and kaempferol and quercetin are the chief representative molecules (Manach et al. 2005). Flavonols are generally present in relatively low concentrations of 15–30 mg/kg fresh weight, but onion (1.2 g/kg fresh weight), leeks, broccoli and blueberries are the richest sources of flavonols (Manach et al. 2004). Flavonols are generally present in glycosylated form with glucose or rhamnose as major sugar.

Flavanols

Flavanols are generally present in two forms, i.e. polymer form known as proanthocyanidins and monomer form known as catechins. Apricot is the richest source of catechins and

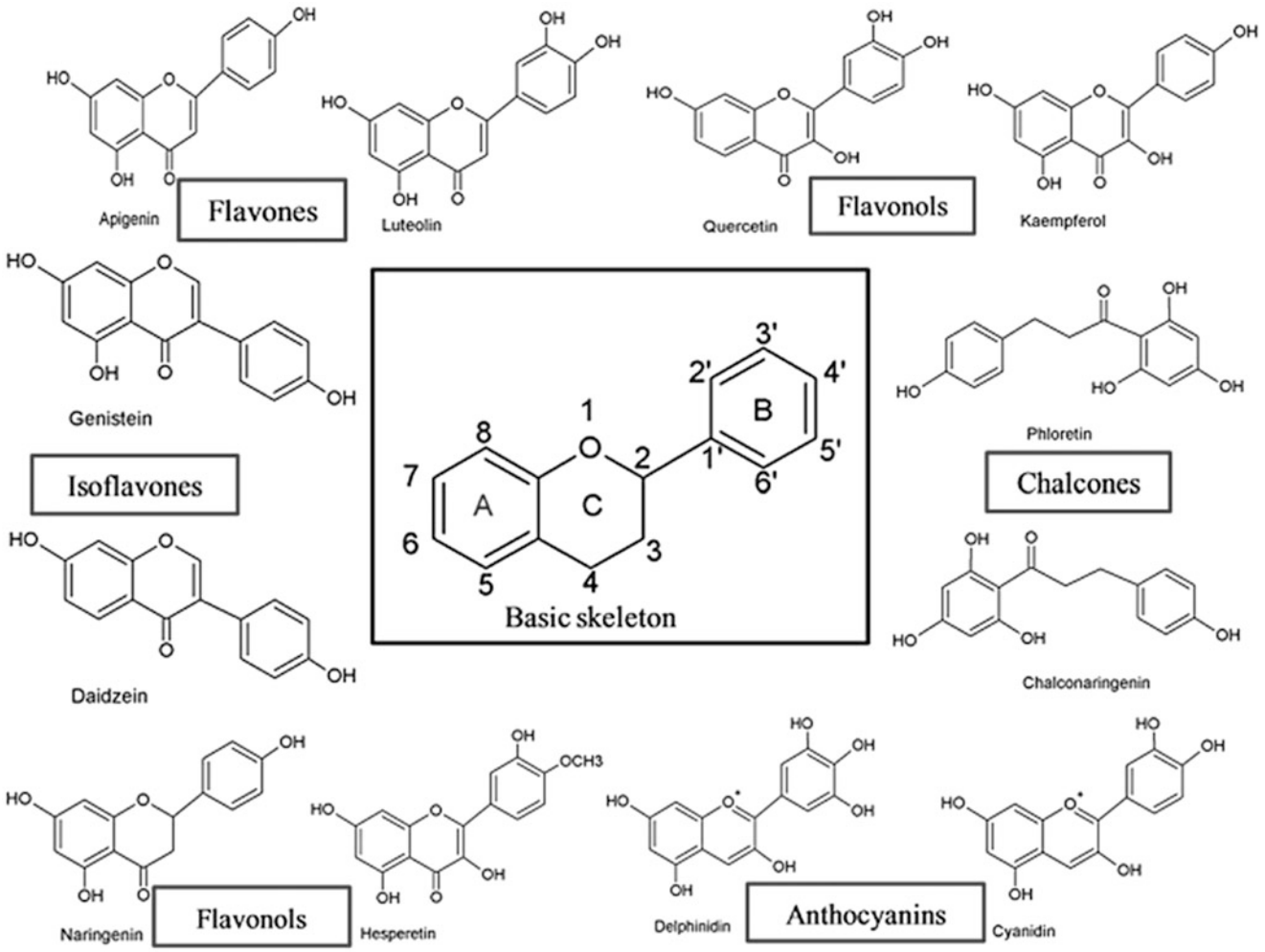


Fig. 3 Basic chemical structures of flavonoids and their classes (Source: Panche et al. 2016)

contains around 250 mg/kg of catechins on fresh weight (Manach et al. 2005). Green tea, red wine and chocolate are also having catechin in great amounts.

Flavones

These are less commonly found in fruits and plants as compared to flavonols and are chiefly consisted of glycosides of luteolin and apigenin. According to Manach et al. (2005), parsley and celery are the only edible sources of flavones. Cereals such as millet and wheat contain C-glycosides of flavones (Erlund et al. 2002; Graefe et al. 2001).

Anthocyanins

These are the colouring pigments present in plants. Cyanidin, malvidin, pelargonidin, delphinidin and peonidin are the most studied anthocyanins. These are commonly present in the outer cellular layers of fruits such as black currants, red grapes, raspberries, strawberries, blueberries, etc. These compounds are generally stable in nature; therefore health benefits along with the stability propel them to be used in nutraceutical industry.

Isoflavones

Soybean-derived products are the richest source of isoflavones. Isoflavones are found in abundance in legumes. Soybeans contain between 580 and 3800 mg isoflavones per kg on fresh weight basis, and soymilk contains between 30 and 175 mg per litre (Hollman and Katan 1997; Moon et al. 2000). Some isoflavones are also present in microorganisms (Matthies et al. 2008).

Chalcones

Chalcones are characterised by the absence of "C ring" in the basic flavonoid skeleton structure, and thus these are termed as "open-chain flavonoids". Examples of chalcones are arbutin, phloridzin, chalconaringenin and phloretin. Chalcones are present in significant amounts in strawberries, pears, tomatoes, bearberries, wheat products, etc.

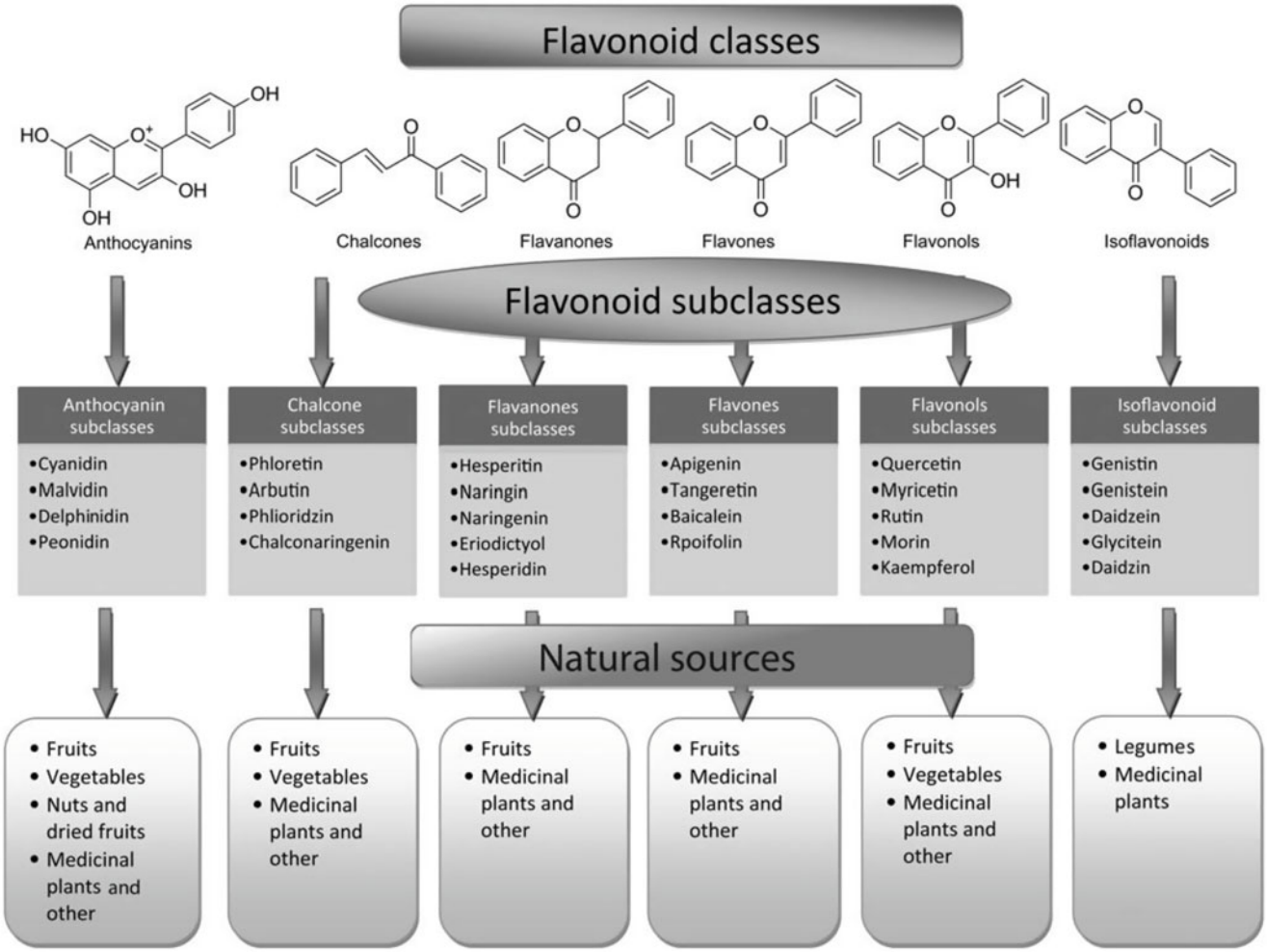


Fig. 4 Classes, subclasses and natural sources of flavonoids (Source: Panche et al. 2016)

2.1.4 Lignans

Lignans are composed of two phenylpropane units. Linseed is the richest dietary source of lignans and contains secoisolariciresinol and matairesinol. Concentration of lignans in linseed is 1000 times more than in other cereals, fruit, grains and certain vegetables (Adlercreutz and Mazur 1997). Lignans are biotransformed by gut microbiota into enterolactone and enterodiol moieties. These two moieties are primarily excreted out in urine, bile and seminal fluids of humans and animals (Wang 2002). These moieties serve as phytoestrogens and thus produce antioestrogenic effects.

2.1.5 Stilbenes

Stilbenes are best exemplified with resveratrol, an antioxidative and antineoplastic agent, found in low quantities in red wine (Bhat and Pezzuto 2002). These are generally found in very low concentrations; hence antineoplastic effect cannot be associated with dietary intake of these molecules.

Phenolic and flavonoid contents in some of the common plants

Source	Total phenolic content {mg gallic acid equivalent/gram on dry weight basis}	Flavonoid content {mg rutin/catechin equivalent/gram on dry weight basis}
Liquorice root	23.65	18.51
Clove	194.47	46.30
Nutmeg	37.26	51.97
Black pepper	17.16	23.57
Mulberry leaf	25.22	21.66
Ginger	21.24	26.21
Peppermint	13.17	27.05
Bamboo leaves	2.75	1.51
Lotus leaf	8.66	12.55
Emblica	26.59	1.35
Ficus	12.36	0.86

(continued)

Source	Total phenolic content {mg gallic acid equivalent/gram on dry weight basis}	Flavonoid content {mg rutin/catechin equivalent/gram on dry weight basis}
Azadirachta indica	12.0	3.14
Terminalia arjuna	12.8	3.49
Acacia nilotica	16.5	4.93

Source: Liu et al. (2008), Sultana et al. (2007), and Pinto and Santos (2017)

2.1.6 Structural Activity Relationship (SAR) of Polyphenols and Flavonoids

Stereochemistry of flavonoids has been closely associated with pharmacological activities/properties. On the basis of known target chemistry, the newer molecules could also be generated following the new dimensions in molecular biology as well as bioinformatics. Thorough understanding of the SAR of flavonoids can help in evolving several newer semi-synthetic and synthetic compounds having promising pharmacological activities and better therapeutic and clinical applications in human and veterinary medicine.

The antioxidant activity of flavonoids is profoundly influenced not only by the position and number of hydroxyl groups on the A and B rings but also by the degree of conjugation between the B and C rings (Sichel et al. 1991; Chen et al. 1996). Antioxidative potential of polyphenols and flavonoids follows the Bors' criteria (Bors et al. 1990) as discussed below:

a. The *o*-dihydroxy (3′,4′-diOH, i.e. catechol) structure in the B ring confers high stability to phenoxyl radicals of the flavonoids via hydrogen bonding or by expanded electron delocalization.
b. The C2–C3 double bond with the 4-oxo group determines the coplanarity of the hetero ring and imparts radical stabilization via electron delocalization over all three ring (A, B and C) systems.
c. 3-OH and 5-OH groups are required for the maximal radical scavenging capacity and the strongest radical absorption.
d. In the absence of *o*-dihydroxy structure in the B ring, hydroxyl substituents in a catechol structure on the A ring are able to compensate and become a larger determinant of the flavonoid antiradical activity (Amic et al. 2007).
e. Hydroxyl group substitution at C-5 in the A ring and C-4′ in the B ring and the methoxyl group substitution at C-3 and C-8 in A ring are quiet essential for DNA gyrase inhibitory action and for inhibition of *Escherichia coli* multiplication (Wu et al. 2013).

3 Classification of Nutraceuticals

Nutraceuticals have been classified as "established and potential nutraceuticals" (Pandey et al. 2010). Polyphenols and flavonoids are considered under "potential nutraceuticals" category in view of their promising pharmacological activities and potential for use in human health and for improving animal health and productivity. Therefore, there is vast scope for exploiting the polyphenols and flavonoids "as nutraceuticals under 'one health mission' too".

4 Significance of Polyphenols in Flora and Fauna and Human Health

Polyphenols have protective role in floral world and also protect humans and plant species from ill and hazardous effects of ultraviolet radiations and microbiological infestations. Due to their antioxidative attributes, these are recognized to have cytoprotective role as these prevent cellular injury.

The popular saying "You are what you eat" predicts the positive correlation between the healthy diet and sound body and mind. Ameliorative potential of different phytochemicals against number of human ailments, such as obesity, cardiovascular disorders, hypertension, hepatomegaly, carcinomas, infectious diseases etc. (Ganesan and Xu 2017) has been well documented, and their protective efficacy has been attributed to modulation/regulation or inhibition of cellular signalling pathways, reduced platelet aggregation, modulation of cholesterol synthesis, decrease in total peripheral resistance and mural tension on blood vessels or antioxidant effects (Vita 2005). Presence of different PCs including polyphenols in plants and their promising therapeutic potential has been the basis of "reverse pharmacology" research which has resulted in discovery of several newer drug molecules during drug development programmes.

4.1 Extraction of Polyphenols and Flavonoids

Although common extraction methods can be employed for extraction of polyphenols and flavonoids, some of the main methods for better extraction of polyphenols and flavonoids are solvent extraction, pressurized liquid extraction, ultrasonic-assisted extraction, microwave-assisted extraction and supercritical extraction. Conventional liquid–liquid and solid–liquid extraction procedures are also frequently used for extraction of polyphenols. In view of the stereochemistry of polyphenols, it is better to use specific extraction protocols for each polyphenol. Time taken for extraction, chemical

structure of the polyphenols and the organic solvents used play very crucial role in extraction procedures and ultimate recovery of the target phytoconstituents.

Phytoconstituents are attached to the cell-wall matrix through glycosidic/ester linkage; therefore, their water solubility is reduced. Studies on extraction procedures have shown that an antioxidant as a basic stabilizer is required in several extraction approaches. Advanced analytical techniques such as high-performance liquid chromatography and/or gas chromatography–MS spectrometry (GC-MS) can also be used for the high-quality extraction of lignans (Brglez 2016).

4.2 Pharmacokinetic Attributes of Polyphenols and Flavonoids

Pharmacokinetic studies of polyphenols and flavonoids have revealed that following ingestion of dietary flavonoids and polyphenols, which predominantly reside in plants as glycoside conjugates, absorption of some of the components, not all, into the circulatory system takes place from the small intestine (Donovan et al. 2006). There are two major pathways of absorption, namely, "lactase-phlorizin hydrolase (LPH)/diffusion" and "transport/cytosolic β-glucosidase (CBG)". Lactase-phlorizin hydrolase (LPH) is present in the brush border of small intestine epithelial cells and exhibits broad substrate specificity for flavonoids, and the released aglycone moieties get passively absorbed through epithelial cells due to its high lipophilicity (Day et al. 2000). CBG-catalyzed hydrolysis is an active process that involves active sodium-dependent glucose transporter (SGLT1) to transport the polar glucosides into epithelial cells (Gee et al. 2000), and this pathway provides an alternative mean of the hydrolytic cleavage of polyphenols and flavonoids within the epithelial cells.

Prior to passage of flavonoids and polyphenols into circulation, aglycones undergo conjugation process, i.e. there are sulphation, methylation and glucuronidation. Additionally, some of the metabolic products might efflux back into the lumen of small intestine, and this efflux might be attributed to the action of ABC [adenosine triphosphate (ATP)-binding cassette] transporters, including multidrug resistance protein (MRP) and P-glycoprotein (P-gp). Then these metabolites undergo phase II metabolism. In addition, there is enterohepatic recycling back to small intestine via biliary excretion (Donovan et al. 2006). Various studies have suggested that after ingestion of the dietary flavonoids, substantial quantities may pass from the small to large intestine (Jaganath et al. 2006; Marks et al. 2009).

Bioavailability is the proportion of the drug or nutrient entity which reaches to systemic circulation in chemically unchanged form. There is no correlation between the concentration of polyphenol and its bioavailability. Prior to absorption, these compounds have to undergo hydrolysis by intestinal enzymes or colonic bacterium. Polyphenols undergo extensive chemical alterations through conjugation reactions in liver and intestine. Methylation, sulphation and/or glucuronidation are the major contributors to conjugation pathways of polyphenols. Polyphenols reach to the tissues, particularly those in which they are metabolized, but these compounds do not possess any accumulation property. Major route of excretion is either via urine or through faeces.

Most of the polyphenols undergo bacterial enzymatic degradation with the help of beta-glucuronidase in distal part of intestine, and the resultant metabolites are reabsorbed into circulation. Thus, enterohepatic recycling may lead to longer half life of polyphenols in body. This microbial catabolism of polyphenols and flavonoids is best exemplified with proanthocyanidins (oligomers and polymers of flavan-3-ols). It results in sequential production of lactones and aromatic and phenolic acids with hydroxylated derivatives depending on the precursor structures as phenylvalerolactones, phenylpropionic acids, phenylacetic acids, phenylvaleric acid, hippuric and benzoic acids (Saura-Calixto et al. 2007).

Absorption percentage of catechins ranges from 47 to 58% and that of aglycones of quercetin is reported to be 4–13%. Glycosylated quercetin moiety showed better bioavailability through improved absorption as compared to the intact quercetin molecules (Hollman and Katan 1997).

Microbiological mode of biotransformation of ellagitannins or hydrolysable tannins, nonflavonoid polymeric molecules, has also been studied during the last almost 10 years (Larrosa et al. 2010). Strawberries, raspberries, walnuts, oak-aged wines, pomegranates, etc. release free ellagic acid after its hydrolysis in lumen of intestinal tract. *Clostridium* and *Eubacteria* are the main bacterial genera which are involved in metabolism of most of the polyphenols and phenolics such as isoflavones (daidzein), flavonols (quercetin and kaempferol), flavones (naringenin and ixoxanthumol) and flavan-3-ols (catechin and epicatechin) (Selma et al. 2009).

Plasma concentration of total polyphenolic metabolites ranged from 0 to 4 μmol/L with dietary intake of 50 mg aglycone equivalents, and the urinary excretion was found to be in the range of 0.3–43% of the ingested dose (Manach et al. 2005). Amongst the 97 well-known polyphenols studied, gallic acid and isoflavones are the most absorbed polyphenols, followed by catechins, flavanones and quercetin glucosides, while proanthocyanidins, the galloylated tea catechins and the anthocyanin were amongst the least absorbed polyphenols.

Despite poor bioavailability of polyphenols, dietary polyphenols (e.g. resveratrol genistein, curcumin and several others) continue to draw enormous attention of the scientific

community due to their promising pharmacological activities like antioxidative, anti-inflammatory, anti-ageing, antineoplastic and antihyperlipidaemic (Farzaei et al. 2016). Crofelemer, an oligomeric proanthocyanidin, has revolutionized the use of polyphenols as pharmaceuticals for managing the side effects of certain HIV drugs, e.g. FDA approval of crofelemer, Polyphenon E, a green tea polyphenol mixture, for the treatment of genital warts in 2006.

Direct and indirect interactions of polyphenols, flavonoids, proteins or polysaccharides or other phytochemicals with human food may alter the pharmacokinetic attributes of the nutraceutical entities and drugs on concurrent administration. Gut pH, intestinal fermentation, biliary excretion, etc. affect the overall gut health and thus may affect absorption of polyphenols (Bohn 2014; Faria et al. 2014; Marin et al. 2015).

The role of concurrently administered xenobiotics and metabolic enzyme inducers and inhibitors cannot be overlooked in terms of their effect in altering the absorption rate. Addition of milk to black tea has been reported to abolish the antioxidant potential (Serafini et al. 1996). But on the contrary, Hollman et al. (2001) showed that addition of milk to black or green tea had no effect on the bioavailability of certain polyphenols and flavonoids as quercetin, catechins or kaempferol in humans, while the alcohol in red wine could improve the intestinal absorption of polyphenols by increasing their dissolution and disintegration, thereby improving solubility. On the contrary, Donovan et al. (1999) have reported that plasma concentrations of catechin metabolites were similar with or without consumption of alcoholised red wine in humans. Existing reports do not suggest marked effect of various diet components on bioavailability of polyphenols in humans and animals.

4.3 Pharmacological Attributes of Polyphenols and Flavonoids

Supplementation of human diet with important bioactive molecules has been reported to enhance the bioavailability of various drugs and modify body functions. Dietary supplements rich in polyphenols and flavonoids have been reported to possess antioxidant, immune booster, antineoplastic and several other activities. Some of the desirable pharmacological properties and mode of action of the polyphenol and flavonoid moieties are described here.

4.3.1 Antioxidant Activity

Cellular damage to body system mainly occurs due to hydrogen abstraction-mediated generation of free radicals; the hyperactive chemical moieties produced include reactive oxygen species (ROS) and reactive nitrogen species (RNS). These ROS and RNS further produce cascade of reactions and damage cellular integrity. Polyphenols protect against oxidative insult-induced cellular damage and also prevent from the ill-effects of radiations or microbiological contamination. The importance of natural dietary antioxidants such as polyphenols and flavonoids as novel therapeutic entity has been suggested in the treatment of several human ailments like neurodegenerative disorders, diabetes, cardiovascular dysfunctions, inflammation and senescence.

Mechanism of Antioxidative Action Polyphenols and flavonoids produce antioxidant action by impeding the hydrogen abstraction and generation of ROS and RNS. Polyphenolic catechins and rutin scavenge the ROS generated due to free radical cascading effect after initiation and propagation of oxidative insult phenomenon (Yang et al. 2008). Metal-chelating property of catechins also stems their potential to combat oxidative insult (Hider et al. 2001; Kumamoto et al. 2001). In addition, polyphenols also produce their antioxidative action by preventing oxidation of LDL lipoprotein, preventing platelet aggregation and damage of red blood cells (Cheynier 2005).

4.3.2 Anti-inflammatory Activity

Cyclooxygenase (COX) is the endogenous enzyme which catalyses the conversion of arachidonic acid into prostaglandins and thromboxanes. COX2 is an inducible enzyme, which is mainly targeted by flavonoids and polyphenols. Depending on the site of origin and also multiple aetiologies, there are different types of inflammation. Rheumatoid arthritis and inflammatory bowel diseases are serious conditions in human medicine and require continuous and long-term use of anti-inflammatory drugs. Rutin, quercetin, resveratrol, catechin, genistein, etc. have been tested and found to be very effective against these conditions in human (Kauss et al. 2008).

Mechanism of Action Polyphenols have shown their efficacy against rheumatoid arthritis and other inflammatory conditions, and the mechanisms include inhibition of macrophage differentiation and osteoclastic function (Kauss et al. 2008), oestrogen modulation (Wang et al. 2008) and antiproliferative and generalised inhibition of migration of inflammatory cells. In allergic rhinitis, bronchitis and asthmatic patients too, the role of flavonoids has been established by Jung et al. (2007). It has been proposed that quercetin and its glycoside rutin helped in lowering the airway resistance and also reduced histamine, phospholipase A2 and endoperoxidase levels. The cellular infiltration was also observed to be inhibited at the site of inflammation.

4.3.3 Antimicrobial Activity

Polyphenols possess antimicrobial activity and also act as clarifying agents (Proestos et al. 2005). Chlorogenic acid

produces bactericidal effect by significantly disrupting the cell membrane, increasing the plasma membrane permeability and ultimately leakage of cytoplasmic macromolecules including nuclear material (Lou et al. 2011; Garg 2016). Antimicrobial activity of honey was found to be potentially augmented in the presence of phenolic acids (Aziz et al. 1998). Researchers have advocated the antibacterial, antifungal and antiviral activities of these polyphenols and flavonoids. Quercetin produces its antibacterial action through inhibition of DNA gyrase. Similarly, sophoraflavone G and (−)-epigallocatechin gallate inhibit cell membrane function and that licochalcones A and C are involved in inhibition of the energy metabolism (Cushnie and Lamb 2005).

5 Polyphenols and Flavonoids in Human Medicine

5.1 Treatment of Neurodegenerative Disorders

Neurodegenerative disorders such as Parkinson's disease are attributed to dysregulation of iron metabolism and oxidative insult conferring production of reactive oxygen species (ROS) from hydrogen peroxide. This in conjunction with inflammatory cytokines and other mediators propagates the cascading events which terminate into apoptosis (programmed cell death) or necrosis.

Mechanism of Neuroprotection Polyphenols and flavonoids produce protective effect against neurodegenerative disorders by altering iron metabolism. Mandel et al. (2004) advocated the iron-chelating property of epigallocatechin-3-gallate (EGCG) to be responsible for its therapeutic efficacy against neurodegenerative disorders. EGCG has been reported to have therapeutic role against Alzheimer's disease by protecting against beta-amyloid-induced neurotoxicity in cultured hippocampal neurons in central nervous system (Choi et al. 2001). The molecular mechanism describes that EGCG modulates the conversion of amyloid precursor protein (APP), through PKC activation, to non-amyloidogenic soluble APP (sAPP), thereby preventing the formation of neurotoxic beta-amyloid protein (Levites et al. 2003). Additionally, *beta*-secretase (BACE1) enzyme is responsible for processing sAPP to beta-amyloid and is also being inhibited by EGCG, thus producing additional inhibitory effect on neurodegenerative processes (Jeon et al. 2003).

5.2 Treatment of Neoplasms

Neoplasia or cancer is defined as uncontrolled cellular growth and metabolism. A therapeutic agent must possess the property of inhibiting the uncontrolled growth and cellular proliferation (Guo et al. 2009). Nutraceuticals have been shown to be effective in reducing the number of cases of neoplasia in vegan and fruit consumers.

Chemical metabolites of polyphenols generated after microbial degradation possess anticancer activity. Forester and Waterhouse (2008) observed that incubating the anthocyanin extract from Cabernet Sauvignon grapes with the large intestine of pigs for 6 h resulted in generation of three identifiable metabolites. Lala et al. (2006) suggested that these metabolic products had the protective potential against colon cancer. In vivo and in vitro studies have also revealed the protective role of ferulic acid against breast cancer (Kampa et al. 2004) and hepatocarcinoma (Lee et al. 2004).

Mechanism of Action Somatic mutation theory of cancer states that DNA alterations result in development of neoplasia (Vogelstein and Kinzler 2004). It has been suggested that low concentrations of kaempferol protect against DNA damage induced by different carcinogens, thus imparting it an anticancer activity (Cemeli et al. 2004). Polyphenols exert protective and therapeutic effects against carcinogenesis by several mechanisms which include removal of the carcinogenic agents (Owen et al. 2000), modulation of cell signalling, cell cycle progression (Corona et al. 2009) and prompting apoptosis (Mantena et al. 2006). Polyphenols also act as metal chelators and alter the enzymatic activities, e.g. resveratrol acts as a chemopreventive agent by inhibiting the ribonucleotide reductase and certain other cellular events involved in carcinogenesis such as initiation, promotion and progression.

Mitogen-activated protein kinase (MAPK) and PI3-kinase signalling pathways have also been targeted as the therapeutic interventional site by some of the flavonoids and polyphenols (Corona et al. 2009). Flavonoids having antioxidant potential also inhibit carcinogenesis (Stefani et al. 1999). Flavonoids such as apigenin, fisetin and luteolin also produce antineoplastic activity by inhibiting cellular proliferation (Fotsis et al. 1997). Possible mechanisms of action of anthocyanin against colon cancer include decrease in colonic cell proliferation, decreased carcinogen-induced aberrant crypt formation and oxidative insult due to antioxidant property (Lala et al. 2006).

5.3 Treatment of Cardiovascular Diseases

Cardiovascular disorders are one of the major causes of mortality in new world. Hypertension is the primary risk factor for stroke, heart disease and renal failure and is one of the most critical issues in human health (Domanski et al. 2002; Lloyd-Jones et al. 2002; Whelton et al. 2002). Endothelial dysfunction is considered an initial stage of

arteriosclerosis with the pivotal role of endothelin as vasoactive substance (Ross 1999). Individual diet schedule and lifestyle monitoring with nutraceutical supplementation can protect against cardiac diseases. Substantial evidence indicate that balanced diet is a boon in prevention of cardiovascular diseases, thus opening the scope of use of polyphenols and flavonoids in functional foods as well as nutraceuticals in human medicine.

Mechanism of Cardioprotective Action Polyphenols ingestion from various sources such as tea, red wine, red clover, etc. results in decrease in levels of total cholesterol, low-density lipoprotein (LDL) and apolipoprotein B and increase in level of good cholesterol, i.e. high-density lipoprotein (HDL) cholesterol and apolipoprotein A-I. Polyphenols thus produce antilipidemic effect and contribute towards cardioprotection.

Cardioprotective efficacy is also attributed to the antioxidant activity of flavonoids and polyphenols. Anthocyanins exert cholesterol-lowering properties, and its mechanism includes anthocyanin-induced activation of AMP-activated protein kinase (AMPK) that inhibits the activity of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-Co-A) reductase enzyme and thereby reduces cholesterol biosynthesis (Guo et al. 2012; Towler and Hardie 2007). Corder et al. (2001) reported that red wine inhibits the synthesis of endothelin-1, a vasoactive peptide, that has role in coronary atherosclerosis. Hence it proves the beneficial and protective efficacy of polyphenols and flavonoids in cardiovascular dysfunctioning.

6 Polyphenols and Flavonoids in Veterinary Medicine

Nutraceuticals have become very popular in human medicine, and their use in human health programmes is increasing at a very fast pace and the market share of nutraceuticals is expected to grow from 4 billion US dollars as of today to 10 billion US dollars by 2022. In view of the promising effects of nutraceuticals in human medicine, there is growing scope for use of nutraceuticals in veterinary medicine too to address the health and production issues related to livestock. Use of antibiotics as growth promoter has been banned in European and certain other countries including India; therefore, these drugs have to be replaced with certain substances which have the property to build biological molecules within the body and trigger growth promotion. Residues of drugs, feed supplements and feed additives in food of animal origin including poultry have further increased the scope for use of nutraceuticals in veterinary medicine as the veterinary nutraceuticals have been jotted down as nondrug substances which are produced either in purified form or extracted form and are administered orally in order to supply elements required for normal body functioning with the aim of improving the health of animals. Quercetin, rutin, resveratrol etc. are amongst the well-studied active moieties. Applications of veterinary nutraceuticals encompass their widespread use in avian, porcine, canine, feline and rodent's production system.

It is reported by Evan et al. (2007) that around 25% dogs over 7 years of age have suffered with cardiac diseases. About 10% of the dogs presented to veterinary clinics have been associated with one or other sort of cardiovascular diseases (Atkins et al. 2009).

Most of the commercially used nutraceuticals include polyphenols and/or flavonoids as one of their major active principles. Quercetin, rutin, stilbene, etc. are the most commonly used components of nutraceuticals, and there is wider scope for inclusion of several other polyphenols and flavonoids in this class.

6.1 Use in Poultry Sector

Quercetin possesses several promising pharmacological properties. Feeding diets supplemented with quercetin counteracted the oxidative stress and improved meat quality of broiler chickens (Rupasinghe et al. 2010). It has also been reported to produce anti-inflammatory effect in birds and animals (Huang et al. 2010). Immune system critically modulates the health and production in animals. Humoral and cellular immune responses regulate the immunological profile in animals. Polyphenols and flavonoids impart better immune health through various pathways such as augmentation of immunoglobulin production, multiplex role in lymphoid organs, enhancing natural killer cell activity, increasing organ weights of immune organs and maintaining balance between the pro- and anti-inflammatory cytokines (Iqbal et al. 2015).

Quercetin has been reported to improve the overall health of broilers and enhance feed conversion efficiency through immunomodulation (Korver 2012). Flavonoids have also been documented to have beneficial effects on various traits such as growth, body weight, feed conversion ratio (FCR), carcass traits, meat and egg quality, immune status and antioxidative profile in animals and birds (Goliomytis et al. 2014).

Flavonoids and polyphenols are also reported to have antidotal property. Quercetin binds to heavy metals like molybdenum, aluminium and nickel and chelates these metals; therefore, there is scope for exploiting this attribute of quercetin not only in treatment of affected animals but also to ameliorate the likely adverse effects of these metals and certain other heavy metals in animals as an antidotal therapy and also to ensure production of quality and residue-free poultry eggs and meat (Maleser and Kuntic 2007).

6.2 Use in Piggery Sector

Nutraceuticals improve the carcass quality and modulate immune system. Pigs are very prone to viral infections. Antimicrobial and immunomodulatory attributes of polyphenols and flavonoids provide extra cushion against these infections and thus reduce neonatal mortality and improve feed conversion ratio. Wein and Wolffram (2014) have advocated the efficacy of quercetin, a polyphenol, in hyperglycaemic pigs by lowering the postprandial glucose level and lipid concentration. Lipid-lowering attribute of the polyphenols, therefore, gives an insight about the scope of use of polyphenols as dietary supplement in pigs in the form of nutraceutical to produce good-quality pork which is low in cholesterol and lipids and can be safely used for human consumption. Soy-genistein at dietary concentrations of 200–400 ppm has been reported to modulate immune response in virally challenged pigs and improve body weight in pigs (Greiner et al. 2001).

Catechin(s), a polyphenol, has also been found to be good antibacterial and reported to promote growth in pigs. Epigallocatechin gallate (EGCG), most abundant catechin of green tea leaves, has proved to be safe and equipotent to other antimicrobials (Caturla et al. 2003). Furthermore, lack of cross-resistance with use of polyphenols and flavonoids seems an added advantage of this phytoconstituents.

Resveratrol also possesses lipid-lowering and antioxidative properties (Resuleo 2016). It reduces the oxidation of low-density lipoproteins and also serves as chelator of copper in blood of pigs (Fremont et al. 1999). Resveratrol has also been used in cosmetic industry as anti-ageing substance based on the studies conducted on porcine as poor absorption from stratum layer and localised beneficial effects (Zhang et al. 2007).

6.3 Use in Companion Animals

Flavonoids are employed as antioxidant and immunomodulator in canines. Flavonoids improve vascularity in canines and reduce the occurrence of purpura and haemorrhages following exposure to radiation (Field and Rekers 1949).

Tea flavonoids have been associated with reduction in atherogenesis process, inhibition of platelet aggregation and also prevention of coronary thrombosis in dogs (Tijburg et al. 1997). Flavonoids and phenolic acids are also reported to inhibit viral replication (Rees et al. 2008; Saha et al. 2009; Kim et al. 2010; Gravina et al. 2011). Antiviral activity of quercetin (30,4,3,5,7-pentahydroxil flavonol) has been documented against adenovirus 3 (AdV-3), herpes simplex virus (HSV) and influenza virus (Choi et al. 2009; Thapa et al. 2011), while morin (20,40,3,5,7-pentahydroxil flavonol) was found to be effective against equine herpesvirus 1 (EHV-1) (Gravina et al. 2011). Rutin (quercetin-3-orutinoside), a glycosidic flavonoid, has also been reported to show antiviral activity against HSV, dengue virus 2 (DENV-2) and human immunodeficiency virus (HIV) (Tao et al. 2007; Zandi et al. 2011), whereas hesperidin is effective against influenza virus (Saha et al. 2009). Antiviral activity of phenolic acids has also been reported against DENV, EHV-1 and HIV (Ichimura et al. 1999; Rees et al. 2008; Gravina et al. 2011).

Dogs are vulnerable to viral infections especially canine distemper (CD). Canine distemper results in disturbances in gastrointestinal (GI) tract, respiratory and nervous systems. Although vaccines are available, the mortality rates have not been curtailed, and even the nervous form of CD is almost incurable. Flavonoids and polyphenols have been reported to prevent virus replication (Kim et al. 2010; Gravina et al. 2011). Antiviral effect of flavonoids and phenolic acids has been shown through the inhibitory mechanisms, which include interaction with the viral envelope glycoproteins (Schnitzler et al. 2008) and/or viral polymerase inhibition and interference with synthesis of viral genome (Formica and Regelson 1995; Cushnie and Lamb 2005).

Biological actions of these active principles have been attributed to their chemical structure, and with the change in chemical structure of flavonoids, change in biological activity and mechanisms have been reported. For instance, isoquercetin, a glycosylated form of quercetin, has been reported to show higher inhibitory activity than quercetin against influenza virus in both in vitro and in vivo experimental models (Kim et al. 2010). Antiviral activity of various derivatives of quercetin with hydroxyl substitutions at C-3, C-30 and C-5 was studied by Thapa et al. (2011), and these synthetic analogues of quercetin were found to show higher therapeutic index than quercetin against influenza virus.

Demrow et al. (1995) suggested that red wine and grape juice reduce platelet activity and thrombosis in stenosed coronary arteries of canine. This effect is attributed to the presence of flavonoids and polyphenols in red wine and grape juices.

Risk of carcinogenesis, especially venereal tumours, is one of the most commonly encountered tumorous conditions in canines. Flavonoids have been reported to be associated with chemopreventive activity in cancer therapy in humans as well as animals. The chalcones, apigenin, luteolin etc. have found to be potent cytotoxic agents against canine cancer cell line DH82 (Silva et al. 2013), thus suggesting their possible use in targeted chemotherapy against malignant and benign tumours in canines and may be in humans too.

Pain management is very challenging and critical both in animals and humans and sometimes even warrants the use of very strong analgesics including narcotic analgesics. Indiscriminate use of analgesics, both narcotics and nonnarcotics, poses great threat to human and animal health due to their

side effects. Gastric impairment, constipation and hepatotoxicity, etc. are the major side effects of nonnarcotic analgesics. The use of flavonoids and polyphenols as nutraceuticals has been suggested to produce relief from degenerative neuropathic pain and osteoarthritis in dogs following supplementation of baicalin and catechin mixture as nutraceutical to dogs through dual inhibition of cyclooxygenase and 5-lipoxygenase by flavonoids (Burnett et al. 2009).

Ocular damage in humans and canines results into nyctalopia terminating into blindness. The flavonoids and polyphenols present in grapefruit have been reported to counteract the damage induced by oxidative insult to canine lens epithelial cells (Barden et al. 2008). Therefore, nutritional supplementation of grapefruit or the flavonoids and polyphenols isolated from this in the diet can be useful in maintaining the corneal visibility for a longer duration. Low et al. (2014) have also suggested efficacy of ferulic acid, an acid having polyphenolic antioxidant activity against halitosis in canines, thus preventing dental caries and indigestion problems in canines. Therefore, ferulic acid can be employed as a constituent in functional feeds/foods for canines.

6.4 Use in Equine Sector

Equines, which include horses, mules and donkeys, are used for race and recreational purposes and carrying load in day-to-day life as well as in difficult areas, not only by general people but also by defence forces. Generally, horses receive hay and green fodder along with some concentrate. Due to racing and weight load especially in difficult terrains, these animals are very prone to excessive wear and tear, inflammatory changes and ageing. Studies on the beneficial effects of polyphenols in equine diet have revealed that age-related inflammation, i.e. inflammageing, is reduced following dietary supplementation of curcumin in equine diet; thereby it reduces the chances of pounder and osteoarthritis in equines (Siard et al. 2016).

In reproduction science also, quercetin has been identified as a new and promising antioxidant to improve vitality of spermatozoa in stored semen as it reduces lipid peroxidation in sperm and maintains internal adenosine triphosphate (ATP) concentration and thereby improve the overall fertility rate by improving capacitation (McNiven and Richardson 2006). Therefore, there is vast scope for use of polyphenols and flavonoids as nutraceuticals in equines.

6.5 Use in Laboratory Animals

Flavonoids and polyphenols are established to possess promising potential against several diseases including degenerative joint diseases; and the use of green tea extract has shown improvement in arthritis due to inhibition of matrix-degrading enzymes/factors at the mRNA level via inhibition of NF-κB pathway (Haqqi et al. 1999). Flavonoids obtained from *Ficus* have been reported to be as effective as vitamin E as nutraceutical and also proven to be a hepatoprotectant against carbon tetrachloride-induced hepatic injury in rats (Augusti et al. 2005).

Anti-inflammatory activity of flavonoids using guinea pig model has been established, and flavonoids have cyclooxygenase (COX) inhibitory potential (Kim et al. 1998). Thus flavonoids have the potential to be used as nutraceuticals against anti-inflammatory conditions. Grape contains polyphenols such as anthocyanins, quercetin, myricetin, kaempferol, resveratrol, etc. These polyphenols as nutraceuticals in guinea pig diet have been proven to alter the hepatic cholesterol metabolism and affect "very low density lipoprotein (VLDL) secretion". This effect results in reduced accumulation of triglycerides and cholesterol in coronary arteries, thereby reducing the chances of coronary heart diseases (Zern et al. 2003). Therefore, these can be used as a constituent in animal feeds to produce healthy and disease-free laboratory animals for biomedical research.

7 Potential Use of Polyphenols and Flavonoid-Rich Plants in Animal Feeding

Moringa oleifera, sea buckthorn, *Curcuma longa*, *Trachyspermum ammi* and several other plants have been widely used either as green fodder or as feed supplements in animal and poultry sector. The pharmacological properties of these plants are attributed to their several active principles especially polyphenols and flavonoids.

Oil and fruit of sea buckthorn have cytoprotective effect against sodium nitroprusside-induced oxidative insult (Geetha et al. 2002). This plant is also known for its potential immunomodulatory action. It improves the cellular arm of immunity by increasing the multiplication of immune cells in respective immune organs such as spleen. Leaves and fruits of sea buckthorn contain flavonoids and exert cardioprotective effect by decreasing myocardial oxygen consumption along with antiplatelet aggregatory effect. Flavonoids in plants also improve cardiac contractions through calcium signal modulation by inhibiting calcium influx and thus prevent congestive heart failure.

Beneficial effects of feeding polyherbal feed supplement (Herbiotic FS) in broilers as reflected by economic returns from broilers have been reported, and the improved FCR was attributed to the three constituent plants-based materials, namely *Trachyspermum ammi*, *Rheuma emodi*, and *Curcuma longa*. These plants are known to be rich in phenolic compounds (Bhushan et al. 2008).

Trachyspermum ammi has been reported to possess antilipedimic, anti-inflammatory, antibacterial and antifungal activities, and these activities have been attributed to the phenolic compounds present in seeds of this plant (Pathak et al. 2010). Bamboo leaves also have lipid-lowering effect in rat model due to presence of flavones (Yang et al. 2015). Additionally, bamboo leaves also possess antimicrobial activity which is attributed to polyphenols and flavonoids (Singh et al. 2012). Aqueous extract of *Nyctanthes arbor-tristis* flowers possesses promising immunomodulatory action by augmenting antibody production and modifying cytokines production (Bharshiv et al. 2016). Similar immunomodulatory effect of *Moringa oleifera* leaf extract has also been reported (Jayanthi et al. 2015), thus suggesting the possible use of leaves and flowers of these plants as biomass in poultry and animal feeding.

Moringa oleifera and *Nyctanthes arbor-tristis* leaves and some other parts of these plants are also rich in flavonoids and polyphenols. Swain et al. (2017) have also shown that addition of *Moringa oleifera* leaf meal at 0.50 kg/100 kg diet as biomass replacer in laying hens improved egg production and feed conversion ratio (FCR) in layers. Divya et al. (2014) reported that powder of *Moringa oleifera* leaves possesses antilipidemic activity and also improves FCR in broilers when used as feed supplement. Alam et al. (2015) reported that feeding neem leaf powder to broilers showed significant improvement in meat yield and overall growth. Thus, it proves the potential of these plants as feed supplements and biomass replacer along with the beneficial effect on health of animals.

8 Toxicity of Polyphenols and Flavonoids

Possibly therapeutic use of polyphenols and flavonoids have been widely investigated, but their toxic effects on animal and human health have not been thoroughly investigated. Flavonoids and polyphenols have been advocated as source of alternative medicine without working out their adverse effects, if any. These substances have pro-oxidant activity that could trigger early lipid peroxidation leading to increase in free radicals and thus increased ROS generation. Green tea catechin and (−)-epigallocatechin-3-gallate (EGCG) are known to trigger production of H_2O_2 and induce oxidative damage to cytosolic DNA in the presence of transition metal ions such as copper. This increase in pro-oxidant activity of EGCG is significantly claiming its role in nitrosamine-induced colon cancer.

Tea is the most commonly used beverage around the globe. Polyphenols and flavonoids in tea prove it to be a nutraceutical drink; however, xanthine content in tea results in variety of toxic signs and symptoms. Commonly encountered toxic effects include irritation to nerves, tonic convulsions, arrhythmia, tachycardia and GI irritation. Additionally, certain varieties of tea also elicit severe allergic reactions such as asthma, nausea, emesis, hay fever, body ache and neck pain in sufferers (Subiza et al. 1989).

Green tea causes hepatotoxicity, mitochondrial toxicity, headache, nausea and oxidative insult in the intoxicated subjects on chronic exposure, whereas black tea causes precipitation of digestive juices and reduction in iron absorption. Excessive consumption of oolong tea can lead to elimination of calcium in urine leading to osteoporosis (Heaney 2002). Hence, it should be limited to only up to three tea cups a day. Further overdosing of oolong tea can lead to hypokalaemia, QT prolongation, atrioventricular block and ventricular tachycardia (Toshiya et al. 1999).

Quercetin is also known to be mutagenic in cultured cells. A procarcinogenic effect of quercetin in rat model of nitrosomethylurea-induced pancreatic cancer or azoxymethane-induced colon cancer has been reported (Barotto et al. 1998). Phase II metabolizing enzymes are the key factors for toxic effects of polyphenols.

Phytoestrogens stimulate the proliferation of oestrogen-responding cells and increase the risks of carcinogenesis (Breinholt et al. 2000). Genistein has been reported to stimulate the proliferation of breast cancer cells implanted in ovariectomized mice (Ju et al. 2001).

With the balance between pro- and antioxidant properties of flavonoids and polyphenols, maximum therapeutic advantage should be exploited from these entities; hence, these should be used with due precaution.

9 Concluding Remarks and Future Directions

Use of phytomedicines in human and animal health in folklore medicine dates back to thousands of years. With advancements in scientific knowledge and scientific-validation methods role of folk medicines and traditional herbal formulary is increasing with passage of time. Polyphenols are a group of vast therapeutic entities that have the potential for use as antioxidant, anti-inflammatory, cardio-protectant, anti-neoplastic, anti-allergic, antibacterial, antiviral, etc. Low bioavailability of flavonoids and polyphenols is a matter of concern. Scientists should evolve methods to improve the pharmacokinetic profile of polyphenols. Encapsulation and nanotechnology tools may prove to be useful in enhancing the bioavailability of these polyphenols and flavonoids.

Efforts should be made for evolving targeted drug delivery systems and to validate molecular insights for stereochemistry-based actions and validate the drug-receptor interaction. Polyphenols and flavonoids have the potential to serve as panacea for modern lifestyle diseases as dietary supplements in human diet apart from their use in the treatment of some of the occupational diseases. These

phytoconstituents should also be exploited for their use in livestock and poultry feeds apart from encouraging the inclusion of biomass of those plants in animal feeding which are rich in polyphenols and flavonoids.

References

Adlercreutz H, Mazur W (1997) Phytoestrogens and western diseases. Ann Med 29:95–120

Alam M, Rakib A, Abdullah-Al-Hasan M et al (2015) Effects of neem leave powder as a growth promoter in broilers. Int J Nat Soc Sci 2:22–26

Amic D, Davidovic A, Beslo D et al (2007) SAR and QSAR of the antioxidant activity of flavonoids. Curr Med Chem 14:827–845

Atkins C, Bonagura J, Ettinger S et al (2009) Guidelines for the diagnosis and treatment of canine chronic valvular heart disease. J Vet Intern Med 23(6):1142–1150

Augusti KT, Prabha SP, Smitha KB et al (2005) Nutraceutical effects of garlic oil, its nonpolar fraction and a *Ficus* flavonoid as compared to vitamin E in CCl4-induced liver damage in rats. Indian J Exp Biol 43 (05):437–444

Aziz NH, Farag SE, Mousa LA et al (1998) Comparative antibacterial and antifungal effects of some phenolic compounds. Microbios 93 (374):43–54

Barden CA, Chandler HL, Lu P et al (2008) Effect of grape polyphenols on oxidative stress in canine lens epithelial cells. Am J Vet Res 69 (1):94–100

Barotto NN, Lopez CB, Eynard AR et al (1998) Quercetin enhances pretumorous lesions in NMU model of rat pancreatic carcinogenesis. Cancer Lett 129:1–6

Belay A, Gholap AV (2009) Characterization and determination of chlorogenic acids (CGA) in coffee beans by UV-VIS spectroscopy. Afr J Pure Appl Chem 3(11):234–240

Bharshiv CK, Garg SK, Bhatia AK (2016) Immunomodulatory activity of aqueous extract of *Nyctanthes* arbor-tristis flowers with particular reference to splenocytes proliferation and cytokines induction. Indian J Pharm 48(4):412–417

Bhat K, Pezzuto JM (2002) Cancer chemopreventive activity of resveratrol. Ann N Y Acad Sci 957:210–229

Bhushan B, Garg SK, Kumar J, Shukla PK (2008) Effect of polyherbal feed supplement on production performance and nutrient utilization in broiler chicks. Indian J Poult Sci 43(1):67–70

Bohn T (2014) Dietary factors affecting polyphenol bioavailability. Nutr Rev 72(7):429–452

Bors W, Heller W, Michel C et al (1990) In: Packer L, Glazer AN (eds) Methods in enzymology, vol 186. Academic Press, San Diego, CA, pp 343–355

Breinholt V, Hossaini A, Svendsen GW et al (2000) Estrogenic activity of flavonoids in mice. The importance of estrogen receptor distribution, metabolism and bioavailability. Food Chem Toxicol 38 (7):555–564

Brglez M (2016) Polyphenols: extraction methods, antioxidative action, bioavailability and anticarcinogenic effects. Molecules 21(7):901

Bull E (2000) What is nutraceutical? Pharm J 265:57–58

Burnett BP, Stenstrom KK, Baarsch MJ et al (2009) A flavonoid mixture, dual inhibitor of cyclooxygenase and 5-lipoxygenase enzymes, shows superiority to glucosamine/chondroitin for pain management in moderate osteoarthritic dogs. Int J Appl Res Vet Med 7(1):1–10

Caturla N, Vera-Samper E, Villalaín J et al (2003) The relationship between the antioxidant and the antibacterial properties of galloylated catechins and the structure of phospholipid model membranes. Free Radic Biol Med 34(6):648–662

Cemeli E, Schmid TE, Anderson D (2004) Modulation by flavonoids of DNA damage induced by estrogen-like compounds. Environ Mol Mutagen 44:420–426

Chen ZY, Chan PT, Ho KY et al (1996) Antioxidant activity of natural flavonoids is governed by number and location of their aromatic hydroxyl groups. Chem Phys Lipids 79:157–163

Cheynier V (2005) Polyphenols in foods are more complex than often thought. Am J Clin Nutr 81:223S–229S

Choi YT, Jung CH, Lee SR et al (2001) The green tea polyphenol(−)-epigallocatechin gallate attenuates b-amyloid-induced neurotoxicity in cultured hippocampal neurons. Life Sci 70:603–614

Choi HJ, Kim JH, Lee CH et al (2009) Antiviral activity of quercetin 7-rhamnoside against porcine epidemic. Antivir Res 81(1):77–80

Clifford MN (1999) Chlorogenic acids and other cinnamates–nature, occurrence and dietary burden. J Sci Food Agric 79:362–372

Clifford MN, Scalbert A (2000) Ellagitannins–nature, occurrence and dietary burden. J Sci Food Agric 80(7):1118–1125

Corder R, Douthwaite JA, Lees DM et al (2001) Health: endothelin-1 synthesis reduced by red wine. Nature 414(6866):863

Corona G, Spencer JP, Dessi MA (2009) Extra virgin olive oil phenolics: absorption, metabolism, and biological activities in the GI tract. Toxicol Ind Health 25:285–293

Cushnie TT, Lamb AJ (2005) Antimicrobial activity of flavonoids. Int J Antimicrob Agents 26(5):343–356

Czapek F (1925) Biochemie der Pflanzen, vol 2. G. Fischer, Switzerland

Day AJ, Canada FJ, Diaz JC et al (2000) Dietary flavonoid and isoflavones glycosides are hydrolysed by the lactase site of lactase phloridzin hydrolase. FEBS Lett 468:166–170

Demrow HS, Slane PR, Folts JD (1995) Administration of wine and grape juice inhibits in vivo platelet activity and thrombosis in stenosed canine coronary arteries. Circulation 91:1182–1188

Divya, Mandal AB, Biswas A et al (2014) Effect of dietary *Moringa oleifera* leaves powder on growth performance, blood chemistry, meat quality and gut microflora of broiler chicks. Anim Nutr Feed Technol 14:349–357

Domanski M, Mitchell G, Pfeffer M et al (2002) Pulse pressure and cardiovascular disease-related mortality: follow-up study of the multiple risk factor intervention trial (MRFIT). JAMA 287:2677–2683

Donovan JL, Bell JR, Kasim KS et al (1999) Catechin is present as metabolites in human plasma after consumption of red wine. J Nutr 129:1662–1668

Donovan JL, Manach C, Faulks RM et al (2006) Absorption and metabolism of dietary secondary metabolites. In: Crozier A, Clifford MN, Ashihara H (eds) Plant secondary metabolites: occurrence, structure and role in the human diet. Blackwell Publishing, Oxford, NY, pp 303–351

Erlund I, Silaste ML, Alfthan G et al (2002) Plasma concentrations of the flavonoids hesperetin, naringenin and quercetin in human subjects following their habitual diets, and diets high or low in fruit and vegetables. Eur J Clin Nutr 56:891–898

Evan SK, Braunwald E, Wood HF (2007) A study of C-reactive protein in the serum of patients with congestive heart failure. Am Heart J 51:533–541

Faria A, Fernandes I, Norberto S et al (2014) Interplay between anthocyanins and gut microbiota. J Agric Food Chem 62:6898–6902

Farzaei MH, Bahramsoltani R, Abdollahi M et al (2016) The role of visceral hypersensitivity in irritable bowel syndrome: pharmacological targets and novel treatments. J Neurogastroenterol Motil 22:558–574

Field J, Rekers PE (1949) Studies of the effects of flavonoids on roentgen irradiation disease. comparison of the protective influence of some flavonoids and vitamin c in dogs. J Clin Invest 28 (4):746–751

Forester SC, Waterhouse AL (2008) Identification of Cabernet Sauvignon anthocyanin gut microflora metabolites. J Agric Food Chem 56(19):9299–9304

Formica JV, Regelson W (1995) Review of biology of quercetin and related bioflavonoids. Food Chem Toxicol 33:1061–1080

Fotsis T, Pepper MS, Aktas E et al (1997) Flavonoids, dietary-derived inhibitors of cell proliferation and in vitro angiogenesis. Cancer Res 57:2916–2921

Fremont L, Belguendouz L, Delpal S (1999) Antioxidant activity of resveratrol and alcohol-free wine polyphenols related to LDL oxidation and polyunsaturated fatty acids. Life Sci 64:2511–2521

Ganesan K, Xu B (2017) A critical review on polyphenols and health benefits of black soybeans. Nutrients 9:455. https://doi.org/10.3390/nu9050455

Garg SK (2016) Green coffee bean. In: Gupta RC (ed) Nutraceuticals: efficacy, safety and toxicity, 1st edn. Academic Press/Elsevier, San Diego, CA, pp 653–667

Gee JM, DuPont SM, Day AJ et al (2000) Intestinal transport of quercetin glycosides in rats involves both deglycosylation and interaction with the hexose transport pathway. J Nutr 130:2765–2771

Geetha S, Singh V, Havazhagan G et al (2002) Antioxidant and immunomodulatory properties of Sea buckthorn—an in vitro study. J Ethnopharmacol 79:373–378

Goliomytis M, Tsoureki D, Simitzis PE et al (2014) The effects of quercetin dietary supplementation on broiler growth performance, meat quality, and oxidative stability. Poult Sci 93:1957–1962

Graefe EU, Wittig J, Mueller S et al (2001) Pharmacokinetics and bioavailability of quercetin glycosides in humans. J Clin Pharmacol 41:492–499

Gravina HD, Tafuri NF, Silva Júnior A et al (2011) In vitro assessment of the antiviral potential of trans cinnamic acid, quercetin and morin against equid herpesvirus 1. Res Vet Sci 91(3):158–162

Greiner LL, Stahl TS, Stabel TJ (2001) The effect of dietary soy genistein on pig growth and viral replication during a viral challenge. J Anim Sci 79(5):1272–1279

Guo Z, Yang X, Sun F et al (2009) A novel androgen receptor splice variant is up-regulated during prostate cancer progression and promotes androgen depletion–resistant growth. Cancer Res 69 (6):2305–2313

Guo H, Xia M, Zou T et al (2012) Cyanidin 3-glucoside attenuates obesity-associated insulin resistance and hepatic steatosis in high-fat diet-fed and db/db mice via the transcription factor FoxO1. J Nutr Biochem 23:349–360

Haqqi TM, Anthony DD, Gupta S et al (1999) Prevention of collagen-induced arthritis in mice by a polyphenolic fraction from green tea. Proc Natl Acad Sci USA 96(8):4524–4529

Hatcher DW, Kruger JE (1997) Simple phenolic acids in flours prepared from Canadian wheat: relationship to ash content, color, and polyphenol oxidase activity. Cereal Chem 74:337–343

Heaney R (2002) Effects of caffeine on bone and calcium economy. Food Chem Toxicol 40:1263–1270

Hider RC, Liu ZD, Khodr HH (2001) Metal chelation of polyphenols. Methods Enzymol 335:190–203

Hollman PC, Katan MB (1997) Absorption, metabolism and health effects of dietary flavonoids in man. Biomed Pharmacother 51:305–310

Hollman PCH, van het Hof KH, Tijburg LBM et al (2001) Addition of milk does not affect the absorption of flavonols from tea in man. Free Radic Res 34:297–300

Huang RY, Yu YL, Cheng WC et al (2010) Immunosuppressive effect of quercetin on dendritic cell activation and function. J Immunol 184 (12):6815–6821

Ichimura T, Otake T, Mori H et al (1999) HIV-1 protease inhibition and anti-HIV effect of natural and synthetic water-soluble lignin-like substances. Biosci Biotechnol Biochem 63(12):2202–2204

Iqbal Z, Kamran Z, Sultan JI et al (2015) Replacement effect of vitamin E with grape polyphenols on antioxidant status, immune, and organs histopathological responses in broilers from 1- to 35-d age. J Appl Poult Res 24(2):127–134

Jaganath IB, Mullen W, Edwards CA et al (2006) The relative contribution of the small and large intestine to the absorption and metabolism of rutin in man. Free Radic Res 40:1035–1046

Jayanthi M, Garg SK, Yadav P et al (2015) Some newer marker phytoconstituents in methanolic extract of *Moringa oleifera* leaves and evaluation of its immunomodulatory and splenocytes proliferation potential in rats. Indian J Pharm 47(5):518–523

Jeon SY, Bae K, Seong YH et al (2003) Green tea catechins as a BACE1 (beta-secretase) inhibitor. Bioorg Med Chem Lett 13(22): 3905–3908

Ju YH, Allred CD, Allred KF et al (2001) Physiological concentrations of dietary genistein dose-dependently stimulate growth of estrogen-dependent human breast cancer (MCF-7) tumors implanted in athymic nude mice. J Nutr 131(11):2957–2962

Jung CH, Lee JY, Cho CH et al (2007) Anti-asthmatic action of quercetin and rutin in conscious guinea-pigs challenged with aerosolized ovalbumin. Arch Pharm Res 30(12):1599–1607

Kampa M, Vassilia-Ismini A, George N et al (2004) Antiproliferative and apoptotic effects of selective phenolic acids on T47D human breast cancer cells: potential mechanisms of action. Breast Cancer Res 6:63–74

Kauss T, Moynet D, Rambert J et al (2008) Rutoside decreases human macrophage-derived inflammatory mediators and improves clinical signs in adjuvant-induced arthritis. Arthritis Res Ther 10(1):R19

Kim HP, Mani I, Iversen L et al (1998) Effects of naturally-occurring flavonoids and bioflavonoid on epidermal cyclooxygenase and lipoxygenase from guinea-pigs. Prostaglandins Leukot Essent Fatty Acids 58(1):17–24

Kim Y, Narayanan S, Chang K (2010) Inhibition of influenza virus replication by plant-derived isoquercetin. Antivir Res 88:227–235

Korver D (2012) Implications of changing immune function through nutrition in poultry. Anim Feed Sci Technol 173:54–64

Kossel A (1891) Uber die Chemische Zusammensetzung der Zelle. Arch Physiol 1891:181–186

Kumamoto M, Sonda T, Nagayama K et al (2001) Effects of pH and metal ions on antioxidative activities of catechins. Biosci Biotechnol Biochem 65(1):126–132

Lala G, Malik M, Zhao C et al (2006) Anthocyanin-rich extracts inhibits multiple biomarkers of colon cancer in rats. Nutr Cancer 54:84–93

Larrosa M, Garcia-Conesa MT, Espín JC et al (2010) Ellagitannins, ellagic acid and vascular health. Mol Asp Med 31(6):513–539

Lee J, Koo N, Min DB (2004) Reactive oxygen species, aging, and antioxidative nutraceuticals. Compr Rev Food Sci Technol 3:21–33

Lempereur I, Rouau X, Abecassis J (1997) Genetic and agronomic variation in arabinoxylan and ferulic acid contents of durum wheat (*Triticum durum* L.) grain and its milling fractions. J Cereal Sci 25:103–110

Levites Y, Amit T, Mandel S et al (2003) Neuroprotection and neurorescue against Abeta toxicity and PKC-dependent release of nonamyloidogenic soluble precursor protein by green tea polyphenol (−)-epigallocatechin-3-gallate. FASEB J 17:952–954

Liu H, Qiu N, Ding H et al (2008) Polyphenols contents and antioxidant capacity of 68 Chinese herbals suitable for medical or food uses. Food Res Int 41(4):363–370

Lloyd-Jones DM, Larson MG, Leip EP et al (2002) Lifetime risk for developing congestive heart failure: the Framingham Heart Study. Circulation 106:3068–3072

Lou Z, Wang H, Zhu S et al (2011) Antibacterial activity and mechanism of action of chlorogenic acid. J Food Sci 76(6):M398–M403

Low S, Peak RM, Smithson CW et al (2014) Evaluation of a topical gel containing a novel combination of essential oils and antioxidants for reducing oral malodor in dogs. Am J Vet Res 75(7):653–657
Maddi VS, Aragade PD, Digge VG et al (2007) Importance of nutraceuticals in health management. Pharmacol Rev 1:377–379
Maleser D, Kuntic V (2007) Investigation of metal-flavonoid chelates and the determination of flavonoids via metal-flavonoid complexing reactions. J Serb Chem Soc 72:921–939
Manach C, Scalbert A, Morand C et al (2004) Polyphenols: food sources and bioavailability. Am J Clin Nutr 79:727–747
Manach C, Williamson G, Morand C et al (2005) Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. Am J Clin Nutr 81:230S–242S
Mandel S, Weinreb O, Amit T et al (2004) Cell signaling pathways in the neuroprotective actions of the green tea polyphenol (−)-epigallocatechin-3-gallate: implications for neurodegenerative diseases. J Neurochem 88:1555–1569
Mantena SK, Baliga MS, Katiyar SK (2006) Grape seed proanthocyanidins induce apoptosis and inhibit metastasis of highly metastatic breast carcinoma cells. Carcinogenesis 27:1682–1691
Marin L, Miguelez EM, Villar CJ et al (2015) Bioavailability of dietary polyphenols and gut microbiota metabolism: antimicrobial properties. Biomed Res Int 2015:905215
Marks SC, Mullen W, Borges G et al (2009) Absorption, metabolism, and excretion of cider dihydrochalcones in healthy humans and subjects with an ileostomy. J Agric Food Chem 57:2009–2015
Matthies A, Clavel T, Gütschow M et al (2008) Conversion of daidzein and genistein by an anaerobic bacterium newly isolated from the mouse intestine. Appl Envrion Microbiol 74:4847–4852
McNiven MA, Richardson GF (2006) Effect of quercetin on capacitation status and lipid peroxidation of stallion spermatozoa. Cell Preserv Technol 4:3. https://doi.org/10.1089/cpt.2006.4.169
Montero R (2016) Alterations in primary and secondary metabolism in *Vitis vinifera* 'Malvasía de Banyalbufar' upon infection with Grapevine leafroll-associated virus 3. Physiol Plant 157(4):442–452
Moon JH, Nakata R, Oshima S et al (2000) Accumulation of quercetin conjugates in blood plasma after the short-term ingestion of onion by women. Am J Phys 279:R461–R467
Owen RW, Giacosa A, Hull WE et al (2000) The antioxidant/anticancer potential of phenolic compounds isolated from olive oil. Eur J Cancer 36:1235–1247
Panche AN, Diwan AD, Chandra SR (2016) Flavonoids: an overview. J Nutr Sci 5(47):1–15
Pandey M, Verma RK, Saraf SA (2010) Nutraceuticals: new era of medicine and health. Asian J Pharm Clin Res 3:11–15
Pathak AK, Nainwal N, Goyal BM et al (2010) Pharmacological activity of *Trachyspermum ammi*: a review. J Pharm Res 3(4):895–899
Pinto P, Santos CN (2017) Worldwide polyphenol intake: assessment methods and identified gaps. Eur J Nutr 56:1393–1408
Proestos C, Bakogiannis A, Psarianos C et al (2005) High performance liquid chromatography analysis of phenolic substances in Greek wines. Food Control 16:319–323
Rees CR, Costin JM, Fink RC et al (2008) In vitro inhibition of dengue virus entry by p-sulfoxy-cinnamic acid and structurally related combinatorial chemistries. Antivir Res 80:135–142
Resuleo G (2016) Resveratrol: multiple activities on the biological functionality of the cell. In: Gupta RC (ed) Nutraceuticals: efficacy, safety and toxicity, 1st edn. Academic Press/Elsevier, San Diego, CA, pp 453–464
Ross R (1999) Atherosclerosis – an inflammatory disease. N Engl J Med 340:115–126
Rupasinghe HP, Ronalds CM, Rathgeber B et al (2010) Absorption and tissue distribution of dietary quercetin and quercetin glycosides of apple skin in broiler chickens. J Sci Food Agric 90:1172–1178
Saha RK, Takahashi T, Suzuki T (2009) Glucosyl hesperidin prevents influenza a virus replication in vitro by inhibition of viral sialidase. Biol Pharm Bull 32(7):1188–1192
Saura-Calixto F, Serrano J, Goñi I (2007) Intake and bioaccessibility of total polyphenols in a whole diet. Food Chem 101:492–501
Schnitzler P, Nolkemper S, Stintzing FC et al (2008) Comparative in vitro study on the anti-herpetic effect of phytochemically characterized aqueous and ethanolic extracts of *Salvia officinalis* grown at two different locations. Phytomedicine 15(1–2):62–70
Selma MV, Espin JC, Tomas-Barberan FA (2009) Interaction between phenolics and gut microbiota: role in human health. J Agric Food Chem 57:6485–6501
Serafini M, Ghiselli A, Ferro-Luzzi A (1996) In vivo antioxidant effect of green and black tea in man. Eur J Clin Nutr 50:28–32
Shahidi F, Naczk M (1995) Food phenolics, sources, chemistry, effects, applications. Technomic Publishing, Lancaster, PA
Siard MH, McMurry KE, Adams AA (2016) Effects of polyphenols including curcuminoids, resveratrol, quercetin, pterostilbene, and hydroxypterostilbene on lymphocyte pro-inflammatory cytokine production of senior horses in vitro. Vet Immunol Immunopathol 173:50–59
Sichel G, Corsaro C, Scalia M et al (1991) In vitro scavenger activity of some flavonoids and melanins against O2-(.). Free Radic Biol Med 11:1–8
Silva G, Fachin AL, Beleboni RO et al (2013) In vitro action of flavonoids in the canine malignant histiocytic cell line DH82. Molecules 18(12):15448–15463
Singh A, Bora TC, Singh NR (2012) Preliminary photochemical analysis and antimicrobial potential of fermented *Bambusa balcooa* shoots. Bioscan 7(3):391–394
Stefani ED, Boffetta P, Deneo-Pellegrini H et al (1999) Dietary antioxidants and lung cancer risk: a case-control study in Uruguay. Nutr Cancer 34:100–110
Subiza J, Subiza JL, Hinojosa M et al (1989) Anaphylactic reaction after the ingestion of chamomile tea: a study of cross-reactivity with other composite pollens. J Allergy Clin Immunol 84:353–358
Sultana B, Anwar F, Przybylski R (2007) Antioxidant activity of phenolic components present in barks of *Azadirachta indica*, *Terminalia arjuna*, *Acacia nilotica*, and *Eugenia jambolana* Lam. trees. Food Chem 104(3):1106–1114
Swain B, Naik PK, Chakurkar EB et al (2017) Effect of Supplementation of *Moringa oleifera* leaf meal (MOLM) on the performance of Vanaraja laying hens. Indian J Anim Sci 87(3):353–355
Tajik N, Tajik M, Mack I et al (2017) The potential effects of chlorogenic acid, the main phenolic components in coffee, on health: a comprehensive review of the literature. Eur J Nutr 56:2215–2244
Tao J, Hu Q, Yang J et al (2007) In vitro anti-HIV and -HSV activity and safety of sodium rutin sulfate as a microbicide candidate. Antivir Res 75:227–233
Thapa M, Kim Y, Desper J et al (2011) Synthesis and antiviral activity of substituted quercetins. Bioorg Med Chem Lett 22(1): 353–356
Tijburg LBM, Mattern T, Folts JD et al (1997) Tea flavonoids and cardiovascular diseases: a review. Crit Rev Food Sci Nutr 37:771–785
Tomas-Barberan FA, Clifford MN (2000) Flavanones, chalcones and dihydrochalcones—nature, occurrence and dietary burden. J Sci Food Agric 80:1073–1080
Toshiya A, Manabu O, Hideyuki H et al (1999) Hypokalemia with syncope caused by habitual drinking of oolong tea. Intern Med 38:252–256
Towler MC, Hardie DG (2007) AMP-activated protein kinase in metabolic control and insulin signaling. Circ Res 100(3):328–341

Valdes KI, Salem AZM, Lopez S et al (2015) Influence of exogenous enzymes in presence of *Salix babylonica* extract on digestibility, microbial protein synthesis and performance of lambs fed maize silage. J Agric Sci 153:732–742

Vita JA (2005) Polyphenols and cardiovascular disease: effects on endothelial and platelet function. Am J Clin Nutr 81:292–297

Vogelstein B, Kinzler KW (2004) Cancer genes and the pathways they control. Nat Med 10:789–799

Wang LQ (2002) Mammalian phytoestrogens: enterodiol and enterolactone. J Chromatogr B Analyt Technol Biomed Life Sci 777 (1–2):289–309

Wang J, Zhang Q, Jin S et al (2008) Genistein modulate immune responses in collagen-induced rheumatoid arthritis model. Maturitas 59:405–412

Wein S, Wolffram S (2014) Concomitant intake of quercetin with a grain-based diet acutely lowers postprandial plasma glucose and lipid concentrations in pigs. Biomed Res Int 2014:748742

Whelton PK, He J, Appel LJ et al (2002) Primary prevention of hypertension: clinical and public health advisory from The National High Blood Pressure Education Program. JAMA 288:1882–1888

Wildman REC (ed) (2001) Handbook of nutraceuticals and functional foods. CRC Press, Boca Raton, pp 13–30

Wu T, Zang X, He M et al (2013) Structure-activity relationship of flavonoids on their anti-*Escherichia coli* activity and inhibition of DNA gyrase. J Agric Food Chem 61(34):8185–8190

Yang J, Guo J, Yuan J (2008) In vitro antioxidant properties of rutin. LWT-Food Sci Technol 41(6):1060–1066

Yang C, Yifan L, Dan L et al (2015) Bamboo leaf flavones and tea polyphenols show a lipid-lowering effect in a rat model of hyperlipidemia. Drug Res (Stuttg) 65(12):668–671

Zandi K, Teoh B, Sam S et al (2011) In vitro antiviral activity of fisetin, rutin and naringenin against dengue virus type-2. J Med Plant Res 4 (23):5534–5539

Zern TL, West KL, Fernandez ML (2003) Grape polyphenols decrease plasma triglycerides and cholesterol accumulation in the aorta of ovariectomized guinea pigs. J Nutr 133(7):2268–2272

Zhang G, Flach CR, Mendelsohn R (2007) Tracking the dephosphorylation of resveratrol triphosphate in skin by confocal Raman microscopy. J Control Release 123(2):141–147

Antioxidants in Prevention and Treatment of Diseases and Toxicity

Dejan Milatovic and Ramesh C. Gupta

Abstract

Oxidative stress reflects an imbalance between the production of reactive oxygen (ROS) and reactive nitrogen species (RNS) and the body's ability to detoxify their toxic effects through antioxidant defense system. Reactive radicals derived from molecular oxygen or nitrogen are generated internally in animal and human body systems or through external sources like environmental pollution, toxic metals, and pesticides. ROS and RNS attack a variety of essential biological molecules, including lipids, cellular proteins, and DNA, cause alterations in normal cell and organ physiology, and activate disease processes. Given the important role of oxidative stress in the cell and tissue injury, several antioxidants have been exploited for their beneficial effect in regulation of ROS/RNS production and neutralization as well as for preservation of equilibrium. This chapter analyzes oxidative stress and its biomarkers and discusses antioxidant systems essential for preventing and ameliorating detrimental effects of ROS and RNS.

Keywords

Oxidative stress · Reactive oxygen species · Biomarkers · Antioxidants

1 Introduction

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are highly reactive molecules with one or more unpaired electron(s) in their external shell. These oxidant species can be generated in a variety of endogenous processes (cellular respiration, phagocytic oxidative bursts, antibacterial defense, and others) or during exposures to various agents (pesticides, metals, xenobiotics, and ionizing radiation) (Chakravarti and Chakravarti 2007; Mangialasche et al. 2009; Il'yasova et al. 2012). The imbalance between the generation of ROS and RNS and the cell's ability to neutralize them by antioxidant defense is defined as oxidative stress. ROS and RNS produced under oxidative stress are known to damage cellular biomolecules, including lipids, sugars, proteins, and polynucleotides, and to initiate detrimental effects (Negre-Salvayre et al. 2010; Roberts et al. 2010; Marrocco et al. 2017).

Free radicals are mostly generated in the cell as superoxide by mitochondrial oxidative phosphorylation (Federico et al. 2012; Trewin et al. 2018; Sas et al. 2018). Superoxide can generate another ROS, hydroxyl radical (HO·), by the Fenton and the Haber–Weiss reactions or RNS by forming peroxynitrite ($ONOO^-$) in reaction with nitric oxide (NO·) (Dix and Aikens 1993; Guéraud et al. 2010; Vatassery 2004).

ROS and RNS are also generated by tightly regulated enzymes or enzyme systems located in cellular membranes or organelles. They are produced by enzymes associated with respiratory chain system, prostaglandin synthesis, phagocytosis, and cytochrome P450 systems (Halliwell and Gutteridge 2007; Bahorun et al. 2006; Kumar and Pandey 2015; Pacher et al. 2007; Pizzino et al. 2017). Enzyme nitric oxide synthase (NOS) catalyzes the production of NO from L-arginine with nicotinamide adenine dinucleotide phosphate (NADPH) and oxygen. There are three major isoforms of NOS: (a) neuronal NOS (nNOS), (b) endothelial NOS (eNOS), and (c) inducible NOS (iNOS) produced by microglia, astrocytes, and neurons. Nitric oxide synthases and nicotinamide adenine dinucleotide phosphate (NADP) oxidase isoforms that produce ROS/RNS are also involved in neuronal transmission, cellular signaling, reactions to stress and various agents, synaptic plasticity, and the induction of mitogenic and apoptotic responses (Beal 2000; Valko et al. 2007; Mangialasche et al. 2009). Therefore, ROS/RNS

D. Milatovic (✉)
Alexandria, VA, USA

R. C. Gupta
Toxicology Department, Breathitt Veterinary Center, Murray State University, Hopkinsville, KY, USA

R. C. Gupta et al. (eds.), *Nutraceuticals in Veterinary Medicine*, https://doi.org/10.1007/978-3-030-04624-8_14

generated by enzyme systems do not only act as toxic by-product but also have controlling function in the cell (Babior 1999; Valko et al. 2007). This chapter describes various biomarkers of oxidative stress, attenuation of oxidative injury, and antioxidant systems.

2 Biomarkers of Oxidative Stress

Products of ROS/RNS-induced modification of lipids, proteins, carbohydrates, and DNA can be used as markers of oxidative stress (Salisbury and Bronas 2015; Frijhoff et al. 2015; Liguori et al. 2018). ROS/RNS may induce protein oxidation, nitrosylation, and hydrolysis of the peptide bond in the presence of proline, affect DNA modifications including nucleotide oxidation, strand breakage, loss of bases, and adduct formation (Berlett and Stadtman 1997; Wiseman and Halliwell 1996; Dizdaroglu et al. 2002; Marrocco et al. 2017), or directly damage lipids containing carbon–carbon double bounds such as cholesterol, phospholipids, and polyunsaturated fatty acids (PUFAs) (Morrow et al. 1990; Montine et al. 2002). Over the years, lipid products of oxidative damage have generated intense interest as in vivo markers of oxidative damage (De Zwart et al. 1999; Milatovic et al. 2011). These compounds include the F_2-isoprostanes (F_2-IsoPs) and F_4-neuroprostanes (F_4-NeuroPs) (Morrow et al. 1990; Yin et al. 2007; Janicka et al. 2010; Milatovic et al. 2011).

F_2-IsoPs are prostaglandin-like compounds that are produced by a noncyclooxygenase free radical-catalyzed mechanism involving the peroxidation of the PUFA, arachidonic acid (AA, C20:4, ω-6). F_2-IsoPs are formed primarily in situ, esterified to phospholipids, and subsequently released by phospholipases (Famm and Morrow 2003; Gao et al. 2006). In contrast to F_2-IsoPs, prostaglandins (PGs) are generated only from free arachidonic acid (Morrow et al. 1990).

F_2-IsoPs analogs termed neuroprostanes (F_4-NeuroPs), due to the high levels of their precursor in the brain, are formed by peroxidation of docosahexaenoic acid (DHA, C22:6, ω-3) (Roberts et al. 1998). While DHA is highly concentrated in neuronal membranes, AA is evenly distributed in all cell types in all tissues (Salem et al. 1986). Therefore, F_2-IsoPs provides an index of global oxidative damage in the brain and determination of F_4-NeuroPs permits the specific quantification of oxidative damage to neuronal membranes. Both prostaglandin-like compounds can be quantified by mass spectrometry-based methods with the lower limit of detection of the F_2-IsoPs to be in the low picogram range (Montine et al. 2004; Milatovic et al. 2005a, b; Milatovic et al. 2011).

Malondialdehyde (MDA) product is also used to quantify oxidative stress. This product is generated by enzymatic and free-radical peroxidation of PUFAs which contain at least three double bonds. MDA can be generated by thromboxane synthase, but also derived from nonenzymatic peroxidative degradation of unsaturated lipids (Kadiiska et al. 2005). MDA-TBA adducts, produced in reaction of MDA with thiobarbituric acid (TBA), are used to spectrophotometrically measure the levels of oxidative stress and consequent lipid peroxidation (Spickett et al. 2010; Fang et al. 2017).

ROS and RON can react with the DNA molecule and induce nitration and deaminations of purines, purine or pyrimidine base or sugar lesions, DNA–DNA, or DNA–protein cross-links (Dizdaroglu et al. 2002). The most investigated DNA adduct, 8-hydroxy-2′-deoxyguanosine (8-OHdG), can be evaluated by multiple techniques including GC-MS, HPLC, LC-MS, immunoassay, and capillary electrophoresis (Lovell and Markesbery 2007; Fang et al. 2017).

ROS and RNS can also attack any amino acid and produce carbonyl derivatives (Stadtman and Levine 2003). Protein carbonyls can be detected with 2,4-dinitrophenylhydrazine (DNPH) and used as biomarkers of oxidative stress (Dalle-Donne et al. 2003). Products of proteins oxidative/nitrosative modification are relatively stable and sensitive assays are available for their detection (Chakravarti and Chakravarti 2007).

3 Oxidative Stress and Neurodegeneration

Oxidative stress plays an important role in neurodegenerative processes such as excitotoxicity and neurotoxicity associated with anticholinesterases or metals. Brain is very sensitive to oxidative stress because of its high metabolic activity, high density of oxidizable substrates, and relative deficiency in antioxidant systems (Garcia-Mesa et al. 2016; Simioni et al. 2018).

Previous studies have supported a role for oxidative stress and excessive generation of ROS and RNS in kainic acid (KA)- and anticholinesterase-induced neurotoxicities (Dettbarn et al. 2001; Yang and Dettbarn 1998; Gupta et al. 2001a, b, 2007; Milatovic et al. 2000a, b, 2001, 2005a; Zaja-Milatovic et al. 2008). Exposure to anticholinesterases, diisopropylphosphorofluoridate (DFP) and carbofuran (CF), significantly suppressed AChE activity in rat brain, induced severe seizure activity, and significantly increased biomarkers of global free radical damage (F_2-IsoPs) and oxidative damage to neuronal membranes (F_4-NeuroPs) (Gupta et al. 2007; Zaja-Milatovic et al. 2009). Following DFP exposure, F_2-IsoPs and F_4-NeuroPs levels are more than twofold and fivefold higher compared to controls, respectively (Zaja-Milatovic et al. 2009). Our studies in mice also showed that KA-induced excitotoxicity caused an increase in biomarkers of oxidative damage, F_2-IsoPs and F_4-NeuroPs (Zaja-Milatovic et al. 2008) (Table 1).

Table 1 Cerebral concentrations of F_2-IsoPs and F_4-NeuroPs and dendritic degeneration of hippocampal pyramidal neurons following KA-induced seizures in mice

	F_2-IsoPs (ng/g)	F_4-NeuroPs (ng/g)	Dendritic length (μm)	Spine density (number/100 μm dendrite)
Control	3.07 ± 0.05	13.89 ± 0.58	1032.10 ± 61.41	16.45 ± 0.55
KA 30 min	4.81 ± 0.19*	34.27 ± 2.71*	363.44 ± 20.78*	8.81 ± 0.55*
KA 60 min	3.40 ± 0.18	18.55 ± 1.26	425.71 ± 23.04*	7.44 ± 0.56*

Data from KA exposed mice were collected 30 min or 60 min post-injection
*One-way ANOVA showed $p < 0.0001$ for each end point. Bonferroni's multiple comparison test showed significant difference ($p < 0.001$) compared to vehicle-injected control

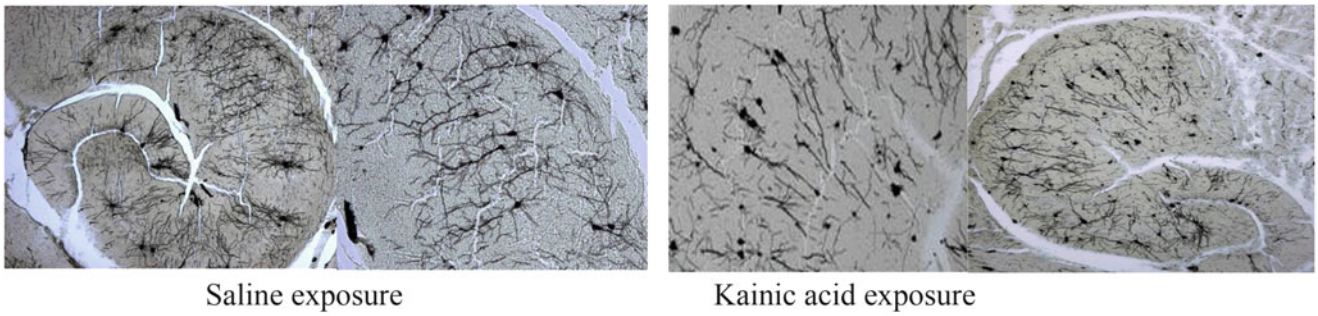


Fig. 1 Photomicrographs of mouse hippocampi with pyramidal neurons from the CA1 hippocampal area of brains 1 h after saline (control) and kainic acid (KA, 1 nmol/5 μl, ICV) injections. Treatment with KA-induced degeneration of the hippocampal dendritic system and decrease in the total length of the dendrite and spine density of hippocampal pyramidal neurons

Furthermore, we have investigated whether seizure-induced cerebral oxidative damage in adult rats and mice is paralleled by alterations in the integrity of the hippocampal CA1 dendritic system. Results from our studies evaluated by Golgi impregnation and Neurolucida-assisted morphometry showed that both KA- and anticholinesterase-induced excitotoxicity targeted the dendritic system with profound degeneration of spines and regression of dendrites in both animal models (Table 1 and Fig. 1) (Zaja-Milatovic et al. 2008, 2009). Together, our studies demonstrate that both models of excitotoxicity lead to profound cerebral oxidative damage and neurodegeneration in the CA1 hippocampal region of brain.

Exposure to some metals, including manganese (Mn), may also lead to oxidative damage and pathological conditions, including neurodegeneration. Early studies demonstrated that mitochondria actively sequester Mn, causing inhibition of oxidative phosphorylation (Cotzias and Greenough 1958; Gavin et al. 1990, 1992). Our studies with primary astrocytes have also shown that Mn exposure induces oxidative damage and a consequent increase in biomarkers of oxidative stress (Milatovic et al. 2007, 2009). Mn concentrations known to elicit neurotoxic effects (100 μM, 500 μM, or 1 mM) in astrocytes induced significant elevations in F_2-IsoPs levels at all investigated exposure times (Milatovic et al. 2007).

Mn-induced oxidative stress and neurotoxicity is also confirmed in our in vivo model. Our study showed that a one-time challenge of mice with Mn (100 mg/kg, s.c.) was sufficient to produce significant increases in cerebral F_2-IsoPs (Milatovic et al. 2009) 24 h following the injection. Our study also demonstrated that Mn exposure altered mice dendritic systems with profound dendrite regression of striatal medium spiny neurons (MSNs). Together, these studies demonstrated that oxidative stress is the underlying mechanism in Mn-induced vulnerability of striatal neurons.

4 Oxidative Stress and Antioxidants in Non-neurodegenerative Diseases

The role of oxidative stress in pathophysiology and antioxidants in the prevention and treatment of diseases associated with cardiac (Wolfram et al. 2005; Willcox et al. 2008; Šterba et al. 2013), respiratory (Janssen 2008; Behndig et al. 2009; Kontakiotis et al. 2011; Nyunoya et al. 2011), hepatic (Jaeschke et al. 2012; Bischoff et al. 2016; Gwaltney-Brant 2016), renal (Tucker et al. 2013; Modaresi et al. 2015), pancreatic (Brownlee 2001), skeletal joints (Gupta 2016) and skeletal muscles (Gupta et al. 2014), and others have been well documented.

5 Antioxidant systems

Antioxidant defense protects biological systems from harmful effects of ROS and RNS. Defense mechanisms are largely based on the presence of antioxidants (exogenous and

endogenous molecules) and the repair or removal of the injured molecules/systems. Antioxidant defense involves a variety of strategies, both enzymatic, such as reductase, superoxide dismutase, or catalase, and nonenzymatic, involving carotene, tocopherols, flavonoids, ascorbate, and glutathione (GSH) (Halliwell 2007).

Antioxidant enzymes, such as superoxide dismutase, glutathione peroxidase, catalase, and reductase, exert synergistic actions in scavenging free radicals. Superoxide dismutase is considered to be the first line of defense against free radical formation. It catalyzes dismutation of superoxide radical into oxygen and hydrogen peroxide. Glutathione peroxidase and reductase are glutathione-dependent enzymes located in the cytoplasm, mitochondria, and nucleus (Gamble et al. 1999). Glutathione peroxidase metabolizes hydrogen peroxide to water using reduced glutathione as a hydrogen donor and is recycled back to glutathione reductase by cofactor NADPH. It plays an important role in the defense mechanism in the erythrocytes against lipid peroxidation damage (Sen 2000; Shah et al. 2014). Superoxide radical may directly inactivate enzymes like glutathione peroxidase and catalase which are needed to eliminate hydrogen peroxide from intracellular medium (Johnson and Giulivi 2005). Another antioxidant enzyme, catalase, is located in peroxisomes (80%) and cytosol (20%) and decomposes hydrogen peroxide to water and oxygen without the production of free radicals (Jones et al. 1981). Catalase does not show significant activity under physiological conditions due to its lower affinity than glutathione peroxidase for hydrogen peroxide but becomes an important enzyme at disease state where concentration of H_2O_2 is elevated (Chance et al. 1979). The antioxidant defense system also involves glutathione-*S*-transferase (Birben et al. 2012).

Glutathione (GSH, L-γ-glutamyl-L-cysteinylglycine), an important element of nonenzymatic antioxidant system, is a tripeptide representing the most abundant nonprotein thiol present in the cell (Wu et al. 2004a; Lu 2009). GSH acts as an antioxidant defense system by its ability to scavenge ROS through the reversible oxidation of GSH. Oxidized form (GSSG) can be enzymatically reduced to GSH by the activity of glutathione reductase and the reducing power of NADPH. In healthy cells and tissues, more than 90% of the total glutathione pool is in the reduced form (GSH) and less than 10% exists in the oxidized form (Schafer and Buettner 2001).

Vitamin E has been identified as one of the most relevant nutritional antioxidants. Vitamin E refers to a group of fat-soluble compounds that include tocopherols and tocotrienols. α-Tocopherol is the most biologically active form, and it has been shown to protect the cells from lipid peroxidation (Azlina et al. 2018; Simioni et al. 2018), oxidative damage of DNA and cellular proteins, and membrane degeneration (Topinka et al. 1989). Vitamin E acts as a radical scavenger and chain breaking antioxidant, protecting cells from peroxidation of PUFA in membrane phospholipids (VanAcker et al. 1993). In addition, vitamin E maintains oxidative phosphorylation in mitochondria, regulates ROS production, and accelerates restitution of high-energy metabolites (Chow et al. 1999; Punz et al. 1998). Decreased levels of vitamin E in response to hyperoxia or treatment with convulsant reported in multiple studies (Mori et al. 2004; Onodera et al. 2003; Rauca et al. 2004) suggest that vitamin E in the brain is consumed to prevent oxidative damage. Vitamin E also exerted anticonvulsive effects by upregulating catalase activity in pilocarpine models of epilepsy (Xavier et al. 2007; Barros et al. 2007; Santos et al. 2008).

The efficacy of the antioxidant vitamin E to suppress an increase in NO and lipid peroxidation and prevent neurodegeneration of hippocampal neurons was also tested in the mice model of KA-induced excitotoxicity (Zaja-Milatovic et al. 2008). Our study showed that vitamin E suppressed KA-induced increases in citrulline and cerebral and neuronal markers of oxidative damage, F_2-IsoPs and F_4-NeuroPs, respectively (Fig. 2) (Zaja-Milatovic et al. 2008). Importantly, vitamin E completely suppressed reduction in both spine density and dendrite length of pyramidal neurons from the CA hippocampal area from KA-exposed mice (Fig. 3). Since vitamin E treatment did not alter severity

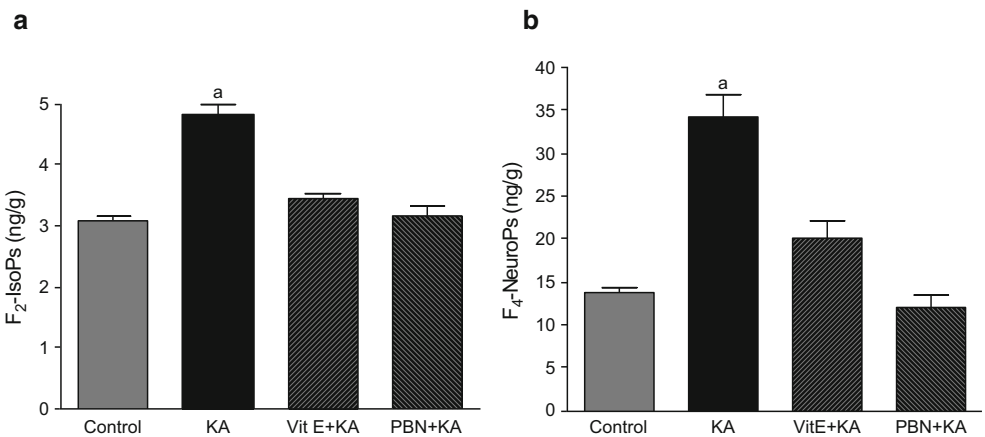


Fig. 2 Ipsilateral cerebral F_2-IsoPs (**a**) and F_4-NeuroPs (**b**) concentrations following i.c.v. KA with or without vitamin E (Vit E) or *N-tert*-butyl-α-phenylnitrone (PBN) pretreatment. Brains from mice exposed to KA were collected 30 min post-injections ($n \geq 5$ for each group). One-way ANOVA had $p < 0.0001$ with Bonferroni's multiple comparison tests significant for KA vs control, Vit E + KA or PBN + KA treatment

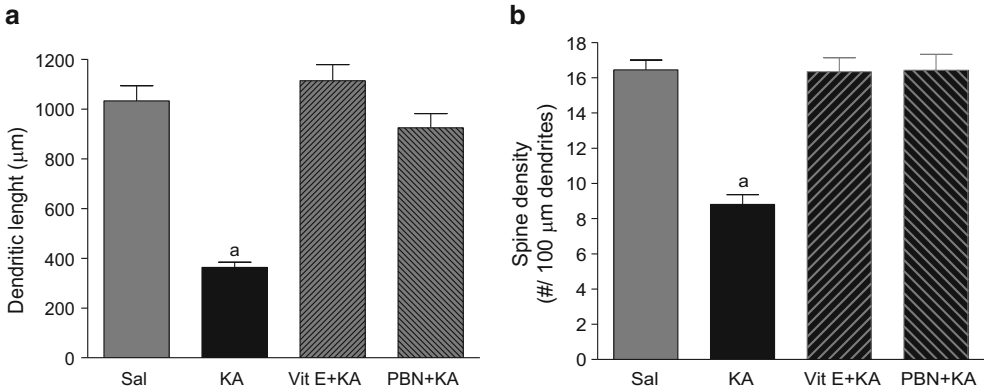


Fig. 3 Dendritic length (**a**) and spine density (**b**) of pyramidal neurons from the CA1 hippocampal area of mice following ICV KA with or without vitamin E or PBN pretreatment. Brains from mice exposed to KA were collected 30-min post-injection ($n \geq 5$ for each group). [a]One-way ANOVA had $p < 0.001$ with Bonferroni's multiple comparison tests significant for KA versus control, vitamin E + KA, or PBN + KA treatment

of kainite-induced seizure, its protective affect is most likely mediated by preventing lipid peroxidation and consequent neuronal damage, but not by its effect on seizures per se (Zaja-Milatovic et al. 2008).

Vitamin C, ascorbic acid, is a water-soluble natural antioxidant and a powerful inhibitor of lipid peroxidation. Vitamin C scavenges free radicals through the formation of ascorbyl radical and thereby prevents damage to macromolecules such as lipids or DNA. This molecule also inhibits propagation of free radicals and reduces α-tocopheroxyl radicals and inhibits propagation of free radicals (Huang et al. 2002). Results from animal models also demonstrated that vitamin C ameliorates edema and hypotension and improves arteriolar responsiveness and capillary blood flow (Tyml et al. 2005; Wu et al. 2004b; Wilson 2009). Vitamin C inhibits the expression of intracellular adhesion molecules and thereby inhibits the intake of immune cells into the microcirculation (Berger and Oudemans-van Straaten 2015). Importantly, vitamin C not only mitigates oxidative stress but also restores vascular responsiveness to vasoconstrictors (Tyml 2017), ameliorates microcirculatory blood flow, preserves endothelial barriers, prevents apoptosis, and augments bacterial defense (Oudemans-van Straaten et al. 2014; Yamamoto et al. 2010).

Vitamin A belongs to a group of unsaturated hydrocarbons and can be found in two main lipid-soluble forms, retinol or retinyl esters and carotene. It has functions in growth and development, good vision, and maintenance of the immune system. The most well-known β-carotene is a potent antioxidant able to quench singlet oxygen (Di Mascio et al. 1991) and reduce lipid peroxidation (Upritchard et al. 2003). Depletion of α-carotene, lycopene, β-cryptoxanthin, and lutein/zeaxanthin has been associated with a variety of diseases including cardiovascular disease (Gaziano et al. 1995) and atherosclerosis (Prince et al. 1988). Together, vitamin C, vitamin E, and carotenoids have shown to synergistically counteract lipid peroxidation (Niki et al. 1995).

Natural food-derived antioxidants have also received great attention in the last two decades. Natural flavonoids, present in fruit, vegetables, and herbs, have shown positive health effect in neurodegenerative disorders, owing to their free radical scavenging activities, preventing lipid peroxidation or chelating metal ions (Lee et al. 2010; Rahal et al. 2014). They are a class of polyphenolic compounds with a benzo-γ-pyrone structure largely represented in plants, responsible for several pharmacological activities (Mahomoodally et al. 2005; Pandey 2007). They account for >85% of the phenolic components in red wine and include different molecules, especially quercetin, and nonflavonoids include mainly resveratrol. Flavonoid affects ROS synthesis suppression, scavenging ROS, and improvement of antioxidant defenses (Halliwell and Gutteridge 1998).

Melatonin, a mammalian hormone synthesized from serotonin, has also shown antioxidant properties (Nishida 2005; Sharman and Bondy 2016). Melatonin exerts its antioxidant capacity by stimulating expression and activity of glutathione peroxidase and superoxide dismutase and inhibiting that of nitric oxide synthase (Pieri et al. 1995; Dato et al. 2013). Melatonin has anti-inflammatory and antiapoptotic effects, but it can also act as an antioxidant scavenger for radical oxygen and nitrogen species (Mahomoodally et al. 2005; Pandey 2007). Several animal studies demonstrated beneficial antioxidant properties of melatonin in septic shock conditions (Heim et al. 2002; Kumar et al. 2013; Kumar and Pandey 2013). Melatonin also suppressed mitochondrial dysfunction as its administration affected mitochondrial NOS activity and restored mitochondrial production of ATP (Escames et al. 2003; Lopez et al. 2006; Mantzarlis et al. 2017).

A synthetic spin trapping agent such as phenyl-*N*-*tert*-butylnitrone (PBN) is also capable of scavenging many types of free radicals. PBN reacts with ROS and forms stable adducts that can be quantified by electron paramagnetic resonance spectrometry. Various in vitro and in vivo studies demonstrated beneficial effects of PBN on the prevention of neuronal degeneration, inhibition of NOS induction (Miyajima and Kotake 1995), in different models of seizures (He et al. 1997; Thomas et al. 1997), protective effects in

models of brain ischemia/reperfusion (Carney and Floyd 1991; Gido et al. 1997; Fetcher et al. 1997), and excitotoxicity (Lancelot et al. 1997; Milatovic et al. 2002). Experimental studies also demonstrated that PBN prevented neurodegeneration in Parkinson's disease (Frederiksson et al. 1997; Sack et al. 1996), anticholinesterase neurotoxicity (Gupta et al. 2001a, b), and Alzheimer's disease (Sack et al. 1996). PBN demonstrated direct effect on striatal function, including inhibition of excitation–contraction coupling (Andersen et al. 1996), Ca^{2+} channel blockade in vascular muscle causing vasodilatation (Anderson et al. 1993), and induction of hypothermia (Pazos et al. 1999). Our studies have demonstrated that PBN treatment prevented KA-induced oxidative damage; suppressed increases in citrulline and cerebral and neuronal markers of oxidative damage, F_2-IsoPs and F_4-NeuroPs (Fig. 2); as well as prevented dendritic degeneration of pyramidal neurons from the CA hippocampal area from KA-exposed mice (Fig. 3) (Zaja-Milatovic et al. 2008). Since antioxidant treatment minimizes lipid peroxidation, then a parallel reduction in neuronal damage provides strong evidence that oxidative stress and lipid peroxidation in a causal way mediate seizure and the corresponding injury.

6 Concluding Remarks and Future Directions

Growing evidence supports the pivotal role played by oxidative stress in tissue injury development. Overproduction of reactive oxygen and nitrogen species, in combination with a lowered antioxidant defense eventually, leads to disturbed cell functions, loss of synapses, neuronal damage, and cell death that gives rise to the clinical symptoms associated with brain disease. Results from multiple studies supported an association between oxidative stress and neurotoxicity and indicated that excitotoxicity leads to profound cerebral oxidative damage and neurodegeneration in the hippocampal brain region. Importantly, our studies have demonstrated that antioxidant therapy, such as vitamin E and PBN, prevented excitotoxicity-induced oxidative damage and dendritic degeneration of pyramidal neurons from the CA hippocampal brain area. Since antioxidant treatment minimize lipid peroxidation, parallel reduction in neuronal damage provides strong evidence that oxidative stress and lipid peroxidation in a causal way mediate seizure and the corresponding injury. The role of oxidative stress and its amelioration by antioxidants in other vital organs have also been well documented. Future studies should be directed at deciphering the mechanisms of protection and guide the development of selective and efficacious antioxidant therapies that target pathways in neuronal and non-neuronal tissues. Antioxidant therapies, including natural antioxidants provided by nutrition, hold great promise.

Acknowledgment Preparation of this chapter is supported by JGMS, Inc.

References

Andersen KA, Diaz PT, Wright VP et al (1996) N-*tert*-butyl-alpha-phenylnitrone: a free radical trap with unanticipated effects on diaphragm function. J Appl Physiol 80:862–868

Anderson DE, Yuan XJ, Tseng CM et al (1993) Nitrone spin traps block calcium channels and induce pulmonary artery relaxation independent of free radicals. Biochem Biophys Res Commun 193:878–885

Azlina MFN, Kamisah Y, Qodriyah MS (2018) Tocopherol and tocotrienol: therapeutic potential in animal models of stress. Curr Drug Targets 19(12):1456–1462

Babior BM (1999) NADPH oxidase: an update. Blood 93(5):1464–1476

Bahorun T, Soobrattee MA, Luximon-Ramma V et al (2006) Free radicals and antioxidants in cardiovascular health and disease. Internet J Med Updat 1:1–17

Barros DO, Xavier SML, Barbosa CO et al (2007) Effects of the vitamin E in catalase activities in hippocampus after status epilepticus induced by pilocarpine in Wistar rats. Neurosci Lett 416(3):227–230

Beal MF (2000) Oxidative metabolism. Ann N Y Acad Sci 924:164–169

Behndig AF, Blomberg A, Helleday R et al (2009) Antioxidant response to acute ozone challenge in the healthy human airway. Inhal Toxicol 21(11):933–942

Berger MM, Oudemans-van Straaten HM (2015) Vitamin C supplementation in the critically ill patient. Curr Opin Clin Nutr Metab Care 18:193–201

Berlett BS, Stadtman ER (1997) Protein oxidation in aging, disease, and oxidative stress. J Biol Chem 272(33):20313–20316

Birben E, Sahiner UM, Sackesen C et al (2012) Oxidative stress and antioxidant defense. World Allergy Organ J 5(1):9–19

Bischoff K, Mukai M, Ramaiah SK (2016) Liver toxicity. In: Gupta RC (ed) Veterinary toxicology basic and clinical principles, 3rd edn. Academic Press/Elsevier, Amsterdam, pp 239–257

Brownlee M (2001) Biochemistry and molecular cell biology of diabetic complications. Nature 414:813–820

Carney JM, Floyd RA (1991) Protection against oxidative damage to CNS by alpha-phenyl-*tert*-butylnitrone (PBN) and other spin-trapping agents: a novel series of nonlipid free radical scavengers. J Mol Neurosci 3:47–57

Chakravarti B, Chakravarti SN (2007) Oxidative modification of proteins: age-related changes. Gerontology 53(3):128–139

Chance B, Sies H, Boveris A (1979) Hydroperoxide metabolism in mammalian organs. Physiol Rev 59:527–605

Chow CK, Ibrahim W, Wei Z et al (1999) Vitamin E regulates mitochondrial hydrogen peroxide generation. Free Radic Biol Med 27:580–587

Cotzias GC, Greenough JJ (1958) The high specificity of the manganese pathway through the body. J Clin Invest 37:1298–1305

Dalle-Donne I, Rossi R, Giustarini D et al (2003) Protein carbonyl groups as biomarkers of oxidative stress. Clin Chim Acta 329:23–38

Dato S, Crocco P, D'Aguila P, de Rango F, Bellizzi D, Rose R, Passarino G (2013) Exploring the role of genetic variability and lifestyle in oxidative stress response for healthy aging and longevity. Int J Mol Sci 14(8):16443–16472

De Zwart LL, Meerman JHN, Commandeur JNM et al (1999) Biomarkers of free radical damage applications in experimental animals and in humans. Free Radic Biol Med 26:202–226

Dettbarn W-D, Milatovic D, Zivin M, Gupta RC (2001) Oxidative stress, acetylcholine and excitoxicity. In: Marwah J, Kanthasamy A (eds) International conference on antioxidants. Prominant Press, Scottsdale, AZ, pp 183–211

Di Mascio P, Kaiser SP, Devasagayam TP et al (1991) Biological significance of active oxygen species: *in vitro* studies on singlet

oxygen-induced DNA damage and on the singlet oxygen quenching ability of carotenoids, tocopherols and thiols. Adv Exp Med Biol 283:71–77

Dix TA, Aikens J (1993) Mechanisms and biological relevance of lipid peroxidation initiation. Chem Res Toxicol 6:2–18

Dizdaroglu M, Jaruga P, Birincioglu M et al (2002) Free radical-induced damage to DNA: mechanisms and measurement. Free Radic Biol Med 32(11):1102–1115

Escames G, Leon J, Macias M et al (2003) Acuna-Castroviejo D. Melatonin counteracts lipopolysaccharide-induced expression and activity of mitochondrial nitric oxide synthase in rats. FASEB J 17(8):932–934

Famm SS, Morrow JD (2003) The isoprostanes: unique products of arachidonic acid oxidation- a review. Curr Med Chem 10:1723–1740

Fang C, Gu L, Smerin D et al (2017) The interrelation between reactive oxygen species and autophagy in neurological disorders. Oxidative Med Cell Longev 2017:1–16

Federico A, Cardaioli E, Pozzo PD (2012) Mitochondria, oxidative stress and neurodegeneration. J Neurol Sci 322:254–262

Fetcher LD, Liu Y, Pearce TA (1997) Cochlear protection from carbon monoxide exposure by free radical blockers in the guinea pig. Toxicol Appl Pharmacol 142:47–55

Frederiksson A, Eriksson P, Archer T (1997) MPTP-induced deficits in motor activity: neuroprotective effects of the spin trapping agents, alpha-phenyl-*tert*-butylnitrone (PBN). J Neural Transm 104:579–592

Frijhoff J, Winyard PG, Zarkovic N et al (2015) Clinical relevance of biomarkers of oxidative stress. Antioxid Redox Signal 23 (14):1144–1170

Gamble SC, Wiseman A, Goldfarb PS (1999) Selenium-dependent glutathione peroxidase and other selenoproteins: their synthesis and biochemical roles. J Chem Technol Biotech 68:123–134

Gao L, Yin H, Milne GL et al (2006) Formation of F-ring isoprostane-like compounds (F_3-isoprostanes) *in vivo* from eicosapentaenoic Acid. J Biol Chem 281:14092–14099

Garcia-Mesa Y, Colie S, Corpas R et al (2016) Oxidative stress is a central target for physical exercise neuroprotection against pathological brain aging. J Gerontol A Biol Sci Med Sci 71:40–49

Gavin CE, Gunter KK, Gunter TE (1990) Manganese and calcium efflux kinetics in brain mitochondria. Relevance to manganese toxicity. Biochem J 266:329–334

Gavin CE, Gunter KK, Gunter TE (1992) Gunter, Mn^{2+} sequestration by mitochondria and inhibition of oxidative phosphorylation. Toxicol Appl Pharmacol 115:1–5

Gaziano JM, Manson JE, Branch LG et al (1995) A prospective study of consumption of carotenoids in fruits and vegetables and decreased cardiovascular mortality in the elderly. Ann Epidemiol 5:255–260

Gido G, Kristian T, Siesjo BK (1997) Extracellular potassium in a neocortical cone area after transient focal ischemia. Stroke 28:206–210

Guéraud F, Atalay M, Bresgen N (2010) Advances in methods for the determination of biologically relevant lipid peroxidation products. Free Radic Res 44:1098–1124

Gupta RC (2016) Nutraceuticals in arthritis. In: Gupta RC (ed) Nutraceuticals: efficacy, safety and toxicity. Academic Press/Elsevier, Amsterdam, pp 161–176

Gupta RC, Milatovic D, Dettbarn W-D (2001a) Depletion of energy metabolites following acetylcholinesterase inhibitor-induced status epilepticus: protection by antioxidants. Neurotoxicology 22:271–282

Gupta RC, Milatovic D, Dettbarn W-D (2001b) Nitric oxide modulates high-energy phosphates in brain regions of rats intoxicated with diisopropylphosphorofluoridate or carbofuran: prevention by N-*tert*- butyl-α- phenylnitrone or vitamin E. Arch Toxicol 75:346–356

Gupta RC, Milatovic S, Dettbarn W-D, Aschner M, Milatovic D (2007) Neuronal oxidative injury and dendritic damage induced by carbofuran: protection by memantine. Toxicol Appl Pharmacol 219:97–105

Gupta RC, Lasher MA, Doss RB, Milatovic D (2014) Skeletal muscle toxicity biomarkers. In: Gupta RC (ed) Biomarkers in toxicology. Academic Press/Elsevier, Amsterdam, pp 291–308

Gwaltney-Brant SM (2016) Nutraceuticals in hepatic diseases. In: Gupta RC (ed) Nutraceuticals: efficacy, safety and toxicity. Academic Press/Elsevier, Amsterdam, pp 87–99

Halliwell B (2007) Oxidative stress and neurodegeneration: where are we now? J Neurochem 97:1634–1658

Halliwell B, Gutteridge JMC (1998) Free radicals in biology and medicine. Oxford University Press, Oxford

Halliwell B, Gutteridge JMC (2007) Free radicals in biology and medicine, 4th edn. Clarendon Press, Oxford

He QP, Smith ML, Li PA et al (1997) Necrosis of the substantia nigra, pars reticulata, influorothyl-induced status epilepticus is ameliorated by the spin trap alpha-phenyl-*N*-*tert*-butylnitrone. Free Radic Biol Med 22:917–922

Heim KE, Tagliaferro AR, Bobilya DJ (2002) Flavonoid antioxidants: chemistry, metabolism and structure-activity relationships. J Nutr Biochem 13(10):572–584

Huang HY, Appel LJ, Croft KD et al (2002) Effects of vitamin C and vitamin E on *in vivo* lipid peroxidation: results of a randomized controlled trial. Am J Clin Nutr 76:549–555

Il'yasova D, Scarbrough P, Spasojevic I (2012) Urinary biomarkers of oxidative stress. Clin Chim Acta 413:1446–1453

Jaeschke H, McGill MR, Ramachandran A (2012) Oxidant stress, mitochondria, and cell death mechanisms in drug-induced liver injury: lessons learned from acetaminophen hepatotoxicity. Drug Metab Rev 44:88–106

Janicka M, Kot-Wasik A, Kot J, Namiesnik J (2010) Isoprostanes-biomarkers of lipid peroxidation: their utility in evaluating oxidative stress and analysis. Int J Mol Sci 11:4631–4659

Janssen LJ (2008) Isoprostanes and lung vascular pathology. Am J Respir Cell Mol Biol 39(4):383–389

Johnson F, Giulivi C (2005) Superoxide dismutases and their impact upon human health. Mol Asp Med 26:340–352

Jones DP, Eklow L, Thor H, Orrenius S (1981) Metabolism of hydrogen peroxide in isolated hepatocytes: relative contributions of catalase and glutathione peroxidase in decomposition of endogenously generated H_2O_2. Arch Biochem Biophys 210:505–516

Kadiiska MB, Gladen BC, Baird DD et al (2005) Biomarkers of oxidative stress study II: are oxidation products of lipids, proteins, and DNA markers of CCl_4 poisoning? Free Radic Biol Med 38:698–710

Kontakiotis T, Katsoulis K, Hagizisi O et al (2011) Bronchoalveolar lavage fluid alteration in antioxidant and inflammatory status in lung cancer patients. Eur J Intern Med 22(5):522–526

Kumar S, Pandey AK (2013) Phenolic content, reducing power and membrane protective activities of *Solanum xanthocarpum* root extracts. Vegetos 26:301–307

Kumar S, Pandey AK (2015) Free radicals: health implications and their mitigation by herbals. Br J Med Med Res 7:438–457

Kumar S, Mishra A, Pandey AK (2013) Antioxidant mediated protective effect of Parthenium hysterophorus against oxidative damage using in vitro models. BMC Complement Altern Med 13:article 120. https://doi.org/10.1186/1472-6882-13-120

Lancelot E, Revaud ML, Boulee RG (1997) Alpha-N-*tert*-butylnitone attenuates excitotoxicity in rat striatum by preventing hydroxyl radical accumulation. Free Radic Biol Med 23:1031–1034

Lee J, Hahm ER, Singh SV (2010) Withaferin A inhibits activation of signal transducer and activator of transcription 3 in human breast cancer cells. Carcinogenesis 31(11):1991–1998

Liguori I, Curcio F, Bulli G et al (2018) Oxidative stress, aging, and diseases. Clin Interv Aging 13:757–772

Lopez LC, Escames G, Ortiz F, Ros E (2006) Acuna-Castroviejo D. Melatonin restores the mitochondrial production of ATP in septic mice. Neuroendocrinol Lett 27(5):623–630

Lovell MA, Markesbery WR (2007) Oxidative DNA damage in mild cognitive impairment and late-stage Alzheimer's disease. Nucleic Acids Res 35(22):7497–7504

Lu SC (2009) Regulation of glutathione synthesis. Mol Asp Med 30 (1–2):42–59

Mahomoodally MF, Gurib-Fakim A, Subratty AH (2005) Antimicrobial activities and phytochemical profiles of endemic medicinal plants of Mauritius. Pharm Biol 43(3):237–242

Mangialasche F, Polidori MC, Monastero R (2009) Biomarkers of oxidative and nitrosative damage in Alzheimer's disease and mild cognitive impairment. Ageing Res Rev 8:285–305

Mantzarlis K, Tsolaki V, Zakynthionoc E (2017) Role of oxidative stress and mitochondrial dysfunction in sepsis and potential therapies. Oxid Med Cell Longev 2017:5985209

Marrocco I, Altieri F, Peluso I (2017) Measurement and clinical significance of biomarkers of oxidative stress in humans. Oxid Med Cell Longev 2017:6501046

Milatovic D, Radic Z, Zivin M, Dettbarn W-D (2000a) Atypical effect of some spin trapping agents: reversible inhibition of acetylcholinesterase. Free Radic Biol Med 28:597–603

Milatovic D, Zivin M, Dettbarn W-D (2000b) The spin trapping agent phenyl-N-*tert*-butyl-nitrone (PBN) prevents excitotoxicity in skeletal muscle. Neurosci Lett 278:25–28

Milatovic D, Zivin M, Gupta RC, Dettbarn W-D (2001) Alterations in cytochrome-c-oxidase and energy metabolites in response to kainic acid-induced status epilepticus. Brain Res 912:67–78

Milatovic D, Gupta RC, Dettbarn W-D (2002) Involvement of nitric oxide in kainic acid-induced excitotoxicity in rat brain. Brain Res 957:330–337

Milatovic D, Gupta RC, Dekundy A et al (2005a) Carbofuran-induced oxidative stress in slow and fast skeletal muscles: prevention by memantine. Toxicology 208:13–24

Milatovic D, VanRollins M, Li K et al (2005b) Suppression of murine cerebral F_2-isoprostanes and F_4-neuroprostanes from excitotoxicity and innate immune response in vivo by alpha- or gamma-tocopherol. J Chromatogr B Anal Technol Biomed Life Sci 827:88–93

Milatovic D, Yin Z, Gupta RC et al (2007) Manganese induces oxidative impairment in cultured rat astrocytes. Toxicol Sci 98:198–205

Milatovic D, Zaja-Milatovic S, Gupta RC et al (2009) Oxidative damage and neurodegeneration in manganese-induced neurotoxicity. Toxicol Appl Pharmacol 240:219–225

Milatovic D, Montine TJ, Aschner M (2011) Measurement of isoprostanes as markers of oxidative stress. Methods Mol Biol 758:195–204

Miyajima T, Kotake Y (1995) Spin trap phenyl-N-*tert*-butylnitone (PBN) inhibits induction of nitric oxide synthase in endotoxin-induction in mice. Free Radic Biol Med 22:463–470

Montine TJ, Milatovic D, Gupta RC et al (2002) Neuronal oxidative damage from activated innate immunity is EP2 receptor-dependent. J Neurochem 83:463–470

Modaresi A, Nafar M, Sahraei Z (2015) Oxidative stress in chronic disease. Iran J Kidney Dis 9(3):165–179

Montine KS, Quinn JF, Zhang J et al (2004) Isoprostanes and related products of lipid peroxidation in neurodegenerative diseases. Chem Phys Lipids 128:117–718

Mori A, Yokoi I, Noda Y et al (2004) Natural antioxidants may prevent posttraumatic epilepsy: a proposal based on experimental animal studies. Acta Med Okayama 58:111–118

Morrow JD, Hill KE, Burk RF et al (1990) A series of prostaglandin F2-like compounds are produced *in vivo* in humans by a non-cyclooxygenase, free radical-catalyzed mechanism. Proc Natl Acad Sci USA 87:9383–9387

Negre-Salvayre A, Auge N, Ayala V et al (2010) Pathological aspects of lipid peroxidation. Free Radic Res 44(10):1125–1171

Niki E, Noguchi N, Tsuchihashi H et al (1995) Interaction among vitamin C, vitamin E, and beta-carotene. Am J Clin Nutr 62:1322S–1326S

Nishida S (2005) Metabolic effects of melatonin on oxidative stress and diabetes mellitus. Endocrine 27:131–136

Nyunoya T, March TH, Tesfaigzi Y et al (2011) Antioxidant diet protects against emphysema, but increases mortality in cigarette smoke-exposed mice. COPD 8(5):362–368

Onodera K, Omoi NO, Fukui K et al (2003) Oxidative damage of rat cerebral cortex and hippocampus, and changes in antioxidative defense systems caused by hyperoxia. Free Radic Res 37:367–372

Oudemans-van Straaten HM, Spoelstra-de Man AM, de Waard MC (2014) Vitamin C revisited. Crit Care 18:460

Pacher P, Beckman JS, Liaudet L (2007) Nitric oxide and peroxynitrite in health and disease. Physiol Rev 87:315–424

Pandey AK (2007) Anti-staphylococcal activity of a pan-tropical aggressive and obnoxious weed *Parihenium histerophorus*: an *in vitro* study. Natl Acad Sci Lett 30(11–12):383–386

Pazos AJ, Green EJ, Busto R et al (1999) Effects of combined post-ischemic hypothermia and delayed N-*tert*-butyl-alpha-phenylnitrone (PBN) administration on histopatological and behavioral deficits associated with transient global ischemia. Brain Res 846:186–195

Pieri C, Marra M, Moroni F et al (1995) The modulation of intracellular glutathione level modulates the mitochondrial response in proliferating rat splenocytes. Arch Gerontol Geriatr 21:115–125

Pizzino G, Irrera N, Cucinotta A et al (2017) Oxidative stress: harms and benefits for human health. Oxid Med Cell Longev 2017:8416763

Prince MR, LaMuraglia GM, MacNichol EF (1988) Increased preferential absorption in human atherosclerotic plaque with oral beta carotene. Implications for laser endarterectomy. Circulation 78:338–344

Punz A, Nanobashvili N, Feugl A et al (1998) Effect of α-tocopherol pretreatment on high energy metabolites in rabbit skeletal muscle after ischemia-reperfusion. Clin Nutr 17:85–87

Rahal A, Kumar A, Singh V et al (2014) Oxidative stress, prooxidants, and antioxidants: the interplay. Biomed Res Int 2014:761264

Rauca C, Wiswedel I, Zerbe R et al (2004) The role of superoxide dismutase and alpha-tocopherol in the development of seizures and kindling induced by pentylenetetrazol—influence of the radical scavenger alpha-phenyl-*N*-*tert*-butyl nitrone. Brain Res 1009:203–212

Roberts LJ, Montine TJ, Markesbery WR et al (1998) Formation of isoprostane-like compounds (neuroprostanes) *in vivo* from docosahexaenoic acid. J Biol Chem 273:13605–13612

Roberts RA, Smith RA, Safe S et al (2010) Toxicological and pathophysiological roles of reactive oxygen and nitrogen species. Toxicology 276(2):85–94

Sack CA, Socci DJ, Crandall BM et al (1996) Antioxidant treatment with phenyl-alpha-*tert*-butylnitrone (PBN) improves the cognitive performance and survival of aging rats. Neurosci Lett 205:181–184

Salem N, Kim HY, Lyergey JA (1986) Docosahexaenoic acid: membrane function and metabolism. In: Martin RE (ed) Health effects of polyunsaturated acids in seafoods. Academic Press, New York, pp 263–317

Salisbury D, Bronas U (2015) Reactive oxygen and nitrogen species: impact on endothelial dysfunction. Nurs Res 64(1):53–66

Santos LFL, Freitas RLM, Xavier SML et al (2008) Neuroprotective actions of vitamin C related to decreased lipid peroxidation and increased catalase activity in adult rats after pilocarpine-induced seizures. Pharmacol Biochem Behav 89(1):1–5

Sas K, Szabó E, Vécsei L (2018) Mitochondria, oxidative stress and the kynurenine system, with a focus on ageing and neuroprotection. Molecules 23:191

Schafer FQ, Buettner GR (2001) Redox environment of the cell as viewed through the redox state of the glutathione disulfide/glutathione couple. Free Radic Biol Med 30(11):1191–1212

Sen CK (2000) Cellular thiols and redox-regulated signal transduction. Curr Top Cell Regul 36:1–30

Shah D, Mahajan N, Paudyal B (2014) Oxidative stress and its biomarkers in systemic lupus erythematosus. J Biomed Sci 21(1):23

Sharman EH, Bondy SC (2016) Melatonin: a safe nutraceutical and clinical agent. In: Gupta RC (ed) Nutraceuticals: efficacy, safety and toxicity. Academic Press/Elsevier, Amsterdam, pp 501–509

Simioni C, Zauli G, Meartelli AM et al (2018) Oxidative stress: role of physical exercise and antioxidant nutraceuticals in adulthood and aging. Oncotarget 9(24):17181–17198

Spickett CM, Wiswedel I, Siems W et al (2010) Advances in methods for the determination of biologically relevant lipid peroxidation products. Free Radic Res 44:1172–1202

Stadtman ER, Levine RL (2003) Free radical-mediated oxidation of free amino acids and amino acids residue in proteins. Amino Acids 25 (3–4):207–218

Šterba M, PopelováO VA et al (2013) Oxidative stress, redox signaling, and metal chelation in anthracycline cardiotoxicity and pharmacological cardioprotection. Antioxid Redox Signal 18(8):899–929

Thomas CE, Ohlweiler DF, Taylor VL et al (1997) Radical trapping and inhibition of iron-dependent CNS damage by cyclic nitrone spin traps. J Neurochem 68:1173–1182

Topinka J, Bincova B, Sram RJ et al (1989) The influence of a-tocopherol and pyritinol on oxidative DNA damage and lipid peroxidation in human lymphocytes. Mutat Res 225:131–136

Trewin AJ, Berry BJ, Wojtovich AP (2018) Exercise and mitochondrial dynamics: keeping in shape with ROS and AMPK. Antioxidants (Basel) 7(1):1–8

Tucker PS, Dalbo VJ, Han T et al (2013) Clinical and research markers of oxidative stress in chronic kidney disease. Biomarkers 18 (2):103–115

Tyml K (2017) Vitamin C and microvascular dysfunction in systemic inflammation. Antioxidants 6:49

Tyml K, Li F, Wilson JX (2005) Delayed ascorbate bolus protects against maldistribution of microvascular blood flow in septic rat skeletal muscle. Crit Care Med 33(8):1823–1828

Upritchard JE, Schuurman CR, Wiersma A et al (2003) Spread supplemented with moderate doses of vitamin E and carotenoids reduces lipid peroxidation in healthy, nonsmoking adults. Am J Clin Nutr 78:985–992

Valko M, Leibfritz D, Moncol J et al (2007) Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol 39:44–84

VanAcker S, Koymans LMH, Bast A (1993) Molecular pharmacology of vitamin E: structural aspects of antioxidant activity. Free Radic Biol Med 15:311–328

Vatassery GT (2004) Impairment of brain mitochondrial oxidative phosphorylation accompanying vitamin E oxidation induced by iron or reactive nitrogen species: a selective review. Neurochem Res 29:1951–1959

Willcox BJ, Curb JD, Rodriguez BL (2008) Antioxidants in cardiovascular health and disease: key lessons from epidemiologic studies. Am J Cardiol 101:75D–86D

Wilson JX (2009) Mechanism of action of vitamin C in sepsis: ascorbate modulates redox signaling in endothelium. Biofactors 35 (1):5–13

Wiseman H, Halliwell B (1996) Damage to DNA by reactive oxygen and nitrogen species: role in inflammatory disease and progression to cancer. Biochem J 313(Part 1):17–29

Wolfram R, Oguogho A, Palumbo B et al (2005) Enhanced oxidative stress in coronary heart disease and chronic heart failure as indicated by an increased 8-epi-PGF (2alpha). Eur J Heart Fail 7:167–172

Wu G, Fang YZ, Yang S et al (2004a) Glutathione metabolism and its implications for health. J Nutr 134(3):489–492

Wu F, Wilson JX, Tyml K (2004b) Ascorbate protects against impaired arteriolar constriction in sepsis by inhibiting inducible nitric oxide synthase expression. Free Radic Biol Med 37(8):1282–1289

Xavier SM, Barbosa CO, Barros DO et al (2007) Vitamin C antioxidant effects in hippocampus of adult Wistar rats after seizures and status epilepticus induced by pilocarpine. Neurosci Lett 420(1):76–79

Yamamoto T, Kinoshita M, Shinomiya N et al (2010) Pretreatment with ascorbic acid prevents lethal gastrointestinal syndrome in mice receiving a massive amount of radiation. J Radiat Res 51:145–156

Yang ZP, Dettbarn W-D (1998) Lipid peroxidation and changes of cytochrome c oxidase and xanthine oxidase in organophosphorous anticholinesterase induced myopathy. Xth International Symposium on Cholinergic Mechanisms. J Physiol 92:157–162

Yin H, Gao L, Tai HH et al (2007) Urinary prostaglandin F2alpha is generated from the isoprostane pathway and not the cyclooxygenase in humans. J Biol Chem 282:329–336

Zaja-Milatovic S, Gupta RC, Aschner M, Montine TJ, Milatovic D (2008) Pharmacologic suppression of oxidative damage and dendritic degeneration following kainic acid-induced excitotoxicity in mouse cerebrum. Neurotoxicology 29:621–627

Zaja-Milatovic S, Gupta RC, Aschner M, Milatovic D (2009) Protection of DFP-induced oxidative damage and neurodegeneration by antioxidants and NMDA receptor antagonist. Toxicol Appl Pharmacol 240:124–131

Resveratrol: Biological Activities and Potential Use in Health and Disease

Gianfranco Risuleo and Camillo La Mesa

Abstract

Resveratrol (RV) is a polyphenol non-flavonoid compound present in strongly pigmented vegetables and fresh fruits as well as dried nuts such as peanuts. High concentrations of this natural compound were found, in the modern occidental world, in the peel of the berries of the red grape *Vitis vinifera*, but usage of this natural drug in popular medicine has been documented much earlier. Resveratrol exhibits diverse biological activities such as antitumor, antioxidant, antiviral, and phytoestrogenic. In particular, as the work reported from our laboratories, the compound shows an inhibitory effect on murine polyomavirus DNA replication, while at higher concentrations, RV shows a significant cytotoxic effect. This complex dose-dependent behavior is not intrinsic to the drug. Other natural substances behave in a similar way, curcumin and a semi-purified fraction of the whole neem oil being two different examples. Most likely, the administration of RV to cultured cells alters the permeability and fluidity of the cell membrane. Also, data presented in literature ascribe to RV an antiproliferative action, thus rendering this drug a good candidate for the control of neoplastic growth. The potential usage of RV both in human and veterinary medicine is also examined in this review.

Keywords

Resveratrol · Nutraceutical · Veterinary nutraceutical · Biological properties · Potential applications · Advanced medicine

"Gianfranco Risuleo" is on retirement.

G. Risuleo (✉)
Department of Biology and Biotechnologies "Charles Darwin", Sapienza University of Rome, Rome, Italy
e-mail: gianfranco.risuleo@uniroma1.it

C. La Mesa
Department of Chemistry "Stanislao Cannizzaro", Sapienza University of Rome, Rome, Italy

1 Prologue

The expression "the research on natural compounds and nutraceuticals is in a continuous and ever growing expansion" may sound overused, if not abused and trivial. But, just to give the reader an example about the validity and timeliness of this saying, we will report on a result easily acquired from a simple and quick literature database search. When one of us (G. R.) was invited to write a chapter for a book on *Nutraceuticals: Efficacy, Safety and Toxicity*, back in 2016 (Risuleo 2016), the number of review articles published, from the 1960s of last century up to 2014, on the general subject "natural substances and their biological activities," was about 1650. Now the same search using simply **resveratrol**, as a keyword, yields an amazing 1560 entries (only in terms of review articles) from 2001 up to the present days. It is easy to assert, therefore, that the general interest on this topic has not at all dwindled but remains a central theme for the research in alternative commodities, foodstuffs, and, last but not least, drugs aimed at therapeutic usages.

In this condensed chapter, we shall focus on resveratrol as a potential medicinal remedy in the human and veterinary field. In any case, an overview on the biological properties of resveratrol is necessary. We deliberately kept the language very plain and comprehensible also to scientists not necessarily or directly engaged in biomolecular/physical research on natural compounds, as well as to the layman and people interested in alternative ways to look at nutrition and health care. As a matter of fact, we are convinced that a subject raising such a vivid attention should be easily accessed by everybody.

2 Introduction

2.1 Phytoalexins in a Nutshell

Phytoalexins are natural compounds endowed of antimicrobial and antioxidative action; they include molecules like terpenoids, glycosteroids, and alkaloids. They are synthesized

R. C. Gupta et al. (eds.), *Nutraceuticals in Veterinary Medicine*, https://doi.org/10.1007/978-3-030-04624-8_15

de novo by plants under stress conditions which may originate, for example, by a pathogenic attack and/or by physical adverse conditions, e.g., drought. Pathogen infection may cause a rapid accumulation of phytotoxic substances, at the site of infection: here, phytoalexins show their character as broad-spectrum inhibitors of the infectious progression. The family of phytoalexins has manifold chemical characteristic and includes terpenoids, glycosteroids, and alkaloids. In any case, the definition of phytoalexin is applied to all types of phytochemical molecules involved in the plant defense: in a very broad sense, their action could be compared to the animal immune system. As a matter of fact, the susceptibility of the plant tissue to infection increases when the biosynthesis phytoalexin is inhibited. With respect to this, authors showed that plant mutants incapable of producing these natural "defenders" are more vulnerable to pathogen colonization as compared to wild type; conversely, host-specific pathogens able to degrade phytoalexins cause a higher virulence toward the plant (Glazebrook and Ausbel 1994; Thomma et al. 1999).

As mentioned above, polyphenols, flavonoids, and chemically related substances play a key role in the defense against fungal and other pathogens. We shall mention here a few examples of these "plant-protective" molecules:

Danielone is a phytoalexin found in the fruit of the papaya plant (*Carica papaya*) showing a potent antifungal activity against *Colletotrichum gloeosporioides*, an infectious pathogenic fungus (Echeverri et al. 1997).

Sakuranetin is a flavanone found in rice and other exotic plant like *Polymnia fruticosa* where it inhibits the germination of the spores of *Magnaporthe grisea* (Molnár et al. 2010; Cho and Lee 2015). Finally, it has been suggested that in *Sorghum*, the interactions with the abovementioned *Colletotrichum* seem to be inhibited by the expression of the SbF3'H2 through the synthesis of the pathogen-specific phytoalexins 3-deoxyanthocyanidin (Shih et al. 2006) whose gene encodes a flavonoid 3′-hydroxylase.

Stilbenes are produced in *Eucalyptus sideroxylon* in case of pathogens attacks. Such compounds can be implied in the hypersensitive response of plants. Actually one of the reasons why some woods show a natural preservation against rot may reside in the presence of high levels of polyphenols as reported by a "classical" work by Hart and Hillis (1974).

Finally, going back to the main subject of our work, also resveratrol belongs to the chemical family of phytoalexins. In particular *trans*-resveratrol in *Vitis vinifera* grapes is produced by the plant to combat the infection and proliferation of fungal pathogens such as *Botrytis cinerea* (Favaron et al. 2009). Figure 1 shows the structures of some common phytoalexins with different chemical features.

From what we have briefly discussed above, it is clear that the importance of these molecules goes beyond the intrinsic scientific interest and derives also by their enormous commercial significance. In fact phytoalexins may be considered very important to counteract plant disease and more in general in pest control.

This brief overview on phytoalexins, however, is far from being exhaustive; the reader who wants to deepen this subject should refer to reviews dealing specifically with this matter. See for instance: (Harborne 1999; Ahuja et al. 2012; Großkinsky et al. 2012; Sanchez Maldonado et al. 2015; Meyer et al. 2016; Oliveira et al. 2016; Burow and Halkier 2017; Santos Silva et al. 2018).

2.2 Resveratrol: The Molecule and Its Synthesis

As mentioned above, resveratrol is a phytoalexin of natural origin produced by both bryophytes and higher plants in response to stimuli of different nature. Resveratrol is a stilbenoid with a low molecular weight (MW = 228,25 dal); it is characterized by two aromatic rings linked by an ethane or ethylene residue. The aromatic rings that compose it present three hydroxyl groups at positions 3′, 4′, and 5′. In the IUPAC nomenclature is classified as 5-[(*E*)-2-(4-idrossiphenil)-ethenil] benzene-1,3-diol, but it is also known as 3,4,5 tri-hydroy-stilbene or 3,4,5 stilbene-triol.

In plants, the synthesis of RV is mediated by the enzyme, resveratrol synthase (Schröder et al. 1988). The drug is normally found in two geometric isomer forms: *cis*-(*Z*) and *trans*-(*E*) resveratrol; both isomers can exist in a free or glucose-bound form. The *cis–trans* isomer transition occurs by photoisomerization, a process mediated by UV-light (Mattivi et al. 1995; Lamuela-Raventos et al. 1995) (Fig. 2).

Resveratrol is water soluble up to a concentration of 16.9 mg/L at 25 °C, but solubility is higher in ethanol. Its melting temperature is 253 °C. In addition, the *trans*-resveratrol powder is stable at 40 °C in the presence of air and in a relatively dry atmosphere (75% humidity). This isomer is also stabilized by the presence of transport proteins (Prokop et al. 2006, see also below). The commercially available preparation consists of a white powder with a yellowish-orange hue.

Resveratrol is synthesized in *Vitis vinifera* following the metabolic pathway of the phenyl-propanoids (Dixon and Paiva 1995) where the starting compound is phenylalanine. The enzyme phenylalanine ammonium lyase mediates the elimination of the NH2 group from the amino acid and catalyzes the formation of cinnamic acid: the series of metabolic steps leading to the synthesis of RV is shown in Fig. 3 (reproduced

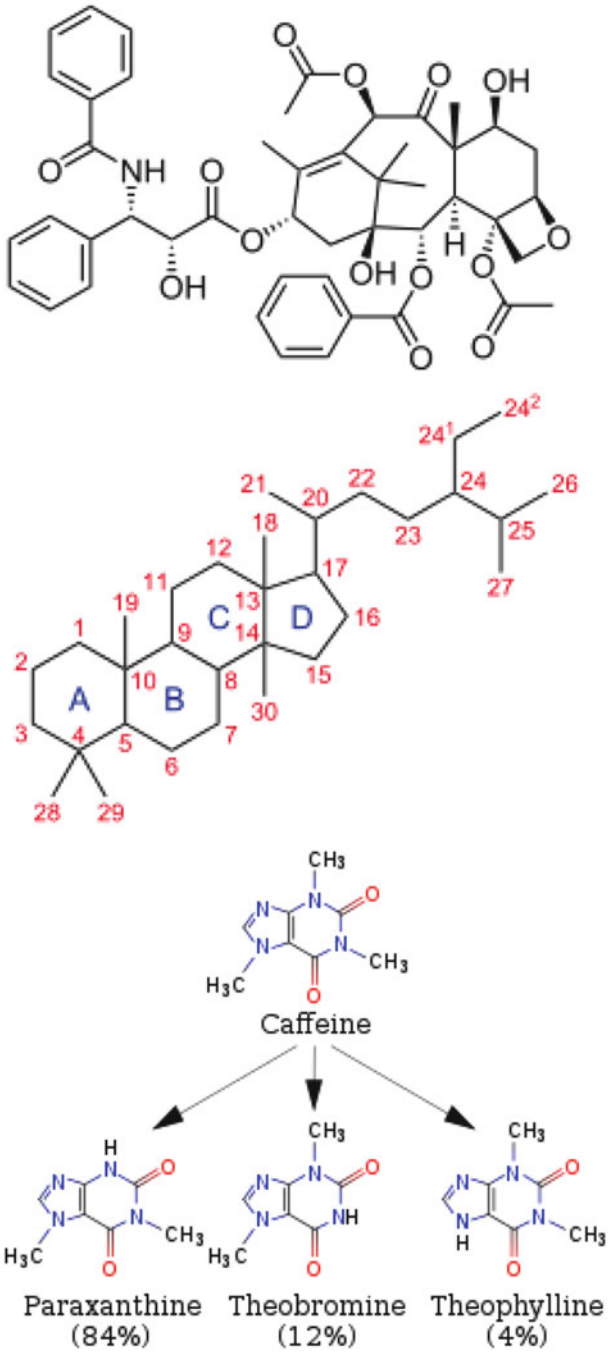


Fig. 1 Structure and function of some common natural substances. Top panel: The terpenoids, also known as isoprenoids, derive from terpenes which are hydrocarbons with additional functional groups, usually containing an oxygen atom. They form a large and diverse class of natural organic chemicals. About 60% of known natural products are terpenoids. The figure reports the chemical structure of the terpenoid taxol, a well-known anticancer drug obtained from the tree *Taxus baccata* and now produced also in laboratory in plant cell culture systems. Center panel: Steroids are biologically active organic compounds with four rings arranged in a specific molecular configuration. They are important components of cell membranes playing a role in membrane fluidity. They may also function as signaling molecules. A large number of steroids are found in plants, fungi, and animals. Bottom panel: Alkaloids can be purified from crude extracts by acid-base extraction by bacteria, fungi, plants, and animals. They show a very broad range of pharmacological activities. Unlike other natural compounds, alkaloids are characterized by a great structural and functional diversity; therefore their univocal classification is almost impossible. Obsolete methods have classified alkaloids by the common natural plant source. This was due mainly to lack of knowledge about their chemical structure. More recent classifications are based on similarity of the carbon skeleton or the biochemical precursor. However, due their diversity the classification of alkaloids is sometimes still uncertain. The picture shows the structure formula of caffeine and its metabolic modifications

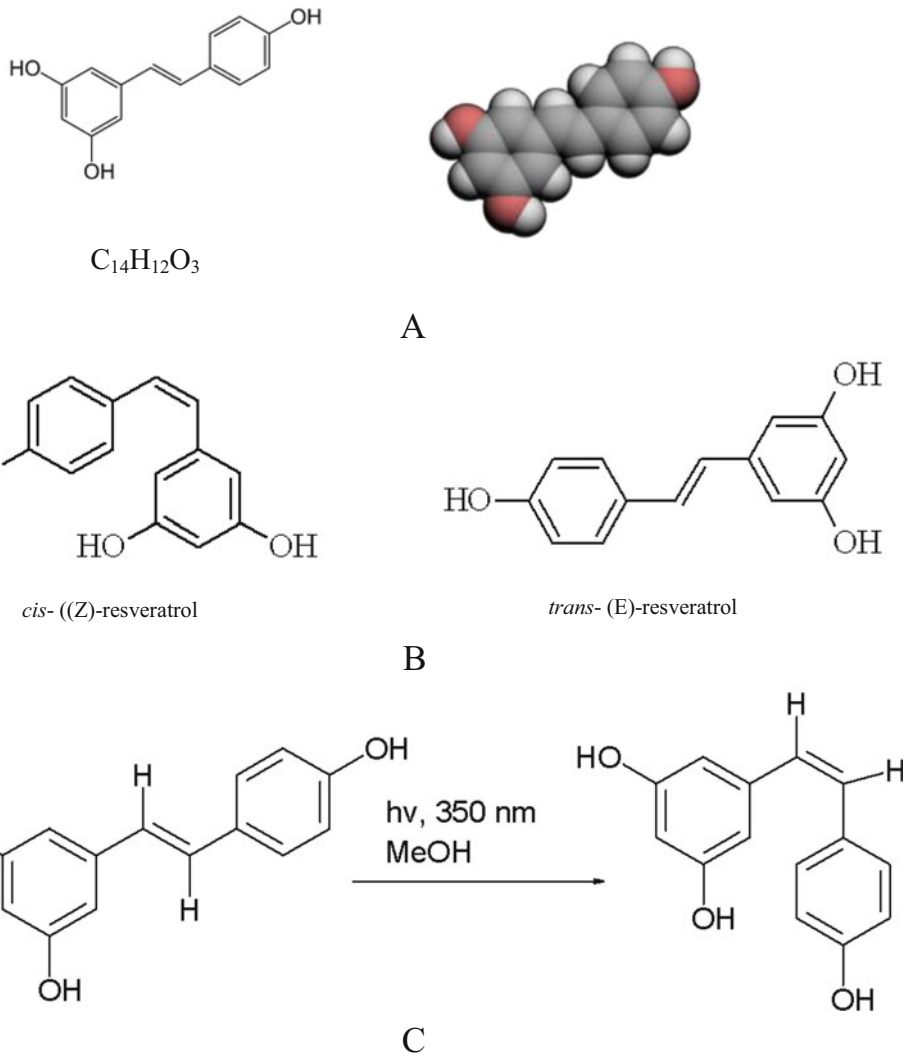


Fig. 2 (**a**) Empirical and structure formulas of *cis*-resveratrol (left and right, respectively). The number of C, H, and O atoms is also given. (**b**) Structure formula of *cis*- and *trans*-resveratrol (left and right, respectively). (**c**) Transition of resveratrol into the two known isomers mediated by UV light

with modifications from: Soleas et al. 1997; Schröder 1999; Jeandet et al. 2002, Cichewicz and Kouzi 2002).

2.3 Occurrence in Nature

Resveratrol was originally found in the berries of the wine grape (*Vitis vinifera*), but it is also present in the roots, seeds, and stock of the plant. The highest concentration is found in the peel of the berries although the content may vary significantly depending upon the fruit source from which resveratrol is extracted and upon the way the fruit is processed. Red wine contains a relevant amount of RV, but it can be also obtained from diverse sources like pea- and pine nuts as well as mulberries: actually the compound can be isolated from all intensely pigmented fruits (Table 1 shows the amount of RV present in various foods and beverages). In any case, this natural product is known since centuries and was used in the Japanese and Chinese traditional medicine: actually, RV was obtained in its crude original form, from the desiccated roots of the Japanese knotweed (*Polygonum cuspidatum*) where is present at a 400-fold higher concentration as compared to grapes or red wine (Fig. 4).

3 Relevant Biological Actions

3.1 Antitumor Activity in Mammals

The main feature of a transformed tumor cell is represented by its unregulated proliferation that determines a significantly increase in duplicative speed as compared to normal tissue cells. There are a number of reasons accounting for this metabolic dysregulation, essentially loss of control of the cell cycle and/or evasion from the apoptotic death mechanisms which represent the principal mode to control differentiation and size of the cell population. With respect to this, cyclins constitute a

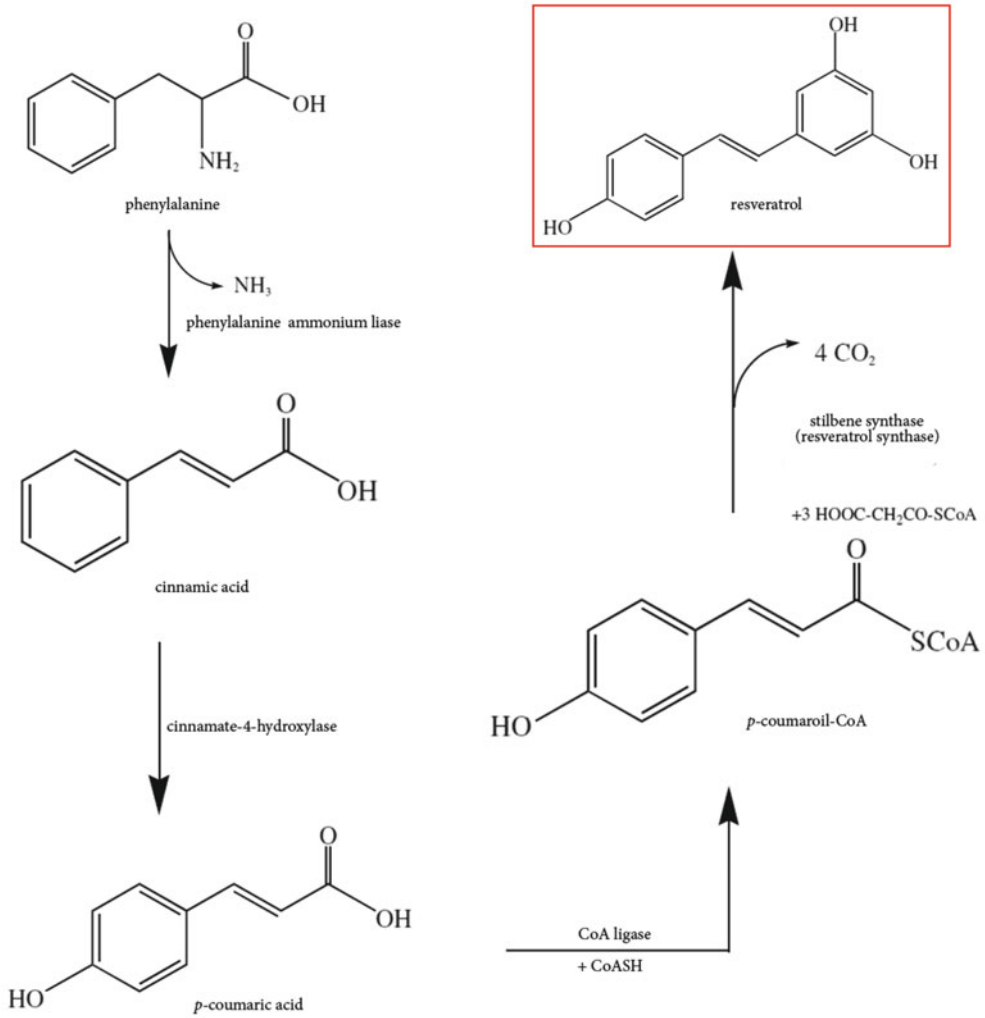


Fig. 3 Schematic illustration of the RV chemical biosynthesis of *Vitis vinifera*. See text for details

Table 1 Resveratrol content in various commercially available commodities

Food	Total resveratrol (mg/100 gm)	Beverages and seasonings	Resveratrol (mg/100 mL)	
			Mean	Range
Peanuts	0.08	Red wine	0.27	0–2.78
Peanut, roasted	0.06	Rosé wine	0.12	5.00×10^{-3} to 0.29
Peanut butter	0.04	White wine	0.04	0.00–0.17
Red grapes	0.24–1.25[a]	Sparkling wine	9.00×10^{-3}	8.00×10^{-3} to 1.00×10^{-2}
Cocoa powder	0.14–0.23[a]	Green grape juice	5.08×10^{-3}	0.00 to 1.00×10^{-2}
Cocoa–chocolate (dark)	0.04[a]	Vinegar	6.86×10^{-3}	0.02–7.75
Pistachio, dehulled	0.11			
Lingonberry, raw	3.00			
Red currant, raw	1.57			
Strawberry, raw	0.35			

Re-elaborated from stilbenes resveratrol in foods and beverages. Phenol-Explorer 2016. Link: http://phenol-explorer.eu/contents/polyphenol/592

[a]These values may vary according to the cultivar of red grapes, the hydration of the powder (cocoa), or its percentage (w/w) in the finished product (chocolate). The same applies to wines and seasonings. Furthermore, the RV content in wine is different from that of grapes since its extraction from the fruit depends on the wine making and fermentation techniques (e.g., the duration of the contact time with the peels of the grape berries). Also, resveratrol in its 3-glucosidic form is hydrolyzed, thus yielding both *trans*- and *cis*-resveratrol. Data for soy sauce and other oriental seasonings as well traditional foods like kosher pickles are not available. Traces of resveratrol and other lycopenes, on the other hand, are present in cosmetics, but the skin adsorption of these compounds deserves further assessment

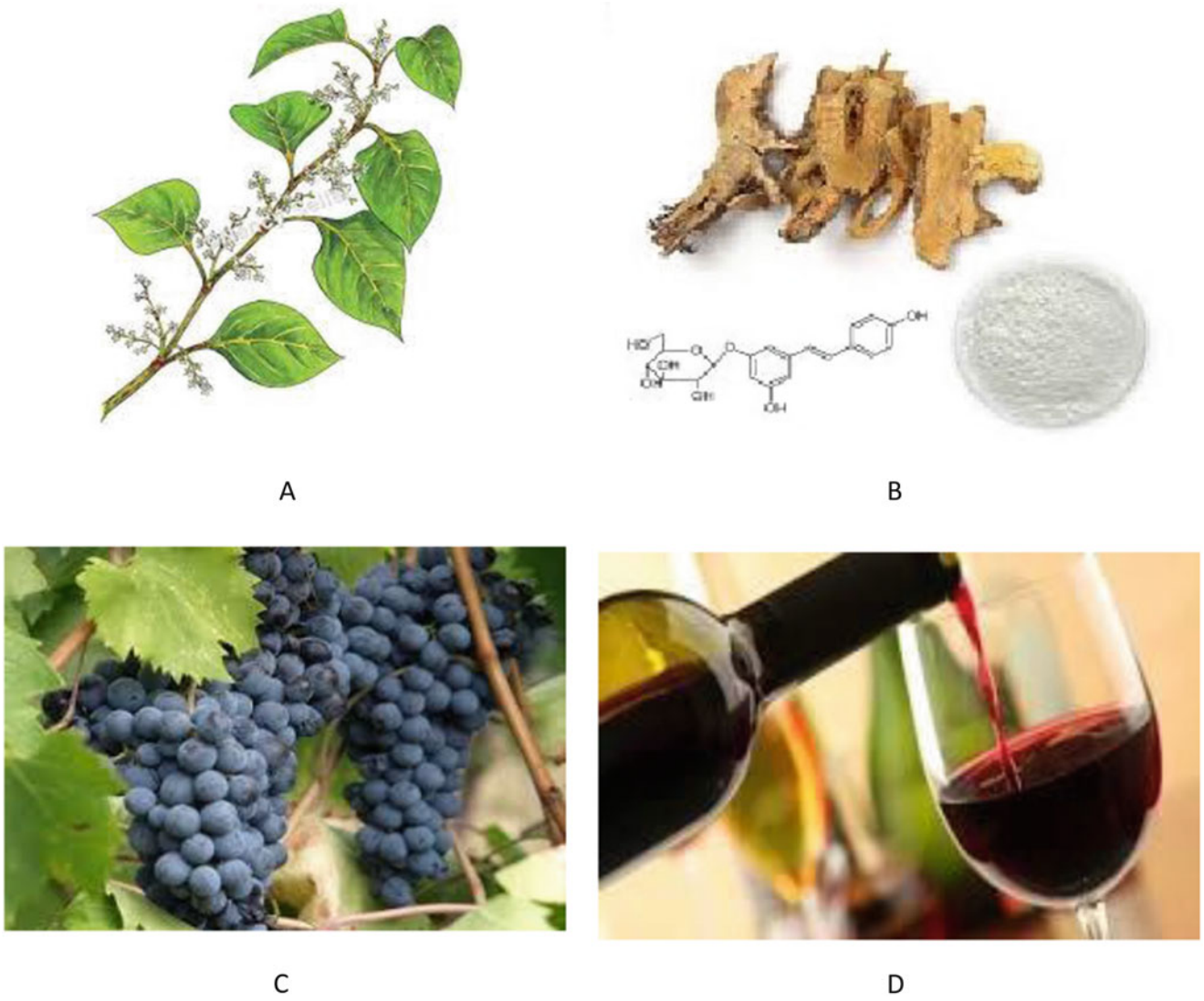


Fig. 4 Top panels. (**a**) Graphic representation of the plant *Polygonum cuspidatum* also known as *Fallopia japonica*. (**b**) Desiccated roots of the plant; *trans*-resveratrol chemical formula; final commercially available powder. (**c**) Bunches of wine blue grapes. (**d**) The final product, red wine. Due to its high content of resveratrol, the moderate assumption of red wine is considered beneficial (the French paradox). However, it should be borne in mind that the "liberal" usage of alcoholic beverages is highly detrimental for the psychophysical human conditions

family of protein factors able to regulate the correct execution and completion of the cell cycle: if the block in G1 phase takes place, the apoptotic pathway is activated. This phenomenon was observed, for instance, in the human epidermal carcinoma (Ahmad et al. 2001), and somewhat more recent data demonstrated the hindrance of progression toward the S phase (Liao et al. 2010). With respect to this, RV has been shown to modulate the cdk-cyclin-dependent mechanisms. This drug has been also involved in the development of the human colon-rectal since it induces clustering of the Fas ligand and its redistribution in sphingolipid matrix of the transformed cells which, in turn, is associated with the formation of DISC (death-inducing signaling complex; Delmas et al. 2011). Studies in a mouse model of epidermal tumor in mammary glands demonstrate that RV counteracts tumorigenesis. Resveratrol also increases the antitumor activity of the mTOR inhibitors, and finally, it represses the activation pathway of tumor-stimulating factors in mammary tumor cell lines (He et al. 2011). The gene product nf-kb is involved in the regulation of a vast number of protein factors modulating very complex biological phenomena such as inflammation, cell proliferation, and carcinogenesis: it has been suggested that RV inhibits the action of this factor (Singh et al. 2011). An interesting consideration, not directly associated to the antitumor property of RV, is that this compound is also a regulator of the human SIRT-1 gene which encodes a protein involved in aging and is considered as a potential factor of longevity (Signorelli and Ghidoni 2005; Singh et al. 2011). However, as of 2018, the evidence that resveratrol has life-prolonging properties in humans remains a matter of debate. Furthermore, the limited

bioavailability of resveratrol may further impede its potential effects (Pallauf et al. 2016; Wahl et al. 2018. See also the section on RV pharmacology in this chapter).

3.2 Antioxidant Properties

The main causative agent of atherosclerosis, and chronic artery inflammation, depends both on the individual lifestyle and environmental conditions. Therefore, tobacco smoking, pollution, hypercholesterolemia, hypertension, and other conditions (e.g., diabetes and obesity) are at the basis of cardiac/coronary problems. One of the final stages in the complex genesis of atherosclerosis is the damage of the vasal endothelium, the deposit of the low-density lipid fraction (LDL) and, finally, the aggregation of platelets. Resveratrol may play a protective role due to its very high antioxidant character (Matos et al. 2012): for instance, treatment of bovine aorta cells with RV delays the formation of the atheroma. Parallel treatment with mitosis-stimulating factors such as PDGF may act synergistically with RV (Araim et al. 2002).

Lipids containing unsaturated fatty acids, or their esters, are oxidized by the molecular oxygen present in cells; lipid peroxidation is a direct consequence of the formation of free reactive oxygen species (ROS). Resveratrol can inhibit the oxidation of the low-density lipid (LDL) fraction circulating in the blood. This oxidation is a primary event in the development of atherosclerosis: the strong antioxidant character of RV attenuates the formation of ROS and, consequently, the cytotoxicity induced by LDL peroxidation as well as the accumulation of intracellular calcium which is also an effect induced by the drug. The final result is the inhibition of caspase-3, an enzyme playing a crucial role in the execution of apoptosis (Shalini et al. 2015). It should be considered that high levels of ROS stimulate platelet aggregation: also in this case, the antioxidant action of the drug and its ability to reduce platelet adhesion to the type I collagen enable *trans*-resveratrol to limit this phenomenon. This ends in an impairment of blood coagulation and establishment of the atherosclerotic process (Zbikowska et al. 1999).

3.3 Cell Death

The strong antioxidant activity of RV with consequent protection from cell damage, as repeatedly stated above, is common knowledge; however the drug may also paradoxically induce apoptosis and autophagy (Kou and Chen 2017). These effects, monitored in a time- and concentration-dependent mode, were demonstrated in different models of cell cultures (Berardi et al. 2009; Whitlock and Baek 2012; Aluyen et al. 2012; Hasima and Ozpolat 2014). Cytofluorimetric analysis also showed activation of the apoptotic marker factors and the occurrence of typical apoptotic morpho-functional alterations (Zhang et al. 2011). In any case the preferential target of RV seems to be represented by the transformed cells (Berardi et al. 2009; Zhang et al. 2012; Risuleo 2016). As far as the cell cycle is concerned, RV can induce an arrest in S phase in murine pre-adipocytes and a significant increase of the intracellular concentration of lactic dehydrogenase (LDH): a hallmark of apoptosis. However the paradoxical effects of the drug which can protect the cell or vice versa induce its death, and its synergistic effects with diverse antioxidant, are a clear sign of the complex interactions of the drug with the target cells. A number of studies demonstrated that this behavior is shared by other natural products (see for instance: De la Lastra and Villegas 2007; Ricci et al. 2009; Chen et al. 2011; Ullah et al. 2015; Wang et al. 2012).

3.4 Antiviral Activity

The first evidence of the antiviral action of RV dates back to 1999 when the inhibition of the replication of herpes simplex virus (HSV) was published (Docherty et al. 1999). Also, its improving effects in a mouse model system of vaginal infection were also shown (Docherty et al. 2005). Subsequently the same authors demonstrated the RV-dependent inhibition of varicella-zoster DNA replication in a human fibroblast cell line. This action is exerted in the early phases of the infection mainly at the level viral mRNA transcription (Docherty et al. 2006). Later studies, however, ascribed this indirect antiviral action to the inhibition by RV of the formation of the NF-kb dimeric complex, an essential prerequisite for DNA synthesis (Gregory et al. 2004); an analogous phenomenon occurs in the case of influenza A virus where the proteins necessary for the assembly and maturation of the viral capsid, after cytoplasmic synthesis, are not efficiently transported to the nucleus (Palamara et al. 2005). Relatively recent work suggested a possible role of RV in the control of the complications after the infection by the H1N1 virus. As reviewed by Uchide and Toyoda, this negative control would be due to the RV-mediated neutralization of the superoxide anion produced by macrophages which would limit the consequences of the viral infection. Resveratrol has also been suggested to influence negatively the vitality of lymphoma cells infected with the Epstein-Barr virus (De Leo et al. 2012) which is involved in different human pathologies (Tooze 1981).

It has been suggested that RV may also have a role in the control of the HIV infection in humans since it potentiates the action of antiviral agents used in anti-HIV therapy; however due to the extremely complicated pathological picture of AIDS, in the opinion of the authors of this chapter, this observation deserves further and deeper evaluation. Finally, in our laboratory, we explored the action of RV on the murine

polyomavirus (Py) replication. Also in this case, RV has shown strong antiviral properties since it reduces the viral DNA synthesis both in murine fibroblasts and in human promyelocytic leukemia cells. The inhibition occurs at low non-cytotoxic doses of the drug which does not seem to impede viral adsorption and entry into the cell. These data combined with other observations from our laboratory on another natural substance strongly suggest that the cell membrane is the main target of RV (Ricci et al. 2009; Aiello et al. 2011). In particular, the role of the membrane permeability with respect to the RV bioactivity will be discussed in further detail in the following section.

3.5 Resveratrol and the Cell Membrane

What discussed previously opens new questions about the mode of action of RV in particular: How does the drug find its target(s)? Is the multifaceted "therapeutic" efficacy also mediated by binding molecular carriers which improve the penetration across the cell membrane and stabilize its intracellular "survival?" Finally, is it possible to construct "cargo" vehicles able to deliver to right cell district thus improving its efficacy? This aspect, in particular, will be the subject of another chapter.

In any case, albumin could act as a good transporter since this protein is able to bind amphiphilic molecules, and in addition to RV, it interacts with other natural substances such as genistein and curcumin (Bourassa 2010). Studies indicate that RV is able to bind one of the major human plasma proteins (HAS). However, the amount of bound drug is inversely proportional to its plasma concentration which makes this result of very difficult interpretation (Jiang 2008). The entry of RV has been suggested to occur through a clathrin-independent process of endocytosis; but lipid rafts seem to play a role since agents that damage their functionality also inhibit penetration of the drug within the cell (Colin et al. 2011). We investigated the mechanism of RV entry into the cell by electrorotation: a biophysical approach which was amply discussed and reviewed (Bonincontro and Risuleo 2015). By this experimental strategy, we were able to evaluate the cell membrane function and intrinsic role in mediating the action of RV. Result from our laboratory demonstrates that the drug is promptly uptaken by the cell population which is not blocked in G1 at a relevant extent (Berardi et al. 2009; Bonincontro et al. 2018).

For a more detailed review on the various and diversified biological properties of resveratrol, the reader should address a previously published work (Risuleo 2016). Table 2 reports a synopsis of the different bioactivities of the drug and the related reference data.

Table 2 Activities/properties exhibited by resveratrol

Action/role	System (s)[a]	Reference/s
Aging	AMS, HS	(Sarubbo et al. 2017; McCubrey et al. 2017)
Autophagy/ cell death	CCS, AMS	(Owen et al. 2017; Tsai et al. 2017; Bhat et al. 2018; Cao et al. 2018; Fan et al. 2018)
Brain	HS, AMS	(Castro et al. 2017; Amro et al. 2018; Lange and Li 2018; Lee et al. 2018)
Cancer	CCS, AMS	(Crooker et al. 2018; Elshaer et al. 2018; Espinoza et al. 2018; Huminiecki and Horbańczuk 2018; Perez-Vizcaino and Fraga 2018; Zhai et al. 2018)
Cardiovascular	AMS, HS/CT	(Treviño-Saldaña and García-Rivas 2017; Bird et al. 2015)
Diabetes	AMS, HS/CT	(Oliveira et al. 2017; Zhu et al. 2017; Popescu et al. 2018)
Skin	HS/CT	(Farris et al. 2013; Ganesan and Choi 2016; Chedea et al. 2011)

These features of the drug are additional to those examined in extenso in the text

[a]Acronyms: AMS, animal model systems; CCS, cell culture systems; HS/CT, human systems/clinical trials

4 Pharmacological Aspects: Pharmacodynamics and Pharmacokinetics

Resveratrol has been identified as a pan-assay interference compound, which produces positive results in many different laboratory assays. The pan-assay-defined compounds may produce false positives, especially in high-throughput screenings, since they tend to react in a nonspecific manner with numerous biological targets within the cell, rather than affecting a specific one (Baell and Walters 2014). Therefore some caution is necessary when analyzing the ample spectrum of actions inhibited by RV. One of the ways to rationalize the multi-target effect of this natural product takes into account its ability to interact with the cell membrane which could cause a cascade of effects in other districts of the cell not directly involved with the membranes (Ingólfsson et al. 2014; Bonincontro and Risuleo 2015, Vang 2015; see also references on this matter reported above in the section: "Resveratrol and the cell membrane"). As a matter of fact, many and very diverse specific biological targets of resveratrol, e.g., hormone receptors, apoptotic factors, and cell cycle regulators, have been identified (Vang 2015). The cell membrane allows the cytoplasm matrix to communicate with the outer world and acts as a highly selective filter. Biological membranes participate to a number of transport mechanisms such as passive osmosis and diffusion. Therefore, their

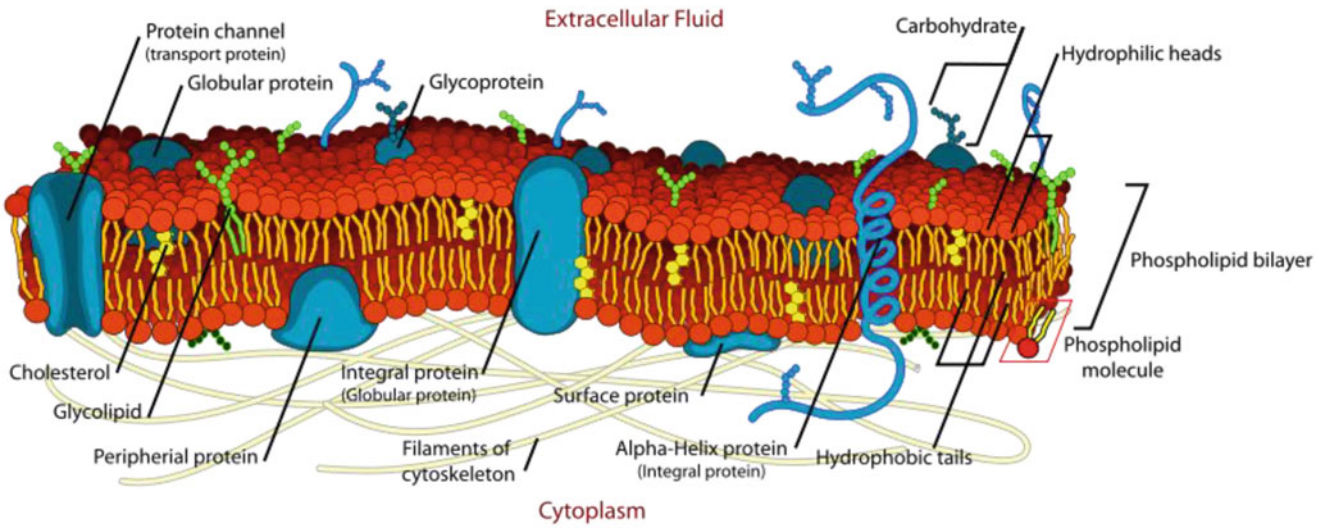


Fig. 5 The cartoon depicts the structure of a typical cell membrane. Its involvement in numerous cell functions and activities is clearly illustrated. The image is freely available on the net and not covered by copyright. We report here the www-link: https://en.wikipedia.org/wiki/Cell_membrane#mediaviewer/File:Cell_membrane_detailed_diagramen.svg. The work of the author of this picture, Lady of Hats, is acknowledged

integrity plays a fundamental role in a number of cell functions such as metabolism, maintenance of the basal homeostasis, and, although in an indirect manner, cell differentiation and development. Recently, it has become clear the role of the cell membrane in "filtering" the paracrine apoptogenic and/or intrinsic death signals; therefore, any agent affecting the efficient function of the cell membrane could play a binary role: helping cell survival and correct proliferation or vice versa triggering mechanisms leading to damage; for a relatively recent review, see Mattetti and Risuleo (2014) and references therein. The complexity and function multiplicity of the cell membrane are depicted in Fig. 5.

Other studies conducted in vitro indicate that resveratrol activates sirtuins. These proteins influence a wide variety of cellular processes such as aging, transcription, apoptosis, inflammation, and stress resistance, as well as energy efficiency and alertness during energy situations such as low-calorie diets. Finally, resveratrol seems to stimulate the production of superoxide dismutase (SOD-2) and GPER activity. This latter is a G protein that in humans interacts with the estrogen receptor-1 GPER and is activated by the female sex hormone. Resveratrol was shown to act in vitro, as an agonist of peroxisome proliferator-activated receptor gamma, a nuclear receptor under pharmacological research as a potential agent for the treatment of type 2 diabetes (Yang et al. 2015).

One way of administering resveratrol in humans is buccal delivery without swallowing, that is, by direct absorption through tissues inside the mouth. One milligram of resveratrol in solution (50% alcohol/water) retained in the mouth for 1 min can be monitored 2 min later in the plasma at concentration of 37 ng/mL (free resveratrol). This same concentration of non-modified native RV is reached after administration of 250 mg of the drug in a pill form (Asensi et al. 2002). However, the relatively low solubility of RV in water limits the amount that can be absorbed through the buccal mucosa. Therefore the viability of the buccal delivery method is questionable due to the relatively low aqueous solubility of the molecule. Therefore further assessment of the efficacy of RV buccal delivery for RV is required (Madhav et al. 2009; Ansari et al. 2011).

According to pharmacological evaluations, about 70% of resveratrol is absorbed after oral administration; however its oral bioavailability is approximately 0.5% due to extensive chemical modifications, such as glucuronidation and sulfation, undergone by the drug: these occur at liver level (Walle 2011). Finally, the production of the RV proprietary formulation SRT-501 was discontinued by GlaxoSmithKline since "the company decided to terminate the Phase 2 trial of SRT501 in multiple myeloma and halt development of the drug as a potential myeloma treatment. The SRT501 formulation of resveratrol may only offer minimal efficacy, while increasing the chances of kidney failure."

See The Myeloma Beacon Staff. Nov 30, 2010 (https://myelomabeacon.org/news/2010/11/30/glaxosmithkline-halts-all-further-development-of-resveratrol-drug-srt501/).

Resveratrol was detected in cerebrospinal fluid in a human study involving oral administration of 500 mg over 13 weeks, (Turner et al. 2015) which suggests that the drug is able to cross the blood-cerebrospinal fluid barrier. Resveratrol is also extensively metabolized in the liver and lungs which are the major sites of its metabolic transformations (Sharan and Nagar 2013).

5 Adverse Effects

A limited number of human studies have shown that resveratrol is generally well-tolerated. Clinical trials showed that one person taking a 1000 mg daily dose developed an itchy rash that was resolved after discontinuation; in the same study, also the blood pressure seemed to be affected. In four of the published trials, people had increased frequency of bowel movements and loose stools in first month of the treatment. In a yearlong Phase 2 trial in people with Alzheimer's, the most frequent adverse effects were diarrhea, weight loss, and nausea (Hausenblas et al. 2014; Fogacci et al. 2018). All in all, these effects do seem to be very important if one considers the potential advantages deriving from the treatment with RV.

6 Concluding Remarks and Future Directions

Control of cell death and senescence seems to be the most relevant effects of the administration of resveratrol. This action is essentially exerted through the interaction of RV with the cell membrane. However, like other natural products, RV may give rise to paradoxical effects; therefore its use as a multi-target therapeutic means should be considered with some *caveats*. For instance, resveratrol is apparently able to cross the membrane without causing serious consequences for the cell viability and survival, but its limited water solubility and bioavailability may reduce the use of this natural substance for therapeutic purposes. This should urge the search for new ways to deliver the drug to the cell population or to the cell district where it may play its role to contrast and/or eliminate pathological phenomena. In any case, the usage of this, as well as other medicaments of natural origin, should be done only after an accurate evaluation of their biocompatibility even though resveratrol does not seem to show significant adverse effects. With respect to the pharmacological use of RV, nanotechnologies may be of great support for an efficient and biocompatible application for the resolution of health problems. As a matter of fact, delivery mediated by nanoparticles such as liposomes, vesicles, or nanotubes may represent the new way for a safe and efficient way to dispatch molecules endowed of therapeutic capacities.

References

Ahmad N, Adhami VM, Afaq F et al (2001) Resveratrol causes WAF-1/p21-mediated G(1)-phase arrest of cell cycle and induction of apoptosis in human epidermoid carcinoma A431 cells. Clin Cancer Res 7:1466–1473

Ahuja I, Kissen R, Bones AM (2012) Phytoalexins in defense against pathogens. Trends Plant Sci 17:73–90

Aiello C, Berardi V, Ricci F, Risuleo G (2011) Biological properties of a methanolic extract of neem oil, a natural oil from the seeds of the Neem tree (*Azadirachta indica* var. A. Juss). In: Preedy VR, Watson RR, Patel VB (eds) Nuts & seeds in health and disease prevention, 1st edn. Academic Press is an imprint of Elsevier, London, Burlington, San Diego, pp 813–821. ISBN: 978-0-12-375688-6

Aluyen JK, Ton QN, Tran T et al (2012) Resveratrol: potential as anticancer agent. J Diet Suppl 9(1):45–56

Amro MS, Teoh SL, Norzana AG et al (2018) The potential role of herbal products in the treatment of Parkinson's disease. Clin Ter 169 (1):e23–e33

Ansari KA, Vavia PR, Trotta F et al (2011) Cyclodextrin-based nanosponges for delivery of resveratrol: in vitro characterisation, stability, cytotoxicity and permeation study. AAPS Pharm Sci Technol 12(1):279–286

Araim O, Ballantyne J, Waterhouse AL et al (2002) Inhibition of vascular smooth muscle cell proliferation with red wine and red wine polyphenols. J Vasc Surg 35(6):1226–1232

Asensi M, Medina I, Ortega A et al (2002) Inhibition of cancer growth by resveratrol is related to its low bioavailability. Free Radic Biol Med 33(3):387–398

Baell J, Walters MA (2014) Chemistry: chemical con artists foil drug discovery. Nature 513:481–483

Berardi V, Ricci F, Castelli M, Galati G, Risuleo G (2009) Resveratrol exhibits a strong cytotoxic activity in cultured cells and has an antiviral action against polyomavirus: potential clinical use. J Exp Clin Cancer Res 28:96–105

Bhat P, Kriel J, Shubha Priya B et al (2018) Modulating autophagy in cancer therapy: advancements and challenges for cancer cell death sensitization. Biochem Pharmacol 147:170–182

Bird JK, Raederstorff D, Weber P et al (2015) Cardiovascular and antiobesity effects of resveratrol mediated through the gut microbiota. Adv Nutr 8:839–849

Bonincontro A, Risuleo G (2015) Electrorotation: a spectroscopic imaging approach to study the alterations of the cytoplasmic membrane. Adv Mol Imaging 5:1–15

Bonincontro A, Domenici F, Milardi GL, Risuleo G (2018) Differential dielectric behavior of the plasma membrane in mouse fibroblasts and human embryo kidney cells. Intl J Sci Res 7:68–71

Bourassa P (2010) Resveratrol, genistein, and curcumin bind bovine serum albumin. J Phys Chem 114:3348–3354

Burow M, Halkier BA (2017) How does a plant orchestrate defense in time and space? Using glucosinolates in *Arabidopsis* as case study. Curr Opin Plant Biol 38:142–147

Cao W, Dou Y, Li A (2018) Resveratrol boosts cognitive function by targeting SIRT1. Neurochem Res 43(9):1705–1713. https://doi.org/10.1007/s11064-018-2586-8

Castro OW, Upadhya D, Kodali M et al (2017) Resveratrol for easing status epilepticus induced brain injury, inflammation, epileptogenesis, and cognitive and memory dysfunction-are we there yet? Front Neurol 13(8):603

Chedea VS, Vicaş SI, Sticozzi C et al (2011) Resveratrol: from diet to topical usage. Food Funct 8:3879–3892

Chen S, Xiao X, Feng X et al (2011) Resveratrol induces Sirt1-dependent apoptosis in 3T3-L1 preadipocytes by activating AMPK and suppressing AKT activity and survivin expression. J Nutr Biochem 23(9):1100–1112

Cho MH, Lee SW (2015) Phenolic phytoalexins in rice: biological functions and biosynthesis. Int J Mol Sci 16(12):29120–29133

Cichewicz RH, Kouzi ESA (2002) Resveratrol oligomers: structure, chemistry, and biological activity. In: Atta-ur-Rahman (ed) Studies in natural products chemistry, vol 26. Elsevier, Amsterdam, pp 507–579

Colin D, Limagne E, Jeanningros-Arnaud S et al (2011) Endocytosis of resveratrol via lipid rafts and activation of downstream signaling pathways in cancer cells. Cancer Prev Res (Phila) 4(7):1095–1106. https://doi.org/10.1158/1940-6207.CAPR-10-0274

Crooker K, Aliani R, Ananth M (2018) A review of promising natural chemopreventive agents for head and neck cancer. Cancer Prev Res (Phila) 11(8):441–450. https://doi.org/10.1158/1940-6207.CAPR-17-0419

De la Lastra CA, Villegas I (2007) Resveratrol as an antioxidant and pro-oxidant agent: mechanisms and clinical implications. Biochem Soc Trans 35(5):1156–1160

De Leo A, Arena G, Lacanna E et al (2012) Resveratrol inhibits Epstein Barr Virus lytic cycle in Burkitt's lymphoma cells by affecting multiple molecular targets. Antivir Res 96(2):196–202

Delmas D, Solary E, Latruffe N (2011) Resveratrol, a phytochemical inducer of multiple cell death pathways: apoptosis, autophagy and mitotic catastrophe. Curr Med Chem 18(8):1100–1121

Dixon RA, Paiva NL (1995) Stress-induced phenylpropanoid metabolism. Plant Cell 7:1085–1097

Docherty JJ, Fu MM, Stiffler BS et al (1999) Resveratrol inhibition of herpes simplex virus replication. Antivir Res 43:145–155

Docherty JJ, Fu MM, Hah JM et al (2005) Effect of resveratrol on herpes simplex virus vaginal infection in the mouse. Antivir Res 67:155–162

Docherty JJ, Sweet TJ, Bailey E et al (2006) Resveratrol inhibition of varicella-zoster virus replication in vitro. Antivir Res 72:171–177

Echeverri F, Torres F, Quinones W et al (1997) Danielone, a phytoalexin from papaya fruit. Phytochemistry 44(2):255–256

Elshaer M, Chen Y, Wang XJ et al (2018) Resveratrol: an overview of its anti-cancer mechanisms. Life Sci 207:340–349

Espinoza JL, Kurokawa Y, Takami A (2018) Rationale for assessing the therapeutic potential of resveratrol in hematological malignancies. Blood Rev S0268–960X(17):30124–30128

Fan Y, Chiu JF, Liu J (2018) Resveratrol induces autophagy-dependent apoptosis in HL-60 cells. BMC Cancer 18(1):581

Farris P, Krutmann J, Li YH (2013) Resveratrol: a unique antioxidant offering a multi-mechanistic approach for treating aging skin. J Drugs Dermatol 12(12):1389–1394

Favaron F, Lucchetta M et al (2009) The role of grape polyphenols on trans-resveratrol activity against *Botrytis cinerea* and of fungal laccase on the solubility of putative grape PR proteins. J Plant Pathol 91(3):579–588

Fogacci F, Tocci G, Presta V et al (2018) Effect of resveratrol on blood pressure: a systematic review and meta-analysis of randomized, controlled, clinical trials. Crit Rev Food Sci Nutr 58(2):1–14

Ganesan P, Choi DK (2016) Current application of phytocompound-based nanocosmeceuticals for beauty and skin therapy. Int J Nanomedicine 11:1987–2007

Glazebrook J, Ausbel FM (1994) Isolation of phytoalexin-deficient mutants of *Arabidopsis thaliana* and characterization of their interactions with bacterial pathogens. PNAS 91:8955–8959

Gregory D, Hargett D, Holmes D et al (2004) Efficient replication by herpes simplex virus type 1 involves activation of the IFkappaB kinase-IkappaB-p65 pathway. J Virol 78(24):13582–13590

Großkinsky DK, van der Graaff E, Roitsch T (2012) Phytoalexin transgenics in crop protection—fairy tale with a happy end? Plant Sci 195:54–70

Harborne JB (1999) The comparative biochemistry of phytoalexin induction in plants. Biochem Syst Ecol 27(4):335–367

Hart JH, Hillis WE (1974) Inhibition of wood-rotting fungi by stilbenes and other polyphenols in *Eucalyptus sideroxylon*. Phytopathology 64:939–948

Hasima N, Ozpolat B (2014) Regulation of autophagy by polyphenolic compounds as a potential therapeutic strategy for cancer. Cell Death Dis 5:e1509. https://doi.org/10.1038/cddis.2014.467

Hausenblas HA, Schoulda JA, Smoliga JM et al (2014) Resveratrol treatment as an adjunct to pharmacological management in type 2 diabetes mellitus-systematic review and meta-analysis. Mol Nutr Food Res 59(1):147–159

He X, Wang Y, Zhu J et al (2011) Resveratrol enhances the anti-tumor activity of the mTOR inhibitor rapamycin in multiple breast cancer cell lines mainly by suppressing rapamycin-induced AKT signaling. Cancer Lett 301(2):168–176

Huminiecki L, Horbańczuk J (2018) The functional genomic studies of resveratrol in respect to its anti-cancer effects. Biotechnol Adv 36 (6):1699–1708

Ingólfsson HI, Thakur P, Herold KF et al (2014) Phytochemicals perturb membranes and promiscuously alter protein function. ACS Chem Biol 9:1788–1798

Jeandet P, Douillet-Breuil AC, Bessis R et al (2002) Phytoalexins from the Vitaceae: biosynthesis, phytoalexin gene expression in transgenic plants, antifungal activity, and metabolism. J Agric Food Chem 50:2731–2741

Jiang J (2008) Design, synthesis and spectroscopic studies of resveratrol aliphatic acid ligands of human serum albumin. Bioorg Med Chem 16:6406–6414

Kou X, Chen N (2017) Resveratrol as a natural autophagy regulator for prevention and treatment of Alzheimer's disease. Nutrients 9(9): E927. https://doi.org/10.3390/nu9090927

Lamuela-Raventos RM, Romero-Perez AI, Waterhouse AL et al (1995) Direct HPLC analysis of *cis*- and *trans*-resveratrol and piceid isomers in Spanish red *Vitis vinifera* wines. J Agric Food Chem 43 (2):281–283

Lange KW, Li S (2018) Resveratrol, pterostilbene, and dementia. Biofactors 44(1):83–90

Lee RHC, Lee MHH, Wu CYC et al (2018) Cerebral ischemia and neuroregeneration. Neural Regen Res 13(3):373–385

Liao PC, Ng LT, Lin LT et al (2010) Resveratrol arrests cell cycle and induces apoptosis in human hepatocellular carcinoma Huh-7 cells. J Med Food 13(6):1

Madhav NV, Shakya AK, Shakya P et al (2009) Orotransmucosal drug delivery systems: a review. J Control Release 140(1):2–11

Matos RS, Baroncini LA, Précoma LB et al (2012) Resveratrol causes antiatherogenic effects in an animal model of atherosclerosis. Arq Bras Cardiol 98(2):136–142

Mattetti A, Risuleo G (2014) Apoptosis: a mode of cell death. Biochem Mol Biol 2:34–39

Mattivi F, Reniero F, Korhammer S (1995) Isolation, characterization, and evolution in red wine vinification of resveratrol monomers. J Agric Food Chem 43(7):1820–1823

McCubrey JA, Lertpiriyapong K, Steelman LS et al (2017) Effects of resveratrol, curcumin, berberine and other nutraceuticals on aging, cancer development, cancer stem cells and micro-RNAs. Aging (Albany NY) 9(6):1477–1536

Meyer J, Murray SL, Berger DK (2016) Signals that stop the rot: regulation of secondary metabolite defences in cereals. Physiol Mol Plant Pathol 94:156–166

Molnár J, Engi H, Hohmann J et al (2010) Reversal of multidrug resistance by natural substances from plants. Curr Top Med Chem 10(17):1757–1768

Oliveira MDM, Varanda CMR, Félix MRF (2016) Induced resistance during the interaction pathogen x plant and the use of resistance inducers. Phytochem Lett 15:152–158

Oliveira ALB, Monteiro VVS, Navegantes-Lima KC et al (2017) Resveratrol role in autoimmune disease-AMini-review. Nutrients 19: E1306

Owen HC, Appiah S, Hasan N et al (2017) Phytochemical modulation of apoptosis and autophagy: strategies to overcome chemoresistance in leukemic stem cells in the bone marrow microenvironment. Int Rev Neurobiol 135:249–278

Palamara AT, Nencioni L, Aquilano K et al (2005) Inhibition of influenza A virus replication by resveratrol. J Infect Dis 191:1719–1729

Pallauf K, Rimbach G, Rupp PM et al (2016) Resveratrol and lifespan in model organisms. Curr Med Chem 23(41):4639–4680

Perez-Vizcaino F, Fraga CG (2018) Research trends in flavonoids and health. Arch Biochem Biophys 646:107–112

Popescu M, Bogdan C, Pintea A et al (2018) Drug Des Devel Ther 12:1985–1996

Prokop J, Abrman P, Seligson AL et al (2006) Resveratrol and its glycon piceid are stable polyphenols. J Med Food 9(1):11–14

Ricci F, Berardi V, Risuleo G (2009) Differential cytotoxicity of MEX: a component of Neem oil whose action is exerted at the cell membrane level. Molecules 14:122–132

Risuleo G (2016) Resveratrol: multiple activities on the biological functionality of the cell. In: Gupta R (ed) Nutraceuticals: efficacy, safety and toxicity. Elsevier/Academic Press, Amsterdam, pp 453–464

Sanchez Maldonado AF, Schieber A, Gänzle MG (2015) Plant defence mechanisms and enzymatic transformation products and their potential applications in food preservation: advantages and limitations. Trends Food Sci Technol 46(1):49–59

Santos Silva M, Barbosa Monteiro Arraes F, de Araújo Campos M et al (2018) Potential biotechnological assets related to plant immunity modulation applicable in engineering disease-resistant crops. Plant Sci 270:72–84

Sarubbo F, Moranta D, Asensio VJ et al (2017) Effects of resveratrol and other polyphenols on the most common brain age-related diseases. Curr Med Chem 24(38):4245–4266

Schröder J (1999) Probing plant polyketide biosynthesis. Nat Struct Biol 6:714–716

Schröder G, Brown JW, Schröder J et al (1988) Molecular analysis of resveratrol synthase. cDNA, genomic clones and relationship with chalcone synthase. Eur J Biochem 172(1):161–169

Shalini S, Dorstyn L, Dawar S et al (2015) Old, new and emerging functions of caspases. Cell Death Differ 22:526–539

Sharan S, Nagar S (2013) Pulmonary metabolism of resveratrol: in vitro and in vivo evidence. Drug Metab Dispos 41(5):1163–1169

Shih C-H, Chu IK, Yip WK et al (2006) Differential expression of two flavonoid 3′-hydroxylase cDNAs involved in biosynthesis of anthocyanin pigments and 3-deoxyanthocyanidin phytoalexins in Sorghum. Plant Cell Physiol 47(10):1412–1419

Signorelli P, Ghidoni R (2005) Resveratrol as an anticancer nutrient: molecular basis, open questions and promises. J Nutr Biochem 16 (8):449–466

Singh NP, Singh UP, Hegde VL et al (2011) Resveratrol (*trans*-3,5,4-′-trihydroxystilbene) suppresses EL4 tumor growth by induction of apoptosis involving reciprocal regulation of SIRT1 and NF-κB. Mol Nutr Food Res 55(8):1207–1218

Soleas GJ, Diamandis EP, Goldberg DM (1997) Resveratrol: a molecule whose time has come? And gone? Clin Biochem 30:91–113

Thomma BP, Nelissen I, Eggermont K et al (1999) Deficiency in phytoalexin production causes enhanced susceptibility of *Arabidopsis thaliana* to the fungus *Alternaria brassicicola*. Plant J 19(2):163–171

Tooze J (ed) (1981) Molecular biology of tumor viruses: DNA tumor viruses part 2. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY

Treviño-Saldaña N, García-Rivas G (2017) Regulation of sirtuin-mediated protein deacetylation by cardioprotective phytochemicals. Oxidative Med Cell Longev:1750306. https://doi.org/10.1155/2017/1750306

Tsai HY, Ho CT, Chen YK (2017) Biological actions and molecular effects of resveratrol, pterostilbene, and 3′-hydroxypterostilbene. J Food Drug Anal 25(1):134–147

Turner RS, Thomas RG, Craft S et al (2015) A randomized, double-blind, placebo-controlled trial of resveratrol for Alzheimer disease. Neurology 85(16):1383–1391

Ullah MF, Bhat SH, Hussain E et al (2015) Ascorbic acid in cancer chemoprevention: translational perspectives and efficacy. Curr Drug Targets 13(14):1757–1771

Vang O (2015) Resveratrol: challenges in analyzing its biological effects. Ann N Y Acad Sci 1348:161–170

Wahl D, Bernier M, Simpson SJ et al (2018) Future directions of resveratrol research. Nutr Health Aging 4(4):287–290

Walle T (2011) Bioavailability of resveratrol. Ann N Y Acad Sci 1215:9–15

Wang Z, Li W, Meng X, Jia B (2012) Resveratrol induces gastric cancer cell apoptosis via reactive oxygen species, but independent of sirtuin1. Clin Exp Pharmacol Physiol 39(3):227–232

Whitlock NC, Baek SJ (2012) The anticancer effects of resveratrol: modulation of transcription factors. Nutr Cancer 64(4):493–502

Yang T, Li S, Zhang X et al (2015) Resveratrol, sirtuins, and viruses. J Rev Med Virol 25(6):431–445

Zbikowska HM, Olas B, Wachowicz B et al (1999) Response of blood platelets to resveratrol. Platelets 10:247–252

Zhai T, Li S, Hu W et al (2018) Potential micronutrients and phytochemicals against the pathogenesis of chronic obstructive pulmonary disease and lung cancer. Nutrients 10(7):E813. https://doi.org/10.3390/nu10070813

Zhang YH, Guo JG, Guo ZH et al (2011) Involvement of p38-p53 signal pathway in resveratrol-induced apoptosis in MCF-7 cells. Yao Xue Xue Bao 46(11):1332–1337

Zhang W, Wang X, Chen T (2012) Resveratrol induces apoptosis via a Bak-mediated intrinsic pathway in human lung adenocarcinoma cells. Cell Signal 24(5):1037–1046

Zhu X, Wu C, Qiu S et al (2017) Effects of resveratrol on glucose controland insulin sensitivity in subjects with type 2 diabetes: systematic review and meta-analysis. Nutr Metab (Lond) 14:60. https://doi.org/10.1186/s12986-017-0217-z

Egg Shell Membranes for Veterinary Uses

Dan DuBourdieu

Abstract

Eggshell membrane (ESM) is a relatively new ingredient on the list of ingredients that have been used to mitigate the effects of joint inflammation in animals. Eggshells contain a thin membrane that coats the inner surface of the shell that is comprised of a wide number of proteins, amino acids, collagen-like proteins, enzymes, and glycosaminoglycans. When ESM is consumed by an animal, the compounds found in ESM will play a role in maintaining joint health. However, ESM also has antimicrobial effects. The antimicrobial effects of ESM can be utilized in animal feeds as an alternative to antibiotics for growth promotion in production animals. Thus, ESM represents a new ingredient for veterinarian use for not only fighting inflammation in joints but also as an alternative to antibiotics for growth promotion activities.

Keywords

Veterinary nutraceuticals · Eggshell membrane

1 Introduction

Animals face many of the same basic issues that humans face when it comes to health. How to treat these health issues is the constant challenge to researchers and manufactures alike. Among the health issues are various joint problems that occur as the animal ages. There are now numerous nutraceutical products on the market that have emerged from the human side to help treat or prevent joint issue in animals. Another such item is ESM. Eggs have been used by humans since time began, but yet, still new ways are being found to exploit the egg. Among the new uses of eggs is the ESM found on the inside of the egg. Besides uses for fighting joint inflammation, a new veterinary use includes as an alternative to antibiotics and for growth promotion in production animals.

2 Eggs and Eggshell Waste

The common bird egg content of yolk and white has been used by humans as a source of food for a very long time. But the shell is typically discarded, and this means a lot of eggshell waste. The US food industry generates more than 24 billion eggshells every year. This generates more than 150,000 tons of shell waste a year (Hecht 1999). However, many landfills are unwilling to take the waste because the shells and the attached membrane attract vermin. Therefore getting rid of the eggshells has presented an issue for disposal. About 25% of waste eggshells are used as fertilizer. This is because eggshells contain calcium that raises, or neutralizes, the pH level of overly acidic soil. But the calcium of the shell can also be used in calcium supplements, food thickeners, and other uses (Yoo et al. 2009). As such, another 25% is used in animal feed ingredients, but still 25% is discarded in municipal dumps, and the rest is used in other ways (Daengprok et al. 2002). But this situation makes for new opportunity to take a waste product and turn it into something useful.

3 Structure of Eggshells and Membranes

From a biological standpoint, the eggshell structure is important for some obvious reasons (Hunton 2005). The shell forms an embryonic chamber for the developing chick, providing mechanical protection and a controlled gas exchange medium. Eggshell consists almost entirely (95–97%) of calcium carbonate ($CaCO_3$) with some magnesium carbonate ($MgCO_3$) present as a columnar structure that creates as many as 17,000 narrow transverse pores between palisade columns of $CaCO_3$. These pores allow air and water

D. DuBourdieu (✉)
Vets Plus Inc., Menomonie, WI, USA
e-mail: dand@vets-plus.com

R. C. Gupta et al. (eds.), *Nutraceuticals in Veterinary Medicine*, https://doi.org/10.1007/978-3-030-04624-8_16

vapor to pass in and out of the egg (Rovenský et al. 2003). Secondly the shell is a container for the market egg, providing protection of the contents and a unique package for a valuable food. But of current interest are the eggshell membranes that coat the inside of the shell (Fig. 1).

4 Eggshell Membranes

There are actually several membranes found in eggs (Fig. 2). The inner shell membrane adheres to the egg whites, while the outer shell membrane adheres on the shell. The inner shell membrane and the outer shell membrane adhere to each other except at the air space. The yolk also is surrounded by a membrane. These membranes are formed before the shell during development since calcium is required to form on the outer membrane.

Fig. 1 Picture of eggshell and eggshell membrane

5 Separation Methods of ESM

Separating ESM from shells by manual hand techniques obviously can be pretty laborious and time-consuming. From a commercial standpoint to generate enough ESM to be useful, there are various ways in which the membrane of an eggshell is separated from the shell. These include chemical, mechanical, steam, and vacuum processes (Vlad 2009; Yoo et al. 2009; MacNeil 2001; Adams 2006; New 2013). The goal of these methods is to use as little degradative force as possible to the membrane. The methods that are of a mechanical nature may be best for maintaining the biological activity of the membrane. Typically, the shell is crushed in some suitable manner such as using a roller apparatus, and then the crushed shells with membranes still attached to shell fragments are placed in a tank of water or other solvent. Stirring by various methods occur to actually separate the membranes from the shells. The shells sink to the bottom of the tank, and membranes float up to achieve separation and subsequent removal. There are many variations of the techniques to separate the membranes from the shells. But once the isolated membrane is dried to produce a powder, it can then be utilized in veterinary products.

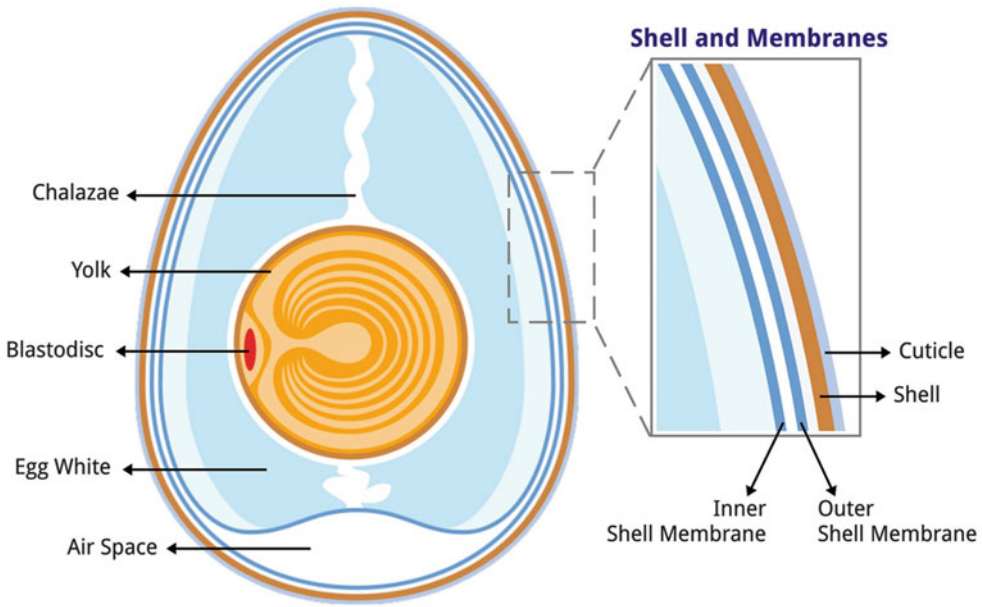


Fig. 2 Structure of eggshell and membranes

6 Components of ESM

The inner and outer shell membranes are the first layers of extracellular matrix covering the egg (yolk and whites) (Creger et al. 1976). The separate layers of the eggshell have been analyzed and found to contain several types of collagen (types X, I, V). The outer membrane is predominantly made of type I collagen, whereas the inner membrane consists mainly of type V collagen (Wong et al. 1984). In addition, type X collagen has been reported to occur in both of these membrane structures (Arias et al. 1991). ESM amino acid profiles have also shown high concentrations of arginine, glutamic acid, histidine, cystine, and proline (Britton and Hale 1977). Additionally, a number of collagen-like proteins (including hydroxyproline, hydroxylysine, desmosine, and isodesmosine) are primary structural components of the membranes (Baker and Balch 1962; Candlish and Scougall 1969; Starcher 1980). The collagens and collagen-like proteins in ESM are of interest for skin care products.

Eggshell membranes have also been shown to contain glycosaminoglycans, such as dermatan sulfate and chondroitin sulfate, and sulfated glycoproteins including hexosamines, such as glucosamine (Picard 1973). ESM has a high content of protein and moderate quantities of glucosamine (up to 1% by dry weight), chondroitin sulfate (up to 2%), hyaluronic acid (up to 2%), and collagen (type I, up to 25%). The glycosaminoglycans (GAGs) are of high interest, as they play key roles in connective tissue (Kjellen and Lindahl 1991). Glucosamine, hyaluronic acid, and chondroitin sulfate are important GAGs in ESM (Nakano 2003; Long et al. 2004). Other components identified in eggshell membranes are hyaluronic acid (Long et al. 2004), sialic acid (Nakano 2003), desmosine and isodesmosine (Starcher 1980), ovotransferrin (Gautron et al. 2001), and enzymes such as lysyl oxidase (Akagawa 1999), lysozyme (Hincke et al. 2000), and β-*N*-acetylglucosaminidase (Ahlborn 2006). With the high protein and enzyme content and naturally occurring GAGs in ESM, scientists and nutritional supplement companies have speculated that it could present a viable alternative to traditional joint disorder and osteoarthritis treatments.

More than 300 proteins are found in ESM. These include fibrous structural proteins made up of collagens and keratins that are generally resistant to conventional gastric proteases. Also abundant are lysozyme, ovotransferrin, ovocleidin, clusterin, ovokeratin, ovodefensin, and many more (Makkar 2016). Many of these proteins and peptides have antimicrobial, antioxidant, and immunomodulatory properties. Among those include lysozyme, ovotransferrin, ovalbumin, globulins, ovomucins, and defensins (Miksík et al. 2007). Many of these proteins are functionally similar to some milk proteins which confer postnatal protection to newborns, help maturation of gut, and shape their microbiome (Lawrence and Pane 2007; Rose and Hincke 2009). Antimicrobial peptides not only provide protection against a wide range of microbes including bacteria and fungi but also can function as adjuvants enhancing immunity against foreign antigens (Brown and Hancock 2006). It should be noted that the membrane components have not yet been wholly characterized; there are likely a number of compounds that have yet to be identified in the membrane. Some of these yet undetected elements may contribute to benefits or improvements seen in joint health or in other uses. Furthermore in view of the need for alternatives to antibiotics in meat animal production (Seal et al. 2013; Thacker 2013), exploring the potential of egg by-products to improve immunity and disease resistance in production animals also makes sense. Factors present in the ESM appear to help modulate immunity and performance of chickens when provided as posthatch nutrient supplements (Makkar 2016).

7 Efficacy (in Humans) for Treating Joint Inflammation

The discovery of eggshell membrane as a natural source of combined collagen, glucosamine, chondroitin, and hyaluronic acid has prompted the evaluation of this material as a potential treatment for joint and connective tissue pain. There is a growing interest in ESM supplementation as a treatment for joint pain, as it does not present the side effects of conventional treatments such as from nonsteroidal anti-inflammatory agents. Clinical reports (Ruff et al. 2009a, b) investigated the timing and effectiveness of ESM supplementation in joint and connective tissue along with osteoarthritis in human patients experiencing severe pain and limited range of motion. These patients received daily 500 mg doses of oral ESM for 4–8 weeks. Rapid (7–10 days) and continuous effects were seen in terms of reduced pain and stiffness, as well as improved flexibility. In these investigations, there were no reports of adverse effects with supplementation. Other reports (Ruff et al. 2009a, b) have shown positive results of 500 mg ESM taken once daily, significantly reduced both joint pain and stiffness compared to placebo at 10, 30, and 60 days. Further reports also have shown ESM to help with exercise-induced joint issues and pain or from osteoarthritis (Ruff et al. 2018; Danesch et al. 2014).

8 Efficacy (in Animals) for Treating Joint Inflammation and Modes of Action

The use of ESM for treating joint inflammation in humans has prompted the same use for animals. Studies in animals have helped elucidate the modes of action of ESM. ESM has been investigated for anti-arthritis activity in rats (Sim et al. 2015).

Arthritis was induced by monosodium iodoacetate and treated by administering ESM. Nitric oxide and cytokines including IL-1β, IL-6, PGE_2, TIMP-1, LTB4, and hs-CRP in serum were decreased in comparison with the controls. The cartilage of patella volume increased significantly. ESM treatment suppressed cartilage deformation and preserved cartilage volume compared to untreated controls. At least part of this, chondroprotection may result from the anti-inflammatory properties of ESM. Serum levels of matrix metalloproteinases (MMP-2 and MMP-9) known to degrade cartilage were also substantially reduced. Other animal studies have also seen a reduction of pro-inflammatory cytokines when ESM has been administered (Ruff and DeVore 2014).

Additional studies (Ruff et al. 2015) have shown that nuclear factor kappa-light-chain-enhancer of activated B-cells (NF-κB) plays role in how ESM works in the body. NF-κB is a signaling protein found in the cytoplasm of nearly all human and animal cell types and is a primary regulator of immune function. ESM hydrolysates significantly activated NF-κB, and increased NF-κB activity might translate into the in vivo efficacy that has been observed with ESM via an "oral tolerance" mechanism. Oral tolerance refers to the phenomenon of a reduced peripheral immune response (tolerance) that results from the repeated exposure of the mucosal immune system in the gut to ingested protein antigens. Oral tolerance to immunogenic peptides that are repeatedly ingested is believed to result from immune surveillance within the gut-associated lymphoid tissue as a way for the body to prevent an inappropriate or unnecessary immune response to proteins normally consumed in the diet. Immune tolerance to cartilage components, particularly type II collagen, from oral supplementation with ESM (which contains types I, V, and X collagen), is a possible explanation for observed reduction in C-telopeptide type II collagen (CTX-II) formation observed with ESM supplementation. It should be noted that CTX-II is a marker of collagen degradation observed in osteoarthritis. Further research has also shown (Wedekind et al. 2017) that oral supplementation with ESM substantially suppressed swelling due to inflammation and markedly lessened cartilage damage, pannus formation, and periarticular bone resorption histologically in rats. In addition, ESM can promote nerve regeneration, which can stem from osteoarthritis (Farjah 2013).

ESM efficacy for joint use has been investigated in other animals besides rats. These animals include cranes (Bauer et al. 2014), camels (Dierenfeld et al. 2014), and horses (Wedekind et al. 2015). Similarly, a chondroprotective effect from ESM treatment in dogs is also seen with naturally occurring joint disease. There were no serious adverse events reported during the study, and subject dog owners reported that ESM was well tolerated by their pet (Ruff et al. 2016). Supplementation with ESM, ~13.5 mg/kg (6 mg/lb) taken once daily, significantly reduced joint pain and improved joint function rapidly as assessed by the Canine Brief Pain Inventory questionnaire. ESM demonstrated a lasting improvement in joint pain leading to an improved quality of life at 6 weeks. Moreover, a profound chondroprotective effect was demonstrated following 6 weeks of supplementation with ESM.

9 ESM as an Alternative to Antibiotics for Growth Promotion in Production Animals

The animal studies previously mention have showed significant anti-arthritis activity when using ESM. However, there are additional uses for ESM that are starting to be recognized. Chief among these new uses of ESM is an alternative to antibiotics used for growth promotion in production animals. There is increasing public and scientific concern about the use of antibiotics as feed additives in animal production. This concern is fueled by the emergence of antibiotic resistance in many human pathogenic bacteria, the release of contaminating residues into the environment (water, soil, etc.), and the risk that growth-promoting antibiotic residues may occur in foods of animal origin. This concern has resulted in the ban of the use of antibiotics as growth promoters in production animals in Europe in 2006. In 2015, the FDA approved a new Veterinary Feed Directive which is an updated guideline that gives instructions to pharmaceutical companies, veterinarians, and producers about how to administer necessary drugs through the animal's feed and water. The FDA has asked drug companies to voluntarily edit its labels to exclude growth promotion as an indication for antibiotic usage. FDA regulations on off-label use prohibit using a drug off-label for non-therapeutic purposes, which would make using the relabeled drug for growth enhancement illegal. The new guidelines took effect on 1 January 2017 (FDA 2017). This has prompted the search for alternatives to antibiotics. Products such as probiotics, prebiotics, bacteriophages, fecal extracts, yolk antibodies, and organic acids have been used to satisfy this need. However, another surprising item to add to the list is eggshell membranes. Research (Makkar 2016) has shown that feeding 0.5% levels of eggshell membrane not only improved the body weight of chickens but also modulated immunoglobulin parameters pro- and anti-inflammatory genes and reduced serum corticosterone levels under endotoxin challenge conditions. In view of the need for alternatives to antibiotics in meat animal production, exploring the potential of egg by-products as nutritional modulator of immunity during posthatch period appears logical.

10 Nutritional Effects of Eggshell Membrane Supplements on Chicken Performance and Immunity

ESM contain a variety of proteins and peptides which help in the development of embryo and provide protection to it. Many of the peptides and proteins associated with ESM have antimicrobial, immune-modulatory, and adjuvant properties. In view of the need for alternatives to antibiotics in meat animal production (Seal et al. 2013; Thacker 2013), exploring the potential of egg by-products to improve immunity and disease resistance in poultry or other production animals makes sense. It was hypothesized (Makkar 2016) that the membrane by-products from egg, provided as posthatch nutritional supplements to chickens, may improve their performance and immunity. To explore its effect, three groups of broiler chicks were fed with feed containing 0%, 0.2%, and 0.4% ESM from day 1 posthatch through 14 days and regular feed thereafter. The chickens in the ESM treated groups showed a statistically significant increase in body weight with no impact on relative organ weights. Compared with controls, the white blood cell and lymphocyte percentage increased in chickens fed 0.4% ESM, whereas the monocyte percentage decreased at both levels of ESM. Except for the serum protein which increased in ESM fed birds, no other metabolic clinical chemistry variables showed any significant change. Both IgM and IgG(Y) levels were elevated, and corticosterone levels reduced in chickens fed ESM supplemented diets. These results suggested that ESM supplements during the early phases of growth may improve immunity and stress variables and enhance their growth performance without any detrimental effect on other physiological parameters.

These studies also showed that the blood corticosterone levels were reduced in chickens fed ESM diets, suggesting a lower levels of stress in these birds, although the mechanism for its decrease is not understood. Stress and inflammation can also cause a loss of body weight and present other signs of sickness such as lethargy that was not observed in ESM fed birds. Low levels of stress can also imply better feeding behavior (Bunnett 2005) that would contribute to increase in BW. IgM is an antibody that fights infection, prevents inflammation, reacts with a variety of foreign antigens including pathogen associated molecules, activates complement, foreruns, and stimulates IgG response. Similarly, an increase in the levels of IgG in ESM fed chickens also suggests a modulation of adaptive immune response. These antibodies play vital roles for protection against a variety of microbial pathogens. Whether the antibody response to ESM is transient or it establishes a lasting resistance to certain infection needs to be verified. The ESM supplement appears to have beneficial effect in chickens while it reduces stress and modulates immunity without sacrificing the growth potential of the birds.

Additional studies were done (Makkar 2016) to determine if ESM could decrease the harmful effects of liposaccharide (LPS) in older chickens. LPS control birds and ESM plus LPS fed birds at 5 weeks of age were used in the study. The ESM was also treated with ethanol to inactivate bacterial factors. The effects of LPS were evaluated at 4 and 24 h of treatment. ESM supplement caused a numerical but nonsignificant weight gain and consistently decreased the blood corticosterone levels. LPS caused a significant loss in body weight at 24 h following its administration, but the ESM supplemented birds showed significantly less body weight loss compared with the control fed birds. The white blood cell, heterophil/lymphocyte ratio, and the levels of IgG were low in chickens fed ESM supplement diet compared with the control fed group. LPS challenge increased the expression of pro-inflammatory cytokine gene IL-6, but the ESM fed birds showed its effect curtailed also, favored the upregulation of some anti-inflammatory genes compared with control fed chickens. Posthatch supplementation of ESM appears to modulate immunity and increase their resistance to endotoxin. The data suggested that ESM supplementation of feed is beneficial to posthatch poultry, and it curtails the harming effects of LPS against common infections. In conclusion, these results show that an ESM supplement can be a sustainable feed additive to improve immunity and health physiology of poultry. Future use of ESM in other production animals as an alternative to antibiotics will be of obvious interest.

11 Other Veterinary Uses of ESM

While ESM has been shown to have efficacy in joint inflammation and as an antimicrobial agent, there are even further uses. This includes uses in human functional cosmetics as an antiaging ingredient to help prevent wrinkle formation since ESM may decrease collagenase activity. The cosmetic effects of ESM such as skin whitening, wound healing, and UV protection appear to help human skin function (Kimoon et al. 2012). ESM may also help retain skin moisture thereby improving the protective ability of the skin. As such, it may be possible that ESM may find new as a material for skin repair for veterinary purposes as well.

12 Conclusion Remarks and Future Directions

ESM, like many nutraceuticals, is one of the newer ingredients on the market that can have multiple uses. ESM was initially found to have efficacy in joint products but has now been found to have efficacy in growth promotion in production animals. ESM supplements during the early phases of growth may improve immunity and stress variables

while enhancing growth performance without any detrimental effect on other physiological parameters. What additional veterinary uses ESM has in the future remains to be seen. But it is clear that ESM will be playing a bigger role as an alternative to antibiotics and as a growth promoter while continuing to be used for fighting inflammation in joints.

References

Adams RG (2006) Eggshell membrane separation method. US Patent 7,017,277, United States: ESM Technologies, LLC

Ahlborn GJ (2006) Identification of eggshell membrane proteins and purification of ovotransferrin and β-NAGase from hen egg white. Protein J 25(1):71–81

Akagawa M (1999) Lysyl oxidase coupled with catalase in egg shell membrane. Biochim Biophys Acta 1434(1):151–160

Arias JL, Fernandez MS, Dennis JE et al (1991) The fabrication and collagenous substructure of the eggshell membrane in the isthmus of the hen oviduct. Matrix 11(5):313–320

Baker JR, Balch DA (1962) A study of the organic material of hen's-egg shell. Biochem J 82:352–361

Bauer KL, Dierenfeld ES, Hartup BK (2014) Evaluation of a nutraceutical joint supplement in cranes. Proc N Am Crane Workshop 12:27–32

Britton WN, Hale KK (1977) Amino acid analysis of shell membranes of young and old hens varying in shell quality. Poult Sci 56:865–871

Brown KL, Hancock RE (2006) Cationic host defense (antimicrobial) peptides. Curr Opin Immunol 18:24–30

Bunnett NW (2005) The stressed gut: contributions of intestinal stress peptides to inflammation and motility. Proc Natl Acad Sci U S A 102:7409–7410

Candlish JK, Scougall RK (1969) L-5-hydroxylysine as a constituent of the shell membranes of the hen's egg. Int J Protein Res 1(4):299–302

Creger CR, Phillips H, Scott IJ (1976) Formation of an eggshell. Poult Sci 55:1717–1723

Daengprok W, Garnjanagoonchorn W, Mine Y (2002) Fermented pork sausage fortified with commercial or hen eggshell calcium lactate. Meat Sci 62:199–204

Danesch U, Seybold M, Rittinghausen R et al (2014) NEM® brand eggshell membrane effective in the treatment of pain associated with knee and hip osteoarthritis: results from a six-center, open-label German Clinical Study. J Arthritis 3(3):136

Dierenfeld ES, Baum D, Hampe L et al (2014) Evaluation of a nutraceutical joint supplement in camels. Am Holist Vet Med Assoc J 39:59–66

Farjah G (2013) Using eggshell membrane as nerve guide channels in peripheral nerve regeneration. Iran J Basic Med Sci 16(8):901–905

FDA (2017) Center for veterinary medicine. FDA's strategy on antimicrobial resistance – questions and answers. Guidance for industry. Accessed March 14, 2017

Gautron J, Hincke MT, Panheleux M et al (2001) Ovotransferrin is a matrix protein of the hen eggshell membranes and basal calcified layer. Connect Tissue Res 42(4):255–267

Hecht J (1999) Eggshells break into collagen market. New Sci 161:6

Hincke MT, Gautron J, Panheleux M et al (2000) Identification and localization of lysozyme as a component of eggshell membranes and eggshell matrix. Matrix Biol 19(5):443–453

Hunton P (2005) Research on eggshell structure and quality: an historical overview. Rev Bras Cienc Avic 7(2):67–71

Kimoon P, Jinhee Y, Youngjae S (2012) Effects of egg shell membrane hydrolysates on skin whitening, wound healing, and UV-protection. Korean J Food Sci Anim Resour 32(3):308–315

Kjellen L, Lindahl U (1991) Proteoglycans: structures and interactions. Annu Rev Biochem 60:443–475

Lawrence RM, Pane CA (2007) Human breast milk: current concepts of immunology and infectious diseases. Curr Probl Pediatr Adolesc Health Care 37:7–36

Long FD, Adams RG, De Vore DP (2004) Preparation of hyaluronic acid from eggshell membrane. 60/453,891(US 2004/0180851 A1)

MacNeil JH (2001) Method and apparatus for separating a protein membrane and shell material in waste egg shells. United States Patent (Foundation TPSR ed, vol 6,176,376). United States: The Penn State Research Foundation

Makkar SK (2016) Proteomic characterization of egg shell membranes and their effect on poultry physiology and immunity. PhD Thesis. Punjabi University, India

Miksík I, Eckhardt A, Sedláková P et al (2007) Proteins of insoluble matrix of avian (*Gallus gallus*) eggshell. Connect Tissue Res 48:1–8

Nakano T (2003) Chemical composition of chicken eggshell and shell membranes. Poult Sci 82:510–514

New L (2013) Eggshell membrane separation process. US Patent 8,448,884 B2

Picard J (1973) Sulfated glycoproteins from egg shell membranes and hen oviduct. Isolation and characterization of sulfated glycopeptides. Biochim Biophys Acta 320:427–441

Rose ML, Hincke MT (2009) Protein constituents of the eggshell: eggshell-specific matrix proteins. Cell Mol Life Sci 66:2707–2719

Rovenský J, Stancíková M, Masaryk P et al (2003) Eggshell calcium in the prevention and treatment of osteoporosis. Int J Clin Pharmacol Res 23:83–92

Ruff KJ, DeVore DP (2014) Reduction of pro-inflammatory cytokines in rats following 7-day oral supplementation with a proprietary eggshell membrane-derived product. Mod Res Inflamm 3(1):19–25

Ruff KJ, DeVore DP, Leu MD et al (2009a) Eggshell membrane: a possible new natural therapeutic for joint and connective tissue disorders. Results from two open-label human clinical studies. Clin Interv Aging 4:235–240

Ruff KJ, Winkler A, Jackson WR et al (2009b) Eggshell membrane in the treatment of pain and stiffness from osteoarthritis of the knee: a randomized, multicenter, double-blind, placebo-controlled clinical study. Clin Rheumatol 28(8):907–914

Ruff KJ, Durham PL, O'Reilly A et al (2015) Eggshell membrane hydrolyzates activate NF-κB in vitro: possible implications for in vivo efficacy. J Inflamm Res 8:49–57

Ruff KJ, Kopp KJ, Von Behrens P et al (2016) Effectiveness of NEM® brand eggshell membrane in the treatment of suboptimal joint function in dogs: a multi-center, randomized, double-blind, placebo-controlled study. Vet Med Res Rep 7:113–121

Ruff KJ, Morrison D, Duncan SA et al (2018) Beneficial effects of natural eggshell membrane versus placebo in exercise-induced joint pain, stiffness, and cartilage turnover in healthy, postmenopausal women. Clin Interv Aging 13:285–295

Seal BS, Lillehoj HS, Donovan DM et al (2013) Alternatives to antibiotics: a symposium on the challenges and solutions for animal production. Anim Health Res Rev 14:78–87

Sim BY, Bak JW, Lee HL et al (2015) Effects of natural eggshell membrane (NEM) on monosodium iodoacetate-induced arthritis in rats. J Nutr Health 48(4):310–318

Starcher BC (1980) The presence of desmosine and isodesmosine in eggshell membrane protein. Connect Tissue Res 8(1):53–55

Thacker PA (2013) Alternatives to antibiotics as growth promoters for use in swine production: a review. J Anim Sci Biotechnol 4:35

Vlad V (2009) Eggshell membrane separation method. US Patent, vol 7534909. United States: Biova, LLC

Wedekind KJ, Coverdale JA, Hampton TR et al (2015) Efficacy of an equine joint supplement, and the synergistic effect of its active ingredients (chelated trace minerals and natural eggshell membrane), as demonstrated in equine, swine, and an osteoarthritis rat model. Open Access Anim Physiol 7:13–27

Wedekind KJ, Ruff KJ, Atwell CA et al (2017) Beneficial effects of natural eggshell membrane (NEM) on multiple indices of arthritis in collagen-induced arthritic rats. Mod Rheumatol 27(5):838–848

Wong M, Hendrix MJ, von der Mark K et al (1984) Collagen in the egg shell membranes of the hen. Dev Biol 104(1):28–36

Yoo S, Kokoszka J, Zou P et al (2009) Utilization of calcium carbonate particles from eggshell waste as coating pigments for ink-jet printing paper [electronic resource]. Bioresour Technol 100:6416–6421

Egg Derived Ovotransferrins and Lactoferrins

Jamil Talukder

Abstract

Proteins and minerals are integral parts of the biological system that harmonize and sustain life. Minerals such as iron binds with protein and is called transferrin and it plays a crucial role in animals. Ovotransferrin (OVTF) and lactoferrin (LF) are avid iron-binding glycoproteins with a molecular weight of 76 and 80 kDa, respectively. They belong to the transferrin family, have a wide range of biofunctions, and are a major component of the mammalian innate immune system. OVTF is present in egg whites, and LF is abundantly present in the colostrum, milk of different species (humans, bovines, and mice), neutrophils, and other body secretions. OVTF shows about a 50% sequence homology with mammalian serum transferrin and LF. The protective effects of OVTF and LF range from direct antimicrobial activities against a large panel of microorganisms to anti-inflammatory and anticancer activities. These extensive activities are made possible by mechanisms of action that utilize not only the capacity of OVTF and LF to bind iron but also the interactions with molecular and cellular components of both host and pathogen. This chapter aims to summarize the current understanding, though incomplete, of the many ways that LF and OVTF influence the complex immune machinery and the known and putative mechanisms that may explain biological functions.

Keywords

Iron-binding protein · Ovalbumin · Milk protein · Ovotransferrin · Lactoferrin · Metalloproteins · Antimicrobial peptides

J. Talukder (✉)
Vets Plus, Inc., Menomonie, WI, USA
e-mail: Jamilt@vets-plus.com

1 Introduction

Since the discovery of iron-binding proteins, OVTF and LF are thought to be the most polyvalent found in vertebrates. Vertebrates have a robust immune system to protect themselves from infectious pathogens and other invaders. The immune system is a highly complex and potentially harmful biological system in which iron-binding proteins not only play antimicrobial roles but also seem to actuate strategic levers to modulate host defense. LF itself has been the object of more than 3000 studies reporting its activities in host defense as well as the mechanisms to explain them. LF appears to have the ability to stimulate the immune system to counteract pathogenic invaders and injuries while preventing overreactions which are harmful to the host. LF is produced by epithelial cells in most exocrine secretions such as seminal fluid, pancreatic exocrine secretions, tears, saliva, and uterine secretions and in milk, where its concentration in humans may vary between 1 and 7 g/L (Bennett and Kokocinski 1978). LF receptors (LFR) are expressed in different tissues including cells of the innate immune system and may locally deliver the molecule to inflammatory sites (Legrand 2012). Neutrophils [polymorphonuclear neutrophils (PMNs)], which represent more than one-half of total white blood cells, produce LF and store it in secondary granules. Upon activation of PMNs, which begins at the very first steps of adhesion to the activated endothelium, LF is released in the blood, where its concentration may rise to 200 mg/L (from about 1 mg/mL under normal conditions), especially in inflamed tissues (Maacks and Wood 1989). Talukder and Harada (2007) demonstrated that bovine LF protects against lipopolysaccharide-induced diarrhea by modulating nitric oxide and prostaglandin E_2 in mice. Furthermore, Talukder et al. (2002) discovered the receptor-mediated transport mechanism of LF in the intestinal cells which are transported into the cerebrospinal fluid via systemic circulation in newborn (Talukder et al. 2002) and young calves (Talukder et al. 2003). In addition, microglial

R. C. Gupta et al. (eds.), *Nutraceuticals in Veterinary Medicine*, https://doi.org/10.1007/978-3-030-04624-8_17

cells, which act as the resident macrophages in the brain, also release LF during inflammation (Fillebeen et al. 2001).

Egg white is the main source of various egg proteins including OVTF. In order of their abundance, the main egg white proteins are ovalbumin (54%), OVTF (12%), ovomucoid (11%), and lysozyme (3.5%), each of which has numerous functional characteristics (Abeyrathne et al. 2013). The minor proteins in egg white include avidin (0.05%), cystatin (0.05%), ovomacroglobulin (0.5%), OVTF (0.8%), ovoglycoprotein (1.0%), and ovoinhibitor (1.5%) (Kovacs-Nolan et al. 2005a, b). The first name given to OVTF was conalbumin. However, after finding that iron can be attached to the protein structure, it was renamed OVTF (Williams 1968).

2 Characteristics of OVTF and LF

OVTF is an acidic glycoprotein with an isoelectric point of 6.0. The overall structure of OVTF is similar to that of human LF and rabbit serum transferrin. It is being folded into two homologous lobes, each containing two dissimilar domains with one Fe^{3+} and one CO_3^{2-} bound at a specific site in each interdomain cleft. It is comprised of 686 amino acids with 15 disulfide bonds to form the 76 kDa structure (Abeyrathne et al. 2014). OVTF carries and transports iron in the animal body and exists in two distinct forms, (1) holo- (iron bound) and (2) apo- (iron-free), each with significantly different physicochemical characteristics (Wu and Acero-Lopez 2012). OVTF is made of two main lobes. The *N*-terminal lobe consisting of amino acid 1–329 and the C-terminal lobe consisting of amino acid 330–686 (Giansanti et al. 2012; Superti et al. 2007). Each lobe contains a site to bind with one mole iron and is further divided into two domains: C1 and C2 and N1 and N2 (Superti et al. 2007). OVTF is capable of binding with other metal cations such as, in order of affinity, $Fe^{3+} > Cr^{2+}$, $Cu^{2+} > Mn^{2+}$, Co^{2+}, and $Cd^{2+} > Zn^{2+} > Ni^{2+}$ (Tan and Woodworth 1969). This protein resembles 50%, 49%, and 51% with LF, human LF, and human transferrin, respectively. Most of the similarities can be found in the C-terminal lobe (Jeltsch et al. 1987). Transferrins are different in their structure of adhered N-glycan (highly mannose) and in the pI, which may influence their biofunctions (Jiang et al. 2014).

LF possesses a greater iron-binding affinity, and it is the only transferrin with the ability to retain this metal over a wide range of pH values, including a resistance to proteolysis. The most striking physicochemical feature of LF is its very high affinity for iron. In both LF and related transferrins, two Fe^{3+} ions are bound very tightly (K ~ 1022 M) but reversibly to LF, with two synergistically bound CO_3^{2-} ions (Aisen and Leibman 1972; Baker 1994). It contains ~ 700 amino acids, with a high homology among species. It is comprised of a simple polypeptide chain folded into two symmetrical lobes (the N-lobe and C-lobe), which are greatly homologous with one another (33–41% homology). The two lobes are connected via a hinge region containing parts of an α-helix between amino acids 333 and 343 in human LF (Öztaş Yeşim and Özgüneş 2005), which confers flexibility to the molecule (Haridas et al. 1995). The polypeptide chain includes amino acids 1–332, comprising the N-lobe, and 344–703, comprising the C-lobe. The chain is also made up of α-helix and β-pleated sheet structures that create two domains within each lobe (domains I and II) (Mazurier and Spik 1995). Each lobe can bind a metal atom in synergy with the carbonate ion (CO_3^{2-}). LF is capable of binding Fe^{2+} or Fe^{3+} ions, but it has also been observed to be bound to Cu^{2+}, Zn^{2+}, and Mn^{2+} ions (Baker et al. 2004). Because of its ability to reversibly bind Fe^{3+}, LF can exist free of Fe^{3+} (apo-Lf) or associated with Fe^{3+} (holo-Lf), and it has a different three-dimensional conformation depending on whether it is bound to Fe^{3+} (Wally and Buchanan 2007). Apo-LF has an open conformation, while holo-LF is a closed molecule with greater resistance to proteolysis (Öztaş Yeşim and Özgüneş 2005). Because of the common structural framework among LFs, it is possible to model their conformations using crystallographic data from other LF species (Fig. 1a). The amino acids directly involved at the iron-binding site in each lobe are Asp60, Tyr92, Tyr192, and His253, while Arg121 is involved in binding the CO_3^{2-} ion (Fig. 1b). LF is a basic, positively charged protein with a pI of 8.0–8.5. The primary structure of LF shows the number and position of Cys residues that allow the formation of intramolecular disulfide bridges. Asn residues in the N- and C-terminal lobes provide several potential N-glycosylation sites (Khan et al. 2001; Anderson et al. 1987).

3 OVTF and LF Receptors

The receptors of OVTF and LF play an important role in the internalization of these metalloproteins. They also facilitate absorption of iron ions. It has been shown that gene expression increases with age in the duodenum and decreases in the jejunum (Liao et al. 2007; Bharadwaj et al. 2009). The moonlighting glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase (GAPDH) has been demonstrated to function as a receptor for LF (Rawat et al. 2012).

4 Genes of OVTF and LF

The Chicken Gene Nomenclature Consortium reported that OVTF genes of *Gallus gallus* are located on chromosome 9 (assembly name, GRCg6a) with a mean length of 7398 bp. Counts and length of annotated features are provided for each

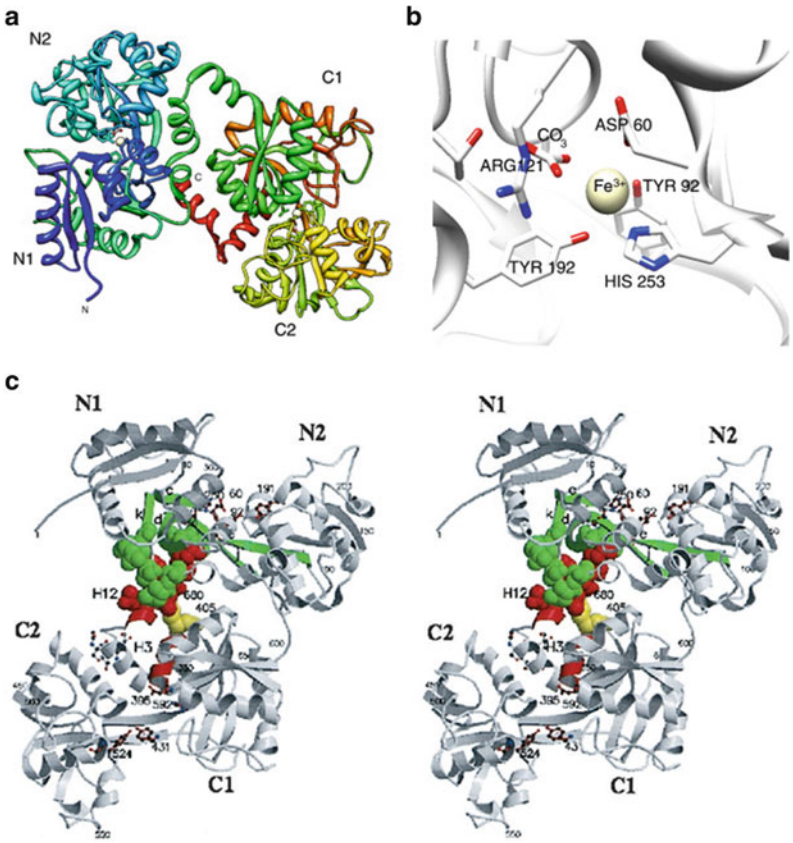


Fig. 1 Predicted structure of LF and OVTF: (**a**) Two-lobe, four-domain polypeptide, (**b**) canonical iron-binding pocket site of lactoferrin. Fe^{3+} (cream), CO_3 (gray and red), and (**c**) two-lobe, four-domain polypeptide of OVTF

assembly gene and pseudogene numbers 24,373; protein coding, 17,477; noncoding, 6534; transcribed pseudogenes, 22; non-transcribed pseudogenes, 240; and genes with variants, 10,544, along with 49,661 mRNAs. The number of transcripts per gene is 2.68, and the number of exons per transcript is 12.8.

LF gene polymorphism between species is much more diverse than the intraspecific polymorphism. About 60 gene sequences of LF have been characterized in 11 species of mammals (Kang et al. 2008). In most species, stop codon is TAA and TGA in *Mus musculus*. Deletions, insertions, and mutations of stop codons affect coding, and their length varies between 2055 and 2190 nucleotide pairs. There are differences in amino acid sequences: 8 in *Homo sapiens*, 6 in *Mus musculus*, 6 in *Capra hircus*, 10 in *Bos taurus*, and 20 in *Sus scrofa*. This variation may indicate functional differences between different types of LF (Kang et al. 2008). In humans, the LF gene is located on the third chromosome in the locus 3q21-q23. In oxen, the coding sequence consists of 17 exons and has a length of about 34,500 nucleotide pairs. Exons of the LF gene in oxen are of similar size to the exons of other genes in the transferrin family, whereas the sizes of introns differ within that family. Similarity in size of exons and their distribution in the domains of the protein molecule indicates that the evolutionary development of the LF gene occurred via duplication (Seyfert et al. 1994). The studying of polymorphisms of genes that encode LF helps in selecting livestock breeds that are resistant to mastitis (O'Halloran et al. 2009).

5 Bioavailability of OVTF and LF

In order to maximize the functions of OVTF and LF, absorption in the digestive system and transportation of these molecules to the target organs or tissues are very crucial. The amount of literature available on the absorption of OVTF in the human body is limited. The majority of investigations have been carried out on its derived peptides. Evenepoel et al. (1999) reported that the amounts of cooked and raw egg proteins which escaped digestion and absorption in the small intestine of healthy volunteers were 5.73% and 35.10%, respectively. It has been perceived that a good amount of protein enters the intestine in various forms, and the majority of it is digested and reabsorbed along with ingested protein (Matthews 1971).

Immunohistochemical and physiochemical investigations on the transportation and absorption of bovine LF in the small

intestine of growing pigs showed that the absorption of LF was mediated by LF-mediated factors on the epithelial cell membrane (Kitagawa et al. 2003). Their results also showed that bovine LF was absorbed through transcytosis at the apical halves of the villi of small intestine as small vesicles via villus columnar epithelial cells. Moreover, it was demonstrated that LF is transported via the lymphatics and the portal vein into systemic circulation (Kitagawa et al. 2003). Talukder et al. (article under review) investigated the absorption of OVTF using rat intestinal epithelial cells. They observed that OVTF is taken up by IEC-6 cells on the transwell membrane as an intact protein from the apical surface and transported to the basolateral surface through the transepithelial exocytosis mechanism. Maximum internalization and exocytosis were observed at 90 min. These processes were time and concentration dependent. It is concluded that absorption and transepithelial transportation of OVTF are accomplished by receptor-mediated transcytosis in the intestinal epithelial cells (Shirkhani et al. 2018).

Mazurier et al. (1985) detected LF receptors with a molecular weight of 100 kD in the microvillus of the enterocyte membrane of human and rabbit small intestine. Immunohistochemical analyses of Kitagawa et al. (2003) demonstrated the presence of LF receptors on the apical villi, striated border, and basolateral membrane of enterocytes.

6 Functional Properties of OVTF and LF

It has been reported that OVTF and LF can demonstrate a wide range of biofunctions. The protective effects of these iron-binding proteins have been shown from anticancer, anti-inflammatory, and immune modulator activities to antimicrobial activities against a large number of microorganisms. The wide range of activities is made possible by the mechanisms of action involving not only the capacity to bind iron but also interactions with molecular and cellular components of both hosts and pathogens (Garcia-Montoya et al. 2012). Therefore, their application in functional foods makes them an attractive compound to enhance human and animal health. Figure 2 shows the shared physiological properties of OVTF and LF with other types of transferrin.

7 Antibacterial Activity

The primary role of LF is to sequester free Fe^{2+} and remove an essential substrate required for bacterial growth (Farnaud and Evans 2003). LF is considered to be a key component of the innate host defense system because it can respond to a variety of physiological and environmental changes (Connely 2001). The structural features of LF provide additional functionalities beyond the Fe^{3+} homeostasis function

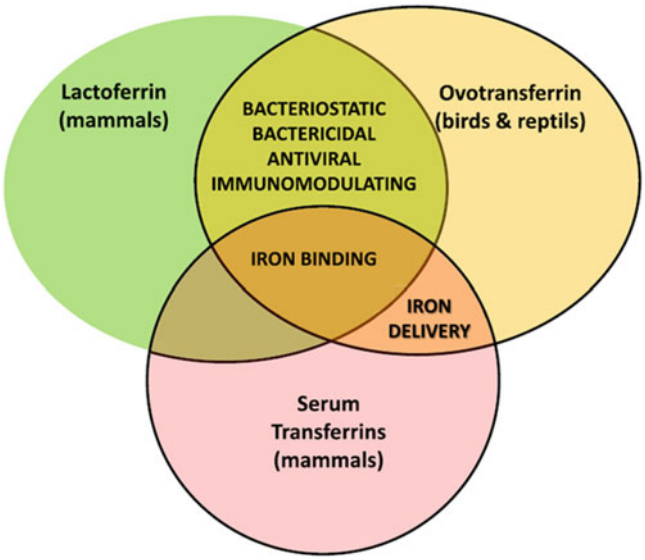


Fig. 2 Physiological properties shared between transferrin family proteins members

common to all transferrins. In particular, LF exhibits strong antimicrobial activity against a broad spectrum of bacteria (Gram positive and Gram negative), fungi, yeasts, viruses (Drago 2006), and parasites (Yamauchi et al. 2006). However, it seems to promote the growth of beneficial bacteria like *Lactobacillus* and *Bifidobacteria* (Sherman et al. 2004). It also exhibits anti-inflammatory and anticarcinogenic activities (Connely 2001) and has several enzymatic functions (Leffell and Spitznagel 1972). LF plays a key role in maintaining cellular iron levels in the body. Sequestering iron from bacterial pathogens is believed to be the sole antimicrobial action of LF because apo-LF possesses antibacterial activity (Kalmar and Arnold 1988; Yamauchi et al. 1993). It was later demonstrated that LF can also kill microorganisms through an iron-independent mechanism (Valenti and Antonini 2005) in which LF interacts directly with the bacterial cell surface (Kalmar and Arnold 1988; Bortner et al. 1989; Farnaud and Evans 2003).

The bacteriostatic characteristics of OVTF are promoted by adding a carbonate ion, enhancing the pH from 6 to 8 and immobilizing VOTF to Sepharose 4B by covalent linkage (Giansanti et al. 2012; Valenti et al. 1982). The most resistant bacterial species against OVTF are *Proteus* spp. and *Klebsiella* spp., whereas the most sensitive ones are *Pseudomonas* spp., *Escherichia coli*, and *Streptococcus mutans* (Valenti et al. 1982). Recently, the current authors observed that OVTF is a potent antimicrobial protein with the ability to kill different types of bacteria in in vitro and clinical applications (Talukder et al. 2018). Bacterial culture of a uterine swab demonstrated the presence of different types of bacteria including Gram positive, Gram negative, α- and β-hemolytic, rods, and cocci. Zone of inhibition studies with

different concentrations of OVTF demonstrated that 5% would be more than enough to kill all types of bacteria found in these studies.

8 Antifungal Activity

Mucosal surfaces can be infected by *Candida*, and it is considered to be analogous to a commensal organism that can also become an opportunistic pathogen. Kirkpatrick et al. (1971) conducted the studies with *Candida* spp. and attributed the antifungal effect of LF to its ability to sequester Fe^{3+} (Kirkpatrick et al. 1971; Viejo-Díaz et al. 2004; Garcia-Montoya et al. 2012). Both human and bovine LF as well as the LF-derived peptide lactoferricin have well-documented in vitro activity toward human pathogenic fungi, especially *Candida albicans* and several other *Candida* species. LF also has antifungal activity (Arnold et al. 1980; Bellamy et al. 1993), and it was observed that LF could kill both *C. albicans* and *C. krusei* by altering the permeability of the cell surface, as it does with bacteria (Wakabayashi et al. 1996; Kuipers et al. 1999). Bovine LF has been shown to be highly fungicidal for *C. tropicalis* and *C. krusei* and somewhat fungicidal for *C. albicans* and *C. guilliermondii*, while *C. glabrata* is almost resistant to LF (Xu et al. 1999). LF exhibited activity against *Cryptococcus neoformans* and *C. albicans* via cytoplasmic and mitochondrial membrane permeabilization (Kondori et al. 2011). Although LF's antifungal mechanism of action is through cell surface interaction rather than iron deprivation (Valenti et al. 1986), several reports demonstrate its ability to cause cell wall damage (Xu et al. 1999; Nikawa et al. 1993, 1995). In addition to direct interaction with the pathogen, Fe^{3+} sequestration is another important mechanism for fungicidal activity. Furthermore, Zarember et al. (2007) showed that Fe^{3+} sequestration by neutrophil apo-LF is important for host defense against *Aspergillus fumigatus* (Zarember et al. 2007). Additionally, the in vitro antifungal activity of two peptides (human LF (1–11 aa), bovine LF N1-domain) derived from human LF was compared, and dose-dependent antifungal activity was observed (Lupetti et al. 2008; van der Kraan et al. 2004). LF shows an interesting antifungal effect on body tinea caused by *Trichophyton mentagrophytes* against which it acts indirectly, facilitating clinical improvement of skin lesions after the peak of symptoms. Treatment of guinea pigs with bovine LF reduces fungal infection on the skin of the back and limbs in *Tinea corporis* and *Tinea pedis*, respectively (Wakabayashi et al. 2000). It has also been demonstrated that LF can mediate its antifungal activity through the stimulation of host cell immune mechanisms both in vitro and in vivo (Viejo-Díaz et al. 2004).

9 Antiparasitic Activity

The ecological niches of microbes often differ from one organism to another and make it difficult to have a clear mechanism and understanding of the antimicrobial activities of LF. The molecular mechanisms of LF as an antiparasitic activity are even more complex. Antiparasitic activities of LF usually involve interference with iron acquisition. This activity has also been shown using peptides derived from the full molecule (Weinberg 1994; Cirioni et al. 2000). There is also evidence supporting the occurrence of a similar mechanism during amebiasis, which is one of the leading causes of diarrhea in children under 5 years of age and is caused by *Entameba histolytica* (León-Sicairos et al. 2006a). In in vitro studies, apo-Lf demonstrated the greatest amebicidal effect against *E. histolytica* because it can bind to lipids on the trophozoite's membrane, causing membrane disruption and damage to the parasite (León-Sicairos et al. 2006b; López-Soto et al. 2010). LF appears to act as a specific iron donor and could be expected to enhance infection by other parasites such as *Tritrichomonas foetus* (Tachezy et al. 1996). It was reported that bovine LF bound to components of *T. brucei* and that bovine LF hydrolysate disrupted the sites responsible for binding to parasite proteins, causing Fe^{3+} deprivation (Tanaka et al. 2004). Other in vitro studies show that serum transferrin as well as human and bovine LF can bind the intracellular parasite *Toxoplasma gondii* responsible for toxoplasmosis in humans and animals. However, LF cannot prevent the parasite from entering the host. The mechanism of action in this case is inhibition of the intracellular growth of *T. gondii* in the host cells (Dzitko et al. 2007). In animal models, lactoferricin reduced infectivity of *T. gondii* and *Eimeria stiedae* sporozoites (Omata et al. 2001). The effect of LF on the hemoparasites *Babesia caballi* and *Babesia equi* depends on whether or not LF is bound to Fe^{3+} (Botteon et al. 2002). *B. caballi* was found to be significantly suppressed by apo-LF but was not inhibited by other types of LF, whereas none of the LF types had an inhibitory effect against *B. equi* (Ikada et al. 2005). In all these, LF demonstrates an additive or synergistic activity with clinically used antiparasitic compounds (Weinberg 1994; León-Sicairos et al. 2006a, b).

10 Antiviral Activity

Rotavirus and norovirus often cause gastroenteritis, a major illness prevalent in winter months. Rotavirus causes gastroenteritis only in children. Norovirus is an extremely important emerging human pathogen that causes a majority of gastroenteritis outbreaks worldwide. The in vitro anti-rotavirus effects of LF have been reported (Superti et al.

1997, 2001; Wakabayashi et al. 2014). The human norovirus remains difficult to study because of the lack of cell cultures and animal models. Instead, feline calicivirus and murine norovirus, which can be cultured and share a number of biochemical properties, similar genomic organization and primary RNA sequences with human norovirus, have been used as a virus surrogate to study human norovirus. A study using feline calicivirus showed that bovine LF inhibits the viral infection of Crandell-Reese feline kidney cells by binding to the cells and lactoferricin B inhibits the infection by binding to the virus (McCann et al. 2003). Bovine LF also decreased murine norovirus infection to murine macrophage cell line Raw264.7 through inhibition of the initial murine norovirus attachment to cells and the subsequent interference with murine norovirus replication (Ishikawa et al. 2013).

The induction of antiviral cytokine interferon (IFN)-α/β expression by LF was involved in the inhibition of viral replication in the infected cells. This is the first report that shows the inhibition of viral replication in the cells and the involvement of IFN-α/β. It has already been reported that oral administration of LF induces IFN-α/β in the small intestine of mice (Kuhara et al. 2006; Wakabayashi et al. 2006). From these findings, IFN-α/β may be a key mediator in the antiviral effects of orally administered LF (Wakabayashi et al. 2014), and the deduced antiviral mechanism of LF is illustrated in Fig. 3.

In a study of rotaviral gastroenteritis in children, daily intake of bovine LF-containing products ameliorated the severity of the disease, although there was no significant benefit in reducing infection incidence (Egashira et al. 2007). The addition of recombinant human LF and lysozyme to a rice-based oral rehydration solution had beneficial effects on children with acute diarrhea in whom rotavirus was identified as a pathogen in 18–19% of stool samples (Zavaleta et al. 2007). The daily administration of LF tablets to children reduced the incidence of noroviral gastroenteritis (Moriuchi and Moriuchi 2009). LF administration exhibited no decrease in diarrhea incidence, but decreased longitudinal prevalence and severity in children where norovirus was isolated as a pathogen in 35% of fecal samples (Moriuchi and Moriuchi 2009). LF inhibits the cytopathic effect of adenovirus in HEp-2 cells (Arnold et al. 2002; Di Biase et al. 2003; Pietrantoni et al. 2003), where the effect of bovine LF has been shown to be more potent than that of human LF. On the other hand, another investigation reported that human LF promotes the binding of adenovirus to human corneal epithelial cells and also infection of the cells by adenovirus (Johansson et al. 2007).

The anti-enteroviral activities of LF are indicated in poliovirus, enterovirus 71, coxsackievirus A16, echovirus 5, and echovirus 6 (Marchetti et al. 1999; Lin et al. 2002; Weng et al. 2005; Furlund et al. 2012; Pietrantoni et al. 2006; Ammendolia et al. 2007). Remarkably, bovine LF induced IFN-α expression of human neuroblastoma cells (SK-N-SH) and inhibited enterovirus 71-induced interleukin (IL)-6 production (Weng et al. 2005). The antiviral activity of bovine LF was not obvious in echovirus 9 (Lin et al. 2002).

Following enterovirus 71 infection, neonatal pups ingesting transgenic milk expressed recombinant porcine LF and showed significantly higher survival rate and heavier body weight compared to wild-type mice (Chen et al. 2008). However, oral supplementation of bovine LF at a dose of 70 mg/day did not show beneficial effects in the prevention of enterovirus 71 or rotavirus infection in children Yen et al. 2011). Herpes simplex virus type 1 and 2 (HSV-1 and HSV-2) establish lifelong latent infections in the host and can re-emerge periodically throughout life, primarily causing facial and genital herpetic lesions, respectively. The in vitro anti-herpes activities of LF have been studied in HSV-1 (Hasegawa et al. 1994; Marchetti et al. 1996, 1998; Siciliano et al. 1999; Seganti et al. 2001; Lampis et al. 2001; Jenssen et al. 2008; Marr et al. 2009) and HSV-2 (Marchetti et al. 1998; Jenssen et al. 2008; Shestakov et al. 2012). The effect of orally administered LF in HSV infection has been reported

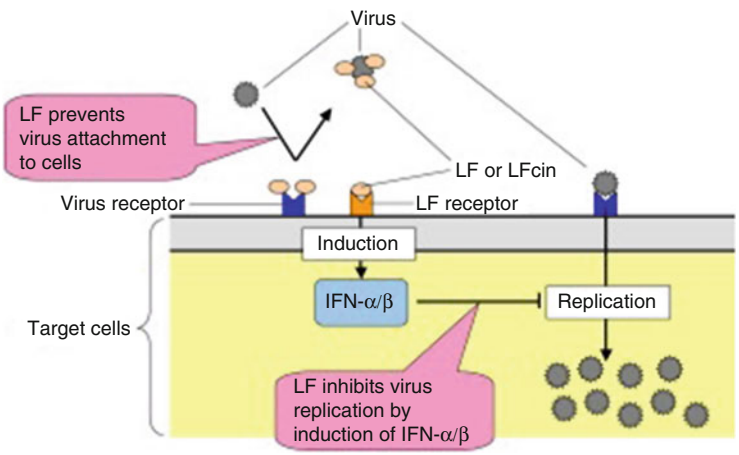


Fig. 3 Deduced mechanism of antiviral effect of lactoferrin (LF). LF or lactoferricin (LFcin) prevent virus attachment to the target cells by binding to the virus receptor on the target cells or binding to the virus. In addition, lactoferrin induces IFN α/β production and thereby inhibits virus replication after entry of the virus into the cells

by Wakabayashi et al. (2004) and demonstrated that LF administration prevents body weight loss and increases the production of Th1 cytokines, including IFN-γ, IL-12, and IL-18, after HSV-1 cutaneous infection in mice. These enhanced Th1 cytokine responses may help host protection against HSV-1 infection.

LF exhibits inhibitory activities against a wide range of viruses in vitro. The effects of LF via oral administration have been studied in various viral infections in animals and humans (Wakabayashi et al. 2014). Being a food component, LF is easily consumed by an individual to prevent these infections. Although the mechanism of action of LF has not been fully elucidated, direct antiviral activities exerted in the gastrointestinal tract and systemic immune modulation seem to be involved in these effects. Thus, LF is a promising candidate to prevent viral infection or diarrhea, and further studies are warranted to establish more reliable evidence.

11 Concluding Remarks and Future Directions

OVTF and LF have many biological functions; the host-protective effects range from direct antimicrobial activities against a large panel of microorganisms, including bacteria, viruses, fungi, and parasites, to anti-inflammatory, antioxidant, and anticancer activities. Thus, these metalloproteins have important therapeutic implications for humans and animals. Further basic and clinical studies will better clarify the usefulness of OVTF and LF.

References

Abeyrathne EDNS, Lee HY, Ahn DU (2013) Egg white proteins and their potential use in food processing or as nutraceutical and pharmaceutical agents—a review. Poult Sci 92(12):3292–3299. https://doi.org/10.3382/ps.2013-03391

Abeyrathne EDNS, Lee HY, Ahn DU (2014) Separation of ovotransferrin and ovomucoid from chicken egg white. Poult Sci 93(4):1010–1017

Aisen P, Leibman A (1972) Lactoferrin and transferrin: a comparative study. Biochim Biophys Acta 257:314–323

Ammendolia MG, Pietrantoni A, Tinari P et al (2007) Bovine lactoferrin inhibits echovirus endocytic pathway by interacting with viral structural polypeptides. Antivir Res 73(2007):151–160

Anderson, B.F., Baker, HM, . Dodson EJ et al (1987). Structure of human lactoferrin at 3.2-A° resolution. Proc Natl Acad Sci USA 84: 1769–1773

Arnold RR, Brewer M, Gauthier JJ (1980) Bactericidal activity of human lactoferrin: sensitivity of a variety of microorganisms. Infect Immun 28:893–898

Arnold D, Di Biase AM, Marchetti M et al (2002) Antiadenovirus activity of milk proteins: lactoferrin prevents viral infection. Antivir Res 53:153–158

Baker EN (1994) Structure and reactivity of transferrins. Adv Inorg Chem 41:389–463

Baker HM, Anderson BF, Baker EN (2004) Dealing with iron: common structural principles in proteins that transport iron and heme. Proc Natl Acad Sci USA 100:3579–3583

Bellamy W, Wakabayashi H, Takase M, Shimamura S et al (1993) Killing of *Candida albicans* by lactoferricin B, a potent antimicrobial peptide derived from the N-terminal region of bovine lactoferrin. Med Microbiol Immunol 182:97–105

Bennett RM, Kokocinski T (1978) Lactoferrin content of peripheral blood cells. Br J Hematol 39:509–521

Bharadwaj S, Naidu AG, Betageri GV et al (2009) Milk ribonuclease-enriched lactoferrin induces positive effects on bone turnover markers in postmenopausal women. Osteoporosis Int 20 (9):1603–1611

Bortner CA, Arnold RR, Miller RD (1989) Bactericidal effect of lactoferrin on *Legionella pneumophila*: effect of the physiological state of the organism. Can J Microbiol 35:1048–1051

Botteon P, Massard C, Botteon R (2002) Seroprevalence of *Babesia equi* in three breeding systems of equines. Parasitol Latinoam (Bras) 57:141–145

Chen H, Wang L, Chang C et al (2008) Recombinant porcine lactoferrin expressed in the milk of transgenic mice protects neonatal mice from a lethal challenge with enterovirus type 71. Vaccine 26:891–898

Cirioni O, Giacometti A, Barchiesi F et al (2000) Inhibition of growth of *Pneumocystis carinii* by lactoferrins alone and in combination with pyrimethamine, clarithromycin and minocycline. J Antimicrob Chemother 46:577–582

Connely OM (2001) Anti-inflammatory activities of lactoferrin. J Am Coll Nutr 438:389S–395S

Di Biase AM, Pietrantoni A, Tinari A et al (2003) Heparin-interacting sites of bovine lactoferrin are involved in anti-adenovirus activity. J Med Virol 69:495–502

Drago SME (2006) Actividades antibacterianas de la lactoferrinaEnferm. Infecc Microbiol 26:58–63

Dzitko K, Dziadek B, Dziadek J et al (2007) *Toxoplasma gondii*: inhibition of the intracellular growth by human lactoferrin. Pol J Microbiol 56(1):25–32

Egashira M, Takayanagi T, Moriuchi M et al (2007) Does daily intake of bovine lactoferrin-containing products ameliorate rotaviral gastroenteritis? Acta Paediatr 96:1238–1244

Evenepoel P, Claus D, Geypens B et al (1999) Amount and fate of egg protein escaping assimilation in the small intestine of humans. Am J Phys 277(5):G935–G943

Farnaud S, Evans RW (2003) Lactoferrin-A multifunctional protein with antimicrobial properties. Mol Immunol 40:395–405

Fillebeen C, Ruchoux MM, Mitchell V et al (2001) Lactoferrin is synthesized by activated microglia in the human substantia nigra and its growing pigs. J Vet Med Sci 65(5):567–572

Furlund CB, Kristoffersen AB, Devold TG et al (2012) Bovine lactoferrin digested with human gastrointestinal enzymes inhibits replication of human echovirus 5 in cell culture. Nutr Res 32:503–513

Garcia-Montoya A, Cendon T, Arevalo-Gallegos S et al (2012) Lactoferrin a multiple bioactive protein: an overview. Biochim Biophys Acta 1820(3):226–236

Giansanti F, Leboffe L, Pitari G et al (2012) Physiological roles of ovotransferrin. Biochim Biophys Acta 1820(3):218–225

Haridas M, Anderson BF, Baker EN (1995) Structure of human diferric lactoferrin refined at 2.2 Å resolution. Acta Crystallogr 51:629–646

Hasegawa K, Motsuchi W, Tanaka S et al (1994) Inhibition with lactoferrin of *in vitro* infection with human herpes virus. Jpn J Med Sci Biol 47:73–85

Ikada H, Tanaka T, Shibahara N (2005) Short report: inhibitory effect of lactoferrin on in vitro growth of Babesia caballi. Am J Trop Med Hyg 73:710–712

Ishikawa H, Awano N, Fukui T et al (2013) The protective effects of lactoferrin against murine norovirus infection through inhibition of both viral attachment and replication. Biochem Biophys Res Commun 434:791–796

Jeltsch JM, Hen R, Maroteaux L et al (1987) Sequence of the chicken ovotransferrin gene. Nucleic Acids Res 15(18):7643–7645

Jenssen H, Sandvik K, Andersen JH et al (2008) Inhibition of HSV cell-to-cell spread by lactoferrin and lactoferricin. Antivir Res 79:192–198

Jiang K, Wang C, Sun Y et al (2014) Comparison of chicken and pheasant ovotransferrin N-glycoforms via electrospray ionization mass spectrometry and liquid chromatography coupled with mass spectrometry. J Agric Food Chem 62(29):7245–7254

Johansson C, Jonsson M, Marttila M et al (2007) Adenoviruses use lactoferrin as a bridge for CAR-independent binding to and infection of epithelial cells. J Virol 81:954–963

Kalmar JR, Arnold RR (1988) Killing of *Actinobacillus actinomycetemcomitans* by human lactoferrin. Infect Immun 56:2552–2557

Kang JF, Li XL, Zhou RY et al (2008) Bioinformatics analysis of lactoferrin gene for several species. Biochem Genet 46 (5–6):312–322

Khan JA, Kumar P, Paramasivam M et al (2001) Camel lactoferrin, a transferrin-cum-lactoferrin: crystal structure of camel apolactoferrin at 2.6 Å resolution and structural basis of its dual role. J Mol Biol 309:751–761

Kirkpatrick CH, Green I, Rich RR et al (1971) Inhibition of growth of *Candida albicans* by iron-unsaturated lactoferrin: Relation to host-defense mechanisms in chronic mucocutaneous candidiasis. J Infect Dis 124:539–544

Kitagawa H, Yoshizawa Y, Yokoyama T et al (2003) Persorption of bovine lactoferrin from the intestinal lumen into the systemic circulation via the portal vein and the mesenteric lymphatics in growing pigs. J Vet Med 65(5):567–572

Kondori N, Baltzer N, Dolphin GT et al (2011) Fungicidal activity of human lactoferrin-derived peptides based on the antimicrobial αβ region. Int J Antimicrob Agents 37:51–57

Kovacs-Nolan J, Phillips M, Mine M (2005a) Advances in the value of eggs and egg components for human health. J Agric Food Chem 53 (22):8421–8431

Kovacs-Nolan J, Phillips M, Mine Y (2005b) Advances in the value synthesis by the human microglial CHME cell line is upregulated by tumor necrosis factor alpha or 1-methyl-4-phenylpyridinium treatment. Brain Res Mol Brain Res 96:103–113

Kuhara T, Yamauchi K, Tamura Y et al (2006) Oral administration of lactoferrin increases NK cell activity in mice via increased production of IL-18 and type I IFN in the small intestine. J Interf Cytokine Res 26:489–499

Kuipers ME, de Vries HG, Eikenboom MC et al (1999) Synergistic fungistatic effects of lactoferrin in combination with antifungal drugs against clinical Candida isolates. Antimicrob Agents Chemother 43:2635–2641

Lampis G, Deidda D, Pinza M et al (2001) Enhancement of anti-herpetic activity of glycyrrhizic acid by physiological proteins. Antivir Chem Chemother 12:125–131

Leffell S, Spitznagel JK (1972) Association of lactoferrin with lysozyme in granules of human polymorphonuclear leukocytes. Infect Immun 6:761–765

Legrand D (2012) Lactoferrin, a key molecule in immune and inflammatory processes. Biochem Cell Biol 90:252–268

León-Sicairos N, Reyes-López M, Ordaz-Pichardo C et al (2006a) Microbicidal action of lactoferrin and lactoferricin and their synergistic effect with metronidazole in *Entameba histolytica*. Biochem Cell Biol 84:327–336

León-Sicairos N, López-Soto SF, Reyes-López M et al (2006b) Amoebicidal activity of milk, apo-lactoferrin, sIgA and lysozyme. Clin Med Res 4:106–113

Liao Y, Lopez V, Shafizadeh TB et al (2007) Cloning of a pig homologue of the human lactoferrin receptor: expression and localization during intestinal maturation in piglets. Comp Biochem Physiol A Mol Integr Physiol 148(3):584–590

Lin T, Chu C, Chiu C (2002) Lactoferrin inhibits enterovirus 71 infection of human embryonal rhabdomyosarcoma cells *in vitro*. J Infect Dis 186:1161–1164

López-Soto F, León-Sicairos N, Nazmi K et al (2010) Microbicidal effect of the lactoferrin peptides lactoferricin 17–30, actoferrampin 265–284, and lactoferrin chimera on the parasite *Entameba histolytica*. Biometals 23:563–568

Lupetti A, Van Dissel JT, Brouwer CPJM et al (2008) Human antimicrobial peptides antifungal activity against *Aspergillus fumigatus*. Eur J Clin Microbiol Infect Dis 27:1125–1129

Maacks SYH, Wood WG (1989) Development and evaluation of luminescence based sandwich assay for plasma lactoferrin as a marker for sepsis and bacterial infections in pediatric medicine. J Biolumin Chemilumin 3:221–226

Marchetti M, Longhi C, Conte MP et al (1996) Lactoferrin inhibits herpes simplex virus type 1 adsorption to vero cells. Antivir Res 29:221–231

Marchetti M, Pisani S, Antonini G et al (1998) Metal complexes of bovine lactoferrin inhibit *in vitro* replication of herpes simplex virus type 1 and 2. Biometals 11:89–94

Marchetti M, Superti F, Ammendolia MG et al (1999) Inhibition of poliovirus type 1 infection by iron-, manganese- and zinc-saturated lactoferrin. Med Microbiol Immunol 187:199–204

Marr AK, Jenssen H, Moniri R et al (2009) Bovine lactoferrin and lactoferricin interfere with intracellular trafficking of *Herpes simplex* virus-1. Biochimie 91:160–164

Matthews DM (1971) Protein absorption. J Clin Pathol s3-5(1):29–40

Mazurier J, Spik G (1995) Comparative study of the iron-binding properties of human transferrins: I. complete and sequential iron saturation and desaturation of the lactotransferrin. Biochim Biophys Acta 629:399–408

Mazurier J, Montreuil J, Spik G (1985) Visualization of lactotransferrin brush-border receptors by ligand-blotting. Biochim Biophys Acta 821(3):453–460

McCann KB, Lee A, Wan J et al (2003) The effect of bovine lactoferrin and lactoferricin B on the ability of feline calicivirus (a norovirus surrogate) and poliovirus to infect cell cultures. J Appl Microbiol 95:1026–1033

Moriuchi M, Moriuchi H (2009) Prevention of norovirus infection in nursery school children by intake of lactoferrin-containing products. In: 50th Japanese Society of clinical virology, p S56

Nikawa H, Samarayanake LP, Tenovuo J et al (1993) The fungicidal effect of human lactoferrin on *Candida albicans* and *Candida krusei*. Arch Oral Biol 38:1057–1063

Nikawa H, Samarayanake LP, Hamada T (1995) Modulation of the anti-Candida activity of apo-lactoferrin by dietary sucrose and tunicamycin in vitro. Arch Oral Biol 40:581–584

O'Halloran F, Bahar B, Buckley F et al (2009) Characterisation of single nucleotide polymorphisms identified in the bovine lactoferrin gene sequences across a range of dairy cow breeds. Biochimie 91 (1):68–75

Omata Y, Satake M, Maeda R et al (2001) Reduction of the infectivity of *Toxoplasma gondii* and *Eimeria stiedae* sporozoites by treatment with bovine lactoferricin. J Vet Med Sci 63:187–190

Öztaş Yeşim ER, Özgüneş N (2005) Lactoferrin: a multifunctional protein. Adv Mol Med 1:149–154

Pietrantoni A, Di Biase AM, Tinari A et al (2003) Bovine lactoferrin inhibits adenovirus infection by interacting with viral structural polypeptide. Antimicrob Agents Chemother 47:2688–2691

Pietrantoni A, Ammendolia MG, Tinari A et al (2006) Bovine lactoferrin peptidic fragments involved in inhibition of echovirus 6 *in vitro* infection. Antivir Res 69:98–106

Rawat P, Kumar S, Sheokand N et al (2012) The multifunctional glycolytic protein glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is a novel macrophage lactoferrin receptor. Biochem Cell Biol 90(3):329–338

Seganti L, Di Biase AM, Rega B et al (2001) Involvement of bovine lactoferrin moieties in the inhibition of herpes simplex virus type 1 infection. Int J Immunopathol Pharmacol 14:71–79

Seyfert HM, Tuckoricz A, Interthal H et al (1994) Structure of the bovine lactoferrin-encoding gene and its promoter. Gene 143 (2):265–269

Sherman MP, Bennett SH, Hwang FF et al (2004) Neonatal small bowel epithelia: enhancing anti-bacterial defense with lactoferrin and Lactobacillus GG. Biometals 17:285–289

Shestakov A, Jenssen H, Nordström I et al (2012) Lactoferricin but not lactoferrin inhibit herpes simplex virus type 2 infection in mice. Antivir Res 93:340–345

Shirkhani RM, Joo Lee E, Talukder J (2018) Mechanism of absorption and transportation of ovotransferrin in the intestine. FASEB J 32 (1 Suppl). (Abstract Number 747.17)

Siciliano R, Rega B, Marchetti M et al (1999) Bovine lactoferrin peptidic fragments involved in inhibition of herpes simplex virus type 1 infection. Biochem Biophys Res Commun 264:19–23

Superti F, Ammendolia MG, Valenti P et al (1997) Antirotaviral activity of milk protein: lactoferrin prevents rotavirus infection in the enterocyte-like cell line HT-29. Med Microbiol Immunol 186:83–91

Superti F, Siciliano R, Rega B et al (2001) Involvement of bovine lactoferrin metal saturation, sialic acid and protein fragments in the inhibition of rotavirus infection. Biochim Biophys Acta 1528:107–115

Superti, F., Ammendolia MG, Berlutti, F., et al (2007). Ovotransferrin. In Bioactive egg compounds (pp. 43–50). Heidelberg: Springer. https://doi.org/10.1007/978-3-540-37885-3_7

Tachezy J, Kulda J, Bahnikova I et al (1996) *Tritrichomonas fetus*: Iron acquisition from lactoferrin and transferrin. Exp Parasitol 83:216–228

Talukder MJR, Harada E (2007) Bovine lactoferrin protects lipopolysaccharide-induced diarrhea modulating nitric oxide and prostaglandin E_2 in mice. Can J Physiol Pharmacol 85(2):200–208

Talukder MJR, Takeuchiand T, Harada E (2002) Transport of colostral macromolecules into the cerebrospinal fluid via plasma in newborn calves. J Dairy Sci 85:514–524

Talukder MJR, Takeuchiand T, Harada E (2003) Receptor mediated transport of lactoferrin into the cerebrospinal fluid via plasma in young calves. J Vet Med Sci 65:957–964

Talukder J, Srivastava A, Ray A, Lall R (2018) Treatment of infectious endometritis with a novel protein, VPI-O22, in cows. FASEB J 32 (1 Suppl). (Abstract Number 882.12)

Tan AT, Woodworth RC (1969) Ultraviolet difference spectral studies of conalbumin complexes with transition metal ions. Biochemistry 8 (9):3711–3716

Tanaka T, Abe Y, Inoue N et al (2004) The detection of bovine lactoferrin binding protein on *Trypanosoma brucei*. J Vet Med Sci 66:619–625

Valenti P, Antonini G (2005) Lactoferrin: an important host defense against microbial and viral attack. Cell Mol Life Sci 62:2576–2587

Valenti P, Antonini G, Fanelli MR et al (1982) Antibacterial activity of matrix-bound ovotransferrin. Antimicrob Agents Chemother 21 (5):840–841

Valenti P, Visca P, Antonini G et al (1986) Interaction between lactoferrin and ovotransferrin and Candida cells. FEMS Microbiol Lett 33:271–275

van der Kraan MIA, Groenink J, Nazmi K et al (2004) Lactoferrampin: a novel antimicrobial peptide in the N1-domain of bovine lactoferrin. Peptides 25:177–183

Viejo-Díaz M, Andres M, Fierro JF (2004) Modulation of *in vitro* fungicidal activity of human lactoferrin against *Candida albicans* by extracellular cation concentration and target cell metabolic activity. Antimicrob Agents Chemother 48:1242–1248

Wakabayashi H, Abe S, Okutomi T et al (1996) Cooperative anti-*Candida* effects of lactoferrin or its peptides in combination with azole antifungal agents. Microbiol Immunol 40:821–825

Wakabayashi H, Uchida K, Yamauchi K et al (2000) Lactoferrin given in food facilitates dermatophytosis cure in guinea pig models. J Antimicrob Chemother 46:595–601

Wakabayashi H, Kurokawa M, Shin K et al (2004) Oral lactoferrin prevents body weight loss and increases cytokine responses during herpes simplex virus type 1 infection of mice. Biosci Biotechnol Biochem 68:537–544

Wakabayashi H, Takakura N, Yamauchi K et al (2006) Modulation of immunity-related gene expression in small intestines of mice by oral administration of lactoferrin. Clin Vaccine Immunol 13:239–245

Wakabayashi H, Oda H, Yamauchi K et al (2014) Lactoferrin for prevention of common viral infections. J Infect Chemother 20 (11):666–671

Wally J, Buchanan SK (2007) A structural comparison of human serum transferrin and human lactoferrin. Biometals 20:249–262

Weinberg GA (1994) Iron chelators as therapeutic agents against *Pneumocystis carinii*. Antimicrob Agents Chemother 38:997–1003

Weng T, Chen L, Shyu H et al (2005) Lactoferrin inhibits enterovirus 71 infection by binding to VP1 protein and host cells. Antivir Res 67:31–37

Williams J (1968) A comparison of glycopeptides from the ovotransferrin and serum transferrin of the hen. Biochem J 108 (1):57–67

Wu J, Acero-Lopez A (2012) Ovotransferrin: Structure, bioactivities, and preparation. Food Res Int 46(2):480–487

Xu YY, Samaranayake YH, Samaranayake LP et al (1999) *In vitro* susceptibility of *Candida* species to lactoferrin. Med Mycol 37:35–41

Yamauchi K, Tomita M, Giehl TJ et al (1993) Antibacterial activity of lactoferrin and a pepsin derived lactoferrin peptide fragment. Infect Immun 61:719–728

Yamauchi K, Wakabayashi H, Shin K et al (2006) Bovine lactoferrin: benefits and mechanism of action against infections. Biochem Cell Biol 84:291–296

Yen M, Chiu C, Huang Y et al (2011) Effects of lactoferrin-containing formula in the prevention of enterovirus and rotavirus infection and impact on serum cytokine levels: a randomized trial. Chang Gung Med J 34:395–402

Zarember KA, Sugui JA, Chang YC et al (2007) Human polymorphonuclear leukocytes inhibit *Aspergillus fumigatus* conidial growth by lactoferrin-mediated iron depletion. J Immunol 178:6367–6373

Zavaleta N, Figueroa D, Rivera J et al (2007) Efficacy of rice-based oral rehydration solution containing recombinant human lactoferrin and lysozyme in Peruvian children with acute diarrhea. J Pediatr Gastroenterol Nutr 44:258–264

Colostrum Antibodies, Egg Antibodies and Monoclonal Antibodies Providing Passive Immunity for Animals

Dan DuBourdieu

Abstract

Passive immunity can be provided to animals by several sources of antibodies including from colostrum, avian eggs, and monoclonal sources. These antibodies have been shown protect production and companion animals from a number of pathogens. This chapter reviews the immune system for the principles of immune response to antigens and the synthesis of immunoglobulins of the five classes of antibodies in the body. Colostrum antibodies are described for passive immunity protection in animals such as calves. Chicken egg antibodies are another source of antibodies for passive immunity. Therapeutic monoclonal antibodies are also used to provide passive immunity in the veterinary field.

Keywords

Passive immunity · Colostrum antibodies · Egg antibodies · Monoclonal antibodies

1 Introduction

The use of antibodies by veterinarians to maintain the health of animals has a long history. Fundamentally, when it comes to the immune system health of production and companion animals, there are little absolute differences in the intended purpose of the immune system. Mammals have the same basic immune system with minor differences between the species. Even the differences between birds and mammals are not so great since the purpose of the immune system is to keep infectious microorganisms, such as certain bacteria, viruses, and fungi, out of the body and to destroy any infectious microorganisms that do invade the body. How veterinarians take advantage of the immune system to maintain health can roughly be defined as taking advantage of the body's inherent method of maintaining health through adaptive immunity provided by vaccinations to generate antibodies inside the animal or by administering preformed antibodies to an animal through a process called passive immunity.

D. DuBourdieu (✉)
Vets Plus Inc., Menomonie, WI, USA
e-mail: dand@vets-plus.com

2 The Immune Response

Evolution has produced an amazing immune system in animals that utilizes various cell types and proteins to protect them from invasive organisms. This system has two broad categories: nonadaptive and adaptive. The nonadaptive immune system is mediated by cells that respond in a non-specific manner to foreign substances. This response includes phagocytosis by macrophages, secretion of lysozymes by lacrimal cells, and cell lysis by natural killer cells. The adaptive immune response is mediated by lymphocytes that produce a set of proteins called antibodies that are either secreted by or found on the surface of the lymphocyte. When the antibodies themselves are created within the animal following vaccination or from exposure to pathogens, the process is called adaptive immunity. When preformed antibodies from a host animal are given to another recipient animal, such as it offspring or even a completely different species animal, the process is called passive immunity.

3 Veterinary Vaccines for Active Immunity

Scientists have long taken advantage of the adaptive immune system by using vaccines.

Vaccines for animal diseases were the first to result from laboratory-based scientific investigation. French chemist Louis Pasteur developed a vaccine for chicken cholera in 1879, and one for anthrax of sheep and cattle in 1881.

R. C. Gupta et al. (eds.), *Nutraceuticals in Veterinary Medicine*, https://doi.org/10.1007/978-3-030-04624-8_18

The major goals of veterinary vaccines are to improve the health and welfare of companion animals, increase production of livestock in a cost-effective manner, and prevent animal-to-human transmission from both domestic animals and wildlife. These diverse aims have led to different approaches in the development of veterinary vaccines from crude but effective whole-pathogen preparations to molecularly defined subunit vaccines, genetically engineered organisms or chimeras, vectored antigen formulations, and naked DNA injections (Meeusen et al. 2007). It has also resulted in various guidelines for vaccinations of companion animals such as for dogs (Ford et al. 2017) and production animals such as swine (Alabama and Auburn 2018), in poultry (Stewart-Brown 2018), cow/calf (Missouri 2018), and other production animals. Successful veterinary vaccines have been produced against viral, bacterial, protozoal, and multicellular pathogens, which in many ways have led the field in the application and adaptation of novel technologies.

4 Passive Immunity

Whereas active immunity refers to the process of exposing the individual to an antigen to generate an adaptive immune response, passive immunity refers to the transfer of antibodies from one individual to another (Marcotte and Hammarström 2015). Passive immunity provides only short-lived protection, lasting from several weeks to up to 3–4 months, but is immediate. Nature intended passive immunity to occur when maternal antibodies are transferred to the fetus through the placenta or from breast milk to the gut of the infant. However, it can also be produced artificially when antibody preparations are derived from sera or secretions of immunized donors and are delivered via oral or systemic routes to nonimmune individuals. Passive immunization is a new approach to providing protection to animals against pathogens because of the emergence of new and drug-resistant microorganisms, diseases that are unresponsive to drug therapy and individuals with an impaired immune system who are unable to respond to conventional vaccines.

5 Antibodies

The immune system can respond specifically to millions of different molecules and is constantly challenged by huge numbers of antigens. A major feature of the immune system is that it can synthesize a vast number of antibodies. Each of these antibodies can bind to a different antigen. This binding is the basis for the molecular specificity of the immune response.

Antibodies are proteins produced by a type of terminally differentiated B lymphocytes. B cells take the B name from chicken bursa cells where they were first discovered (Gitlin and Nussenzweig 2015). Antibodies are produced in response to the presence of foreign molecules in the body. The antibodies circulate throughout the blood and lymph where they bind to the foreign antigens. Once bound, these antibody-antigen complexes are removed from circulation, primarily through phagocytosis by macrophages. This is the basis for antibodies protecting the animal against pathogens.

Antibodies are a large family of glycoproteins that share key structural and functional features. From a structure standpoint, antibodies look like a Y-shaped molecule (Fig. 1). Each Y contains four polypeptides. Two of the polypeptides are identical and called heavy chain. The other two, also identical, are called light chain and are connected by disulfide bonds. There are five classes of antibodies, IgG, IgM, IgA, IgE, and IgD, that are classified based on the number of Y-like units and the type of heavy-chain polypeptide they contain (Fig. 2).

6 Antibody Classes

IgM is the largest antibody, and it is the first to appear in response to initial antigen exposure. The spleen, where plasma cells responsible for antibody production reside, is the major site of specific IgM production (Capolunghi et al. 2013; Marchalonis et al. 2002). IgG is the main type of antibody found in blood and extracellular fluid allowing it

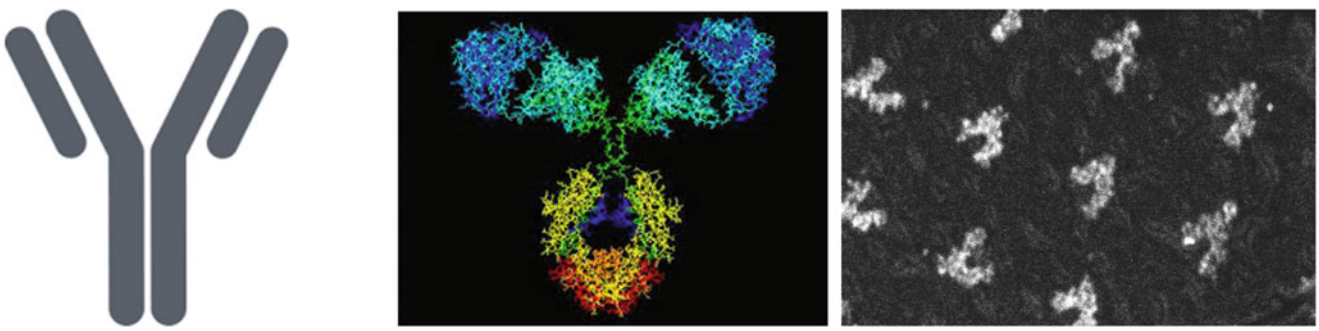


Fig. 1 Antibody structure as drawn, by protein model and by electron microscopy

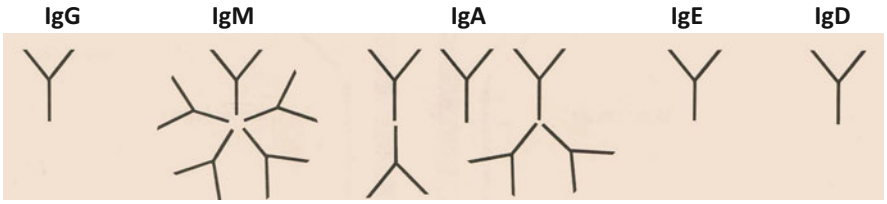


Fig. 2 Structures of the five classes of antibodies

to control infection within body tissues. Approximately 80% of all antibodies in humans and companion animals are of the IgG class. Immunoglobulin A (IgA) plays a crucial role in the immune function of mucous membranes. The amount of IgA produced in association with mucosal membranes is greater than all other types of antibodies combined (Fagarasan and Honjo 2003; Holmgren and Czerkinsky 2005; Snoeck et al. 2006). IgD was initially thought to be a recently evolved antibody class because it was only detected in primates, mice, rats, and dogs and not guinea pigs, swine, cattle, sheep, and frogs (Preud'homme et al. 2000). However, recent discoveries of IgD in ancient vertebrates suggest that IgD has been preserved in evolution from fish to humans for important immunological functions (Chen and Cerutt 2011). Immunoglobulin E (IgE) has only been identified in mammals. IgE's main function is immunity against parasites such as helminths (Erb 2007) like *Schistosoma mansoni*, *Trichinella spiralis*, and *Fasciola hepatica* (Watanabe et al. 2005; Pfister et al. 1983). IgE also has an essential role in type I hypersensitivity (Gould et al. 2003) which manifests in various allergic diseases, such as allergic asthma, most types of sinusitis, allergic rhinitis, food allergies, and specific types of chronic urticaria and atopic dermatitis (Mueller et al. 2016).

7 Antibody-Antigen Interactions

Antibodies bind antigens at the upper tips of the Y molecule. The region of antigen where binding occurs is called the epitope (Fig. 3).

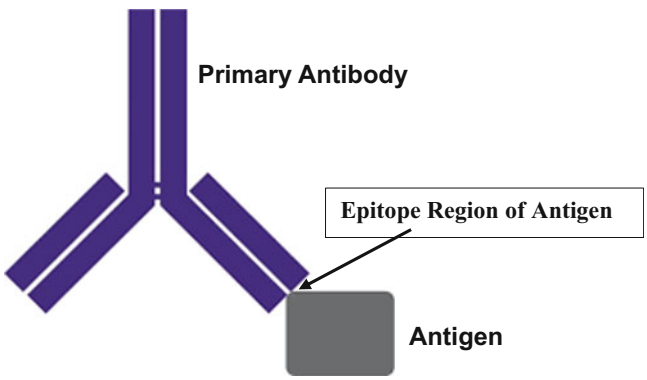


Fig. 3 Antibody binding to an antigen

Antibodies can bind to a wide range of chemical structures and can discriminate among related compounds. How well the antibody binds to an antigen is known as affinity. This affinity can range from low to high.

8 Colostrum and Passive Immunity

Mammals are born without a fully functional adaptive immune system even though the basic elements are present. When a mammal is born, it emerges from the sterile uterus into an environment where it is immediately exposed to a host of microorganisms. The gastrointestinal tract (GIT) acquires a complex microbial flora within hours. If it is to survive, the newborn animal must be able to control this microbial invasion. In practice, the adaptive immune system takes some time to become fully functional, and innate mechanisms are responsible for the initial resistance to infection. In some species with a short gestation period, such as mice, the adaptive immune system may not even be fully developed at birth. In animals with a long gestation period, such as domestic mammals, the adaptive immune system is fully developed at birth but cannot function at adult levels for several weeks. The complete development of active immunity depends on antigenic stimulation. The proper development of B cells and B-cell receptor diversity requires clonal selection and antigen-driven cell multiplication. Thus, newborn mammals are vulnerable to infection for the first few weeks of life. They need assistance in defending themselves at this juncture. Temporary help is provided by the mother in the form of colostrum, which contains antibodies. The passive transfer of immunity from mother to newborn is essential for survival.

Calves are born without an active immune system and rely on the consumption of antibodies for protection from disease such as scours and pneumonia. The cow provides its calf with nutrients for growth and development during gestation, but the cow cannot directly provide the calf with antibodies to protect it from diseases. Fortunately, immunoglobulins form an important component of the immunological activity found in milk and colostrum. While humans have a large amount of IgA in their colostrum, the colostrum from most other animals contains a high percentage of IgG (Hurley and Theil 2011) (Fig. 4). Immunoglobulins found in mammary secretions arise from systemic and local sources. In the case

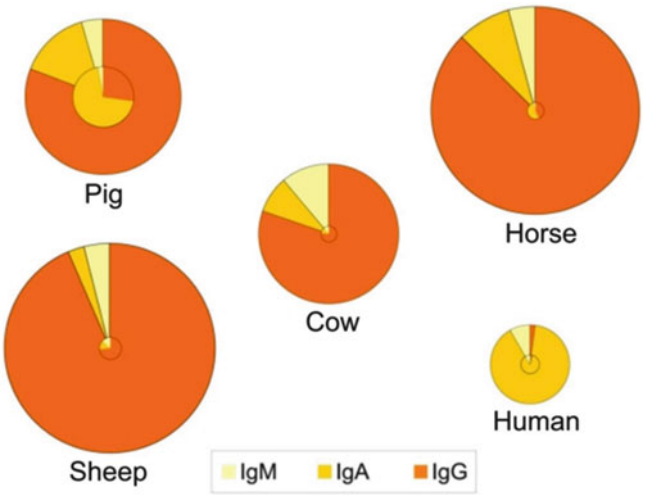


Fig. 4 Relative distribution of IgG, IgA, and IgM in colostrum (outer circle) and in milk (inner circle) of five species

of IgG in milk, the major portion comes from the serum (Mayer et al. 2005). While plasma cells producing IgG may occur within the mammary tissue, their contribution to the IgG in colostrum is minor compared with the IgG derived from serum.

The other major classes of immunoglobulins transported into colostrum and milk are IgA and IgM. Immunoglobulin A (IgA) is the major immunoglobulin in human colostrum and milk; however, it is also present in milk of most other species. Colostrum and milk IgA and IgM are found in the form of secretory IgA, or sIgA, and sIgM. Much of this is produced by plasma cells in the mammary tissue. The plasma cells are part of the gut-associated lymphoid tissue (GALT), the largest immune organ of an organism, which includes the Peyer's patches, lymphoid and myeloid cells in the lamina propria, and intraepithelial lymphocytes (Ishikawa et al. 2005). Interestingly enough, more than 70% of the immune system is located in the gastro intestinal tract, the site where many oral pathogens first interact with an animal (Vighi et al. 2008). GALT is a part of the mucosa-associated lymphoid tissue and works in the immune system to protect animals from invasion of pathogens in the gut. One of the physiological functions of the mucosa in the gut is for food absorption. However, of equal importance of the GALT is in the body's defense, due to its large population of plasma cells whose number exceeds the number of plasma cells in the spleen, lymph nodes, and bone marrow combined (Nagler-Anderson 2001).

Lymphocytes from the GALT system will move to the mammary gland and provide a direct link between the antigen exposure response in the mother's mucosa system and the secretory immunoglobulins of the mammary gland (Brandtzaeg 2010). As such, this means that maternal colostrum and milk will contain antibodies specific for pathogens that may be encountered by the neonate's intestine and other mucosal tissues. This provides a rationale for the observations that bovine colostrum from nonimmunized cows may also afford passive immune protection against human pathogens in both humans and animals (Li-Chan et al. 1994; Yolken et al. 1985) and opens the door to new technology to provide veterinarians another way to protect animals from pathogens that does not involve antibiotics.

Antibodies must be obtained by drinking colostrum within the first couple of hours after birth as part of the passive immunization system in order to maximize antibody absorption (Pakkanen and Aalto 1997). Like other animals, antibodies are generated by healthy cows as a result of every day exposure to infectious agents. Antibodies can also be the result of specific vaccination programs. The cow's natural antibodies to these infectious agents are passed from the cow to the calf through colostrum. The level of antibodies transmitted from the cow through the colostrum can be elevated by a pre-calving vaccination program (Thomas 2017).

When the calf drinks colostrum, the maternal derived antibodies are absorbed from the calf's GIT into the blood stream. Some of the immunoglobulins also remain in the gut where they can neutralize pathogenic bacteria and help prevent the development of diarrhea. The absorption of antibodies from the GIT into the bloodstream is called passive transfer. Failure of passive transfer (FPT) in dairy calves is defined by a blood IgG level of <10 mg/mL 24–48 h after birth (Stilwell and Carvalho 2011). Calves that experience FPT are more likely to become sick or die in the first 2 months of life than calves with adequate immunity. Many factors can contribute to FPT, but colostrum and the management of colostrum feeding are often involved. To successfully obtain passive transfer and provide the calf with protection from diseases, it is thought that the calf needs to consume a minimum of 150–200 g of immunoglobulins (Meganck et al. 2014).

The pathway between the gastrointestinal tract and the bloodstream is only open for a short window of time. Research shows that this pathway starts to close shortly after birth, and after 8–12 h, approximately 50% of the calf's ability to absorb colostrum antibodies is gone. It has therefore been recommended to feed calves as much colostrum as they want by bottle within 1–4 h after birth and at 12 h of age to substantially reduce the probability of FPT (Chigerwe et al. 2009; Trotz-Williams et al. 2008).

Colostrum also provides the calf with protein, energy in the form of fat and sugar, and vitamins (Quigley and Drewry 1998). Some vitamins do not cross the placental barrier, and colostrum is the primary source of these nutrients for the calf after birth. Energy is required for all metabolic functions including maintenance of body temperature. One of the leading causes of death in dairy calves is failure to initiate breathing and metabolic processes in the first hours of life. The

newborn calf only has a few hours of energy reserves in stored fat and therefore needs the energy from colostrum. Research also confirms that the sooner a calf consumes colostrum, the more maternal antibodies it can utilize.

The quality of colostrum is a major issue that the dairy industry faces on a regular basis. Generally speaking, quality of colostrum is related to the amount of antibody that is present. Colostral IgG concentration is an important factor that affects whether calves receive sufficient passive immunity (Godden et al. 2012). Unfortunately, the amount of IgG in maternal colostrum varies dramatically among cows (< 1–235 g/l) with 29.4–57.8% of samples that do not reach the desired amount of 50 g IgG/l (Gulliksen et al. 2008). Colostral quality is difficult to estimate by the farmer based on produced volume or appearance of the colostrum. There are many variables that impact colostrum quality, including nutrition, the time the cow is milked, heat stress, and stage of lactation. A study from Iowa State University suggests that a minimum 30% of dairy calves in the USA are currently being fed colostrum classified below industry standards for IgG content and are at a greater risk of FPT, mortality, and morbidity (Morrill et al. 2011).

Other production mammals such as piglets face the same issues as calves for colostrum quality. Studies have shown that on average 25% of pigs with a colostrum intake below 200 g usually die. In pigs with a colostrum intake below 100 g, mortality was as high as 65% (Devillers et al. 2011). Giving spray-dried bovine colostrum to other animals such piglets has been shown (Sty et al. 2006) to help protect against gut dysfunction and inflammation. It may be possible that using bovine colostrum for piglets could help supplement sow colostrum. Research has also been done in foals with an enhanced bovine colostrum supplementation (Fenger et al. 2016).

9 Colostrum Programs

Since up to 50% of cows have colostrum with an IgG level below 50 mg/mL, which will not prevent FPT, a variety of programs and protocols have been implemented by dairy producers. High-quality maternal colostrum is still the gold standard for feeding newborn calves. However, colostrum supplement and replacer products can be valuable tools to increase calf immunity when colostrum supplies are limited or disease eradication is desired. Colostrum products that contain IgG are regulated by the USDA Center for Veterinary Biologics and are available in bolus, gel, and powder formats. Supplement products are unable to raise the blood concentration of IgG above the species standard, which is 10 mg/mL for calves. Any product that is able to raise serum IgG concentration above 10 mg/mL may be called a colostrum replacer (Penn State 2017). Supplements do not contain sufficient quantities of antibodies to raise the blood IgG level in calves beyond what average quality colostrum will do. Colostrum replacer contains greater levels of IgG and other nutrients and provides an effective, convenient method of providing passive immunity to calves when maternal colostrum is not available.

Colostrum supplements available today are made from dried bovine colostrum or serum and contain 40–60 g of IgG per dose (9–13% globulin protein). The fat content of these products ranges from 0.5 to 15%. Spray-dried colostrum with high concentrations of immunoglobulin may be produced economically and used as an effective and convenient colostrum replacer in newborn calves (Chelack et al. 1993). Numerous products designed to replace colostrum are now on the market. These products are made from bovine colostrum or serum and contain 100–150 g of IgG per dose. These products also provide fat, protein, vitamins, and minerals needed by the newborn calf, although the amount varies between products. A summary (Pennsylvania State University 2017) of treatment means from 26 published studies investigating colostrum products indicated that replacer products provided an average of 157 g of IgG, with an absorption efficiency of 31%, and serum IgG of 12 mg/mL. Supplement products (fed in addition to colostrum) provided 136 g of IgG with 19% absorption efficiency and resulted in serum IgG of 9 mg/mL.

It has been recognized that while antibodies found in colostrum can certainly reduce diseases in animals via passive immunity when given to a newborn, they will only work if the colostrum donor animal has been exposed either naturally to the disease or given a vaccine to the disease, in order to have specific antibodies produced. Bovine colostrum that is typically spray-dried for supplements or replacer will contain only the antibodies that the cow may have encountered naturally. Therefore, the colostrum used may not have specific antibodies against particular diseases that a producer might be interested in. The animal industry has recognized this issue and has developed methods to produce specific antibodies in high titer against specific diseases that can be delivered in colostrum products. This is achieved by hyperimmunizing animals such as cows against specific animal diseases, collecting colostrum and processing it into powders such as by freeze-drying methods and then giving to a newborn animal in gels or boluses. A number of diseases including bacterial diseases like *E. coli* (Selim et al. 1995) and viral diseases such as rotavirus and coronavirus (Combs et al. 1993) or *coccidial* diseases such as *giardia* or *cryptosporidia* (Graczyk et al. 1999; Fayer et al. 1989; Naciri et al. 1994) have been researched for hyperimmunized colostrum efficacy. The hyperimmunized colostrum is collected, processed, and given to newborn calves. To varying degrees of success, these hyperimmunized colostrum antibodies have been proven to be successful in providing passive immunization.

Besides calves, research has been done in a variety of production animals using bovine or other sources of colostrum. For example, lambs have been supplemented with ewe colostrum as well as hyperimmunized serum from sheep against *E. coli* (Pommer 2010). Other research has examined vaccination of cows with clostridial antigens and passive transfer of clostridial antibodies from bovine colostrum to lambs (Clarkson et al. 1985). Piglets have also been given passive protection against porcine epidemic diarrhea by hyperimmune bovine colostrum (Shibata et al. 2001).

Specific antibodies in a hyperimmunized colostrum-derived product can be used while complementing early colostrum feeding and can be delivered at the same time as colostrum. There are some advantages to this strategy as specific immunoglobulins immediately fight at the gut level to protect against diseases that destroy the intestinal lining while also allowing for antibodies to be absorbed into the bloodstream. It's important to protect the intestinal lining because if the cells that line the digestive tract become damaged, milk cannot be digested or absorbed by the calf.

10 Vaccination of Newborn Animals While Receiving Colostrum

Besides the quality of the colostrum itself, researchers first believed that calves could not be vaccinated effectively while they had circulating maternal antibodies from the colostrum in their system. Preweaning calves can respond to vaccination stimulation as early as 1 month of age. The maternal antibodies absorbed from colostrum, however, cannot distinguish between the antigens of a natural challenge and the antigens in a vaccine. Therefore, colostrum antibodies can interfere with the immune response to a vaccination (Niewiesk 2014). Work continues to be done to develop ways to circumvent maternal antibody interference.

Vaccination of calves in the face of maternal antibodies (IFOMA) often does not result in seroconversion as maternally derived immunity interferes with the activation of adequate antibody responses to vaccination. However, it can prime T- and B-cell memory responses that protect calves against clinical disease when maternal immunity has decayed. The activation of B- and T-cell memory responses in calves vaccinated IFOMA varies and is affected by several factors, including age, level of maternal immunity, type of vaccine, and route of administration. These factors influence the adequate priming of humoral and cell-mediated immune responses and the outcome of vaccination. Failure to adequately prime immune memory after vaccination IFOMA could result in lack of clinical protection and an increased risk of viremia and/or virus shedding (Chamorro et al. 2016).

There is obviously some controversy about whether newborn calves should be vaccinated (Cook et al. 2003). It is thought that the process of the calf mounting an immune response to a vaccine requires energy that could better be used to fight off disease and gain weight and the response could actually be detrimental to the early health of that calf. On the other hand, while maternal antibodies can block response to vaccination, sometimes they do not (Woolums 2007). The exact immunologic outcome in calves vaccinated IFOMA can vary, and this variation likely depends on many factors. These factors are not well characterized but likely include the nature of the vaccine administered, number of doses administered, age of the calf and level of maternal antibody present in the calf, and the means by which a protective response is defined. Guidelines for vaccinating newborn animals such as calves require additional research to clarify the IFOMA vaccination reactions.

11 Antibody Products

Fortunately, producers also have the option of using antibody products in order to generate immediate protection in situations where colostrum quality is poor. Antibody products complement colostrum feeding because they can be fed at the same time. These products are available in bolus, gel, and powder form. They also are included in some colostrum replacer and supplement formulas for added value. Typically, the antibody products are from hyperimmunized cows, and the specific antibody is found in the colostrum. The colostrum is processed and typically fractionated into a semi-purified preparation of antibody that is freeze-dried and put into capsules or boluses. Because antibody boluses can be fed in conjunction with colostrum, they can be a tool to help the calf not only achieve adequate passive transfer but also provide enough specific antibodies to protect against the most common early calf hood diseases.

Antibody products do not require the calf to react to a vaccine in order to develop antibodies. Rather specific antibodies against various diseases are already present, measured, and verified to be at a high enough level to protect the calf from scour-related diseases, and they can be fed as close to birth as possible. United States Department of Agriculture (USDA)-approved antibody products are available on the market that can be fed in conjunction with colostrum and provide the calf with immediate immunity. These antibodies go to the gut to immediately bind and neutralize diarrhea antigens while also being absorbed into the blood stream for extended protection (Combs et al. 1993; Chamorro et al. 2014). Another advantage of this approach is that providing specific antibody can potentially avoid vaccine stimulation.

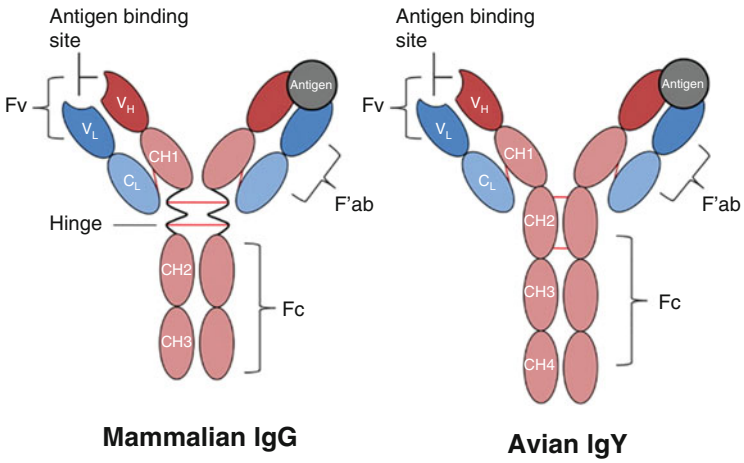


Fig. 5 Structure of mammalian IgG compared to avian IgY

Avoiding vaccine stimulation can allow a calf to conserve its minimal supply of fat and nutrients that are critical to get the calf through its first few days of life.

12 Passive Immunity by Egg Antibodies

Cows pass their immunity to their offspring by colostrum, and various colostrum products are on the market to achieve passive immunity. Additional sources of antibody products can be utilized in a similar manner to achieve passive immunity. These are from avian sources such as chickens. In birds, passive transfer of immunity occurs through the egg. By hyperimmunizing chickens over a period of time with inactivated multivalent bacterial or viral vaccines, this procedure results in the production of polyclonal immunoglobulins of the IgY class (specific to avians) directed against the stimulating organisms.

Oral consumption of the "immune" eggs containing specific IgY antibodies protects the animal against the specific organism(s) with which the hen was stimulated. Unfortunately, the eggs really cannot be cooked since heat dentures the antibodies found in the eggs. Other physical parameters such as an acidic pH will also destroy the antibodies to a certain extent. However, enough orally administrated IgY may survive passage through the GIT and, after excretion, still retain a great deal of its antigen-binding ability indicating that orally administrated IgYs are useful for passive immunity.

13 Eggs as a Natural Source of Immunoregulatory Factors

Just as immune protection is transferred in utero in mammals or passively by a lactating mother via colostrum, hens passively transfer protection to their young by secreting immunoglobulin and other immune factors into their eggs for use by the hatching chick. The transfer of chicken immunoglobulins from the hen's serum to the yolk and from the yolk to the chick is analogous to cross-placental transfer of IgG from the mammalian mother to its offspring. While IgM and IgA are found in chicken eggs, the principle immunoglobulin is IgY which is found in the yolk of the egg (Hamal et al. 2006). IgY (Y stands for yolk) is an immunoglobulin class specific to avians and analogous in function to that of mammalian immunoglobulins. IgY has a similar structure as mammalian IgG with some minor differences in the heavy chains (Fig. 5).

Both eggs and milk (including breast milk) contain naturally occurring antibodies, and there are reports of immunomodulatory factors in milk as well (Li et al. 2017). However, immunoglobulin levels in eggs can be significantly higher than levels found in serum or milk (Woolley and Landon 1995). This may not be surprising since mammals have a considerably longer time of weeks or months during which they may passively transfer immunoglobulin and immune factors, while the hen has a single opportunity (the egg) to transfer all necessary survival components to its offspring. All the aspects that the chick needs to survive must be in the egg. Because egg products are a common source of protein in human diets and eggs contain antibodies and immune factors, it was obvious to utilize egg antibodies to provide passive immunity to animals. As such, this has resulted in a number of commercial egg antibody products on the market for production animals as well as companion animals to prevent various diseases. With few exceptions, oral consumption of specific antibodies has been reported (Diraviyam et al. 2014) to protect both humans and animals. Furthermore, in vitro studies with specific IgY antibodies have been found to inhibit processes associated with bacterial growth, adhesion to intestinal cells, and toxin production (Sugita-Konishi et al. 1996).

Meta-analysis has demonstrated the beneficial effects of IgY (Diraviyam et al. 2014) for a variety of animals. This

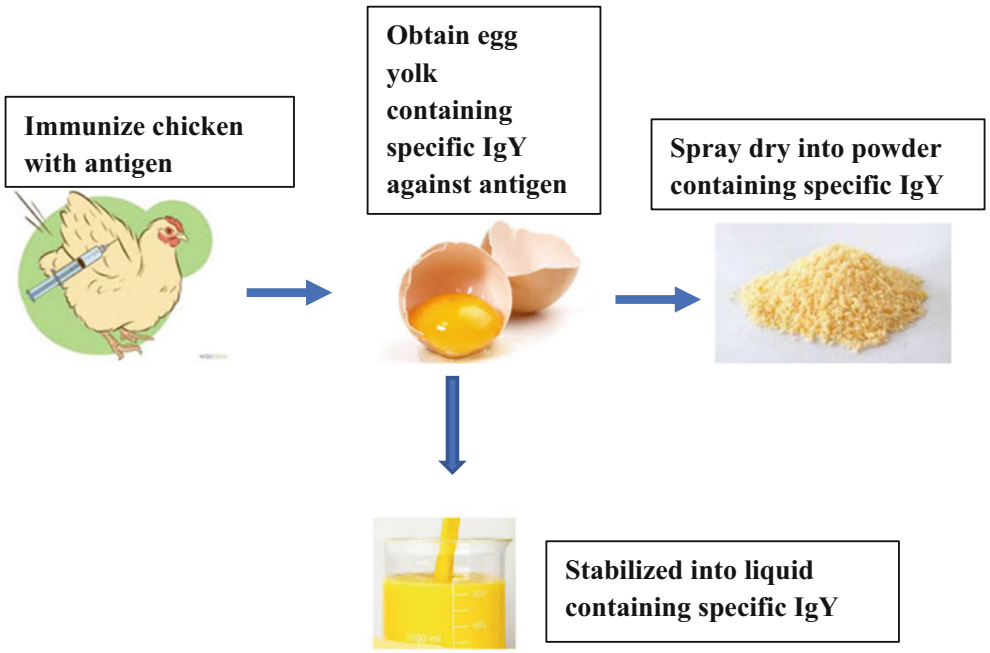


Fig. 6 Vaccination for specific antibodies and processing egg yolk strategies

analysis supports the opinion that IgY is useful for prophylaxis and treatment. Currently, the oral passive immunization using chicken IgY has been focused as an alternative to antibiotics for the treatment and control of diarrhea. IgY has been demonstrated to be effective in controlling and preventing diarrhea in humans and in animals including piglets (Cui et al. 2012; Vega et al. 2012), mice (Buragohain et al. 2012), poultry (El-Ghany 2011; Farooq et al. 2012), and calves (Cook et al. 2005; Germine et al. 2011; Vega et al. 2011).

Most commercially available chickens are immunized at birth to protect them from avian diseases. The only difference between such supermarket eggs and "immune" eggs is that the latter are from chickens that have received additional proprietary vaccinations with other inactivated pathogens known to be the etiologic agents of animal infections. A wide range of bacteria, viruses, and coccidia have been used in vaccines for producing commercial IgY products. A partial list includes *Shigella dysenteriae*, *Staphylococcus epidermidis*, *Escherichia coli*, *E. coli* K99, *Salmonella enteritidis*, and *S. typhimurium*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Haemophilus influenzae*, species of *Streptococcus*, *Salmonella Dublin*, *Salmonella anatum*, *Clostridium Perfringens* type A and type C toxoids, rotavirus types 1 and 2, coronavirus, reovirus, parvovirus, and *Cryptosporidium parvum* (Schade et al. 2005). Only the chicken (not the egg) is exposed to inactivated pathogens. Immunoglobulins and other immune factors are passively transferred to the egg from the serum for use by the chick and, more importantly, for passive immunity for other animals.

After appropriate times following vaccination, eggs are collected from the specially designated chicken flocks, washed and broken, and the yolk and egg white are typically dried to a fine proteinaceous powder. Various processes have been developed to help minimize heat damage to egg antibodies and immunoregulatory factors during the spray-drying procedure. However, spray-drying eggs can denature the IgY to an extent, due to at least some heating during the process. Keeping the egg away from heat is an optimal way of maintaining IgY titer levels and efficacy. While freeze-drying is an expensive option for maintaining antibody titer levels, simply feeding fresh immunized eggs is another method which exists. This is to keep the immunized eggs in a stabilized liquid (DuBourdieu 2014). This stabilized liquid format is a practical and inexpensive delivery method that allows the specific IgY to be delivered in watering systems to production animals or incorporation into other delivery formats such as soft chews that can be readily given to companion animals (Fig. 6).

The effective mechanism by which avian IgY's work is effective is by the same mechanism that mammalian IgG antibodies work in the animal to provide passive immunity. For example, bacteria *E. coli* strain K88 typically causes problems in production animals that result in diarrhea and possible death. Therefore, a vaccination program is used in chickens to create specific anti-*E. coli* K88 IgY antibodies. These specific antibodies found in the yolk of the egg are given orally to animals to prevent *E. coli* K88 from causing disease. This occurs when specific anti-*E. coli* K88 IgY antibodies bind to the pathogenic bacteria. This binding blocks the ability of the pathogen to bind to the mucin layer

in the GI tract of the animal. The pathogens are flushed out of the GI tract with the feces since they have been rendered unable to bind.

The total immunoglobulin content of eggs from hyperimmunized hens is identical to the total level of immunoglobulins found in conventional table eggs. However, the quantities of immunoglobulins to selected antigens are different in the two varieties of eggs. Additionally, both the table egg and the "immune" egg contain immunoregulatory factors, but eggs from hyperimmunized chickens may contain many times greater concentrations of individual factors as compared to regular eggs. Besides immunoglobulins, eggs contain a number of bioactive components, including phospholipids, cholesterol, lutein, zeaxanthin, and proteins, that possess a variety of pro- and/or anti-inflammatory properties. Two major categories of immune components are found in "hyperimmune" egg: (1) the immunoglobulins with neutralizing specificities against the stimulating pathogens and (2) the immunoregulatory factors that modulate cellular functions. The immunoglobulins provide local protection against gastrointestinal intoxication. The immunomodulatory mediators act directly on gastrointestinal surfaces and circulate systemically, affecting every immune, physical, metabolic, and neuroendocrine pathway in the body. These may have important implications for the pathophysiology of numerous chronic diseases and immune responses to acute injury (Andersen 2015). Given the essentiality of pro-inflammatory responses in normal immune defense against pathogens, further research into the role of egg intake on immunity is warranted and may lead to further commercial uses of eggs and IgY technology.

14 Monoclonal Antibodies in Veterinary Use

Biological medicine in humans, an intervention pioneered in the last 30 years, is now on the horizon for companion animals. This strategy includes the use of monoclonal antibodies (mAbs) to selectively target proteins such as cellular receptors or soluble molecules involved in disease pathogenesis. Such treatment holds the potential for targeted therapies of chronic diseases such as osteoarthritis, atopic dermatitis, or lymphomas in dogs and cats.

Monoclonal antibodies are antibodies that are made by identical immune cells that are all clones of a unique parent cell. Monoclonal antibodies have monovalent affinity, in that they bind to the same epitope. In contrast, polyclonal antibodies bind to multiple epitopes and are usually made by several different plasma cell lineages (Fig. 7).

Given almost any substance, it is possible to produce monoclonal antibodies that specifically bind to that substance; they can then serve to detect or purify that substance. This has become an important tool in biochemistry, molecular biology, and medicine. Therapeutic mAbs can be used medically to block disease-relevant proteins (e.g., cytokines or receptors on cells) and cancer and have gained significant use in humans. They can also be used to target viruses or bacteria and aid in their destruction and elimination. Veterinary medicine is only now incorporating this tool.

Despite the success of mAbs in human medicine, there are considerably fewer in the pipeline for veterinary medicine. This may be due to uncertain regulatory guidance in both Europe and the USA. These regulatory documents are written from the perspective of human medicine-based risk assessment and development. It is recognized that this may present a challenge to veterinary mAbs manufacturers to achieve the extent of characterization/quality control testing typically required for human mAbs. (EMA 2017). Regardless, the pet medicine industry is making strides to put mAbs into the market through basic research.

Early human therapeutic mAbs contained a high proportion of mouse-derived sequences (fully mouse or mouse/human chimeric mAbs) that were recognized by the human immune system as foreign. This immune response triggered production of anti-mAbs, leading to reduced therapeutic efficacy. Subsequently, the design of humanized and fully human mAbs has resulted in a vast reduction in their immunogenicity, although most therapeutic mAbs may have some remaining immunogenicity that is not followed by apparent adverse clinical manifestations. These same issues hold true

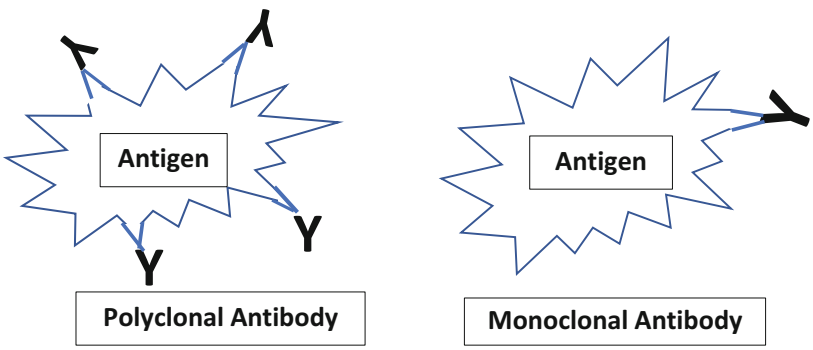


Fig. 7 Polyclonal antibody compared to monoclonal antibody binding of antigens

for making veterinary mAbs and utilizing canine mAbs for dogs that helps efficacy. It has been found that caninized anti-IgE mAbs reduce IgE hypersensitivity in mite-sensitized beagles (Gearing et al. 2013). Caninized anti-nerve growth factor mAb (Webster 2014) significantly reduced pain scores (Webster et al. 2014) in dogs, and mAbs that neutralize the pruritogenic cytokine IL-31 in dogs reduced the pruritic response for 3 weeks after injection (Dunham et al. 2014).

One of the aspects regarding commercializing therapeutic antibodies involves regulation from the USDA. In 2015, two USDA-approved monoclonal antibody treatments for B-cell and T-cell lymphomas in dogs were granted. These mAbs fight lymphoma by targeting the protein CD20, which is commonly expressed in B-cell lymphoma (Ogilvie et al. 2014; Bulman-Fleming et al. 2014). While these particular mAbs were not commercially successful, they are part of a new approach for using therapeutic antibodies in veterinary medicine. Other companies are developing veterinary mAbs for cancer, allergies, and chronic inflammatory disease such as atopic dermatitis and for the control of pain associated with osteoarthritis in dogs and cats (Webster et al. 2014). The first mAb approved for veterinary use was in the European Union (BMJ 2017). It treats the clinical signs of atopic dermatitis in dogs, including itch and inflammation, for up to 1 month. The mAb treatment works by mimicking the activity of natural antibodies to selectively bind to and neutralize interleukin-31 (IL-31), a key protein involved in cell communication which triggers itching associated with atopic dermatitis in dogs. Because it neutralizes IL-31, it has been demonstrated not to interfere with the immune response, meaning that it does not induce unintended immunosuppression or enhancement.

Most therapeutic mAbs are delivered via intravenous, intramuscular, or subcutaneous injection. This is because antibodies can't be delivered orally because of breakdown in the stomach by acids or other factors found there. Various encapsulation methodologies are required in order to overcome this basic issue, and these methods should be forthcoming. Once injected, most therapeutic mAbs, like natural antibodies, have a long half-life (about 21 days). The absolute half-life for each is unique, depending upon its concentration, distribution of its target, and, if the mAb is directed to a cell-surface receptor, clearance and elimination of the target receptor. Therapeutic mAbs have two main safety advantages: (1) they have very specific targets, and (2) they don't have intracellular activity. As a result, there are few anticipated side effects and reactions although they can occur (Catapanoab and Papadopoulosc 2013). mAbs are eventually eliminated via intracellular catabolism in the lysosome, where they are broken down into peptides or amino acids that can be either reused for synthesis of new proteins or excreted via the urine.

15 Concluding Remarks and Future Directions

Antibodies for veterinary use have great potential for the future. Passive antibody therapy in the treatment of infectious diseases is a concept which dates back more than 120 years, to the 1890s, when the use of serum from immunized animals provided the first effective treatment options against infections with *Clostridium tetani* and *Corynebacterium diphtheriae* (Hey 2015). However, due to the discovery of penicillin by Fleming in 1928, and the subsequent introduction of the much cheaper and safer antibiotics in the 1930s, serum therapy was largely abandoned. But in more recent times, the broad and general use of antibiotics in human and veterinary medicine has resulted in the development of multiresistant strains of bacteria with limited or no response to existing treatments and thus a need for alternative treatment options. This situation can be partially attributed to the overuse of antibiotics as growth promoters for production animals and other indiscriminate use.

The combined specificity and flexibility of antibody-based treatments in providing passive immunity makes them very valuable tools for designing specific antibody treatments to infectious agents. These attributes have already caused a revolution in new antibody-based treatments in oncology and inflammatory diseases, with many approved products for human use. However, only very few mAbs are approved for veterinary use. mAbs therapies are expensive, and this has been a barrier for their development in the presence of inexpensive antibiotics. The use of antibiotics as growth promoters came to an end in 2017 in the USA with the rest of the world already limiting their use for this purpose manner (FDA 2015). This opens the door to new technologies and antibodies from monoclonal sources, chicken eggs, cow colostrum, or other sources to be among the chief contenders for limiting diseases in a safe manner and potentially to act as growth promoters. For that purpose, antibodies and antibody-derived treatments offer very attractive tools and attributes to neutralize infectious agents or modulate the immune system to enable effector cells to escape immunosuppressed conditions and contribute to the elimination of infections. The ability to raise antibodies to any target, and the ability to modulate effector functions, half-life, and size of the treatment units, makes antibodies ideal for tailoring treatments for specific infectious agents. However, more research into the use of antibodies as growth promoters will need to occur.

One area that researchers are looking to make better use of antibody treatments for veterinary use includes more use of mAbs. It has been predicted that minimal amino acid changes are needed to adapt an antibody from one species to another

to avoid immune rejection. Using libraries of genetic information and algorithms to make sure that key amino acid sequences are recognized as "self" or "native" by the target species' immune system can reduce the chance of undesirable immune reactions. This will increase the advantages typical of mAbs for potency, safety, and a prolonged elimination half-life. Therefore, these second- and third-generation mAbs will be at the forefront of veterinary antibody technology along with IgY technology.

Molecular targets for therapeutic mAbs, IgY, and colostrum IgG in animals should (1) be involved in clinical signs or disease mechanism and (2) not have redundant pathways compensating for blockade of the intended target. The validity of blocking a molecule or eliminating a cell type must also be weighed against the importance of this protein or cell for desirable normal body functions. It is possible to speculate on uses of these antibodies in companion animals. These might include immune-mediated hemolytic anemias/thrombopenias, myasthenia gravis, and autoimmune blistering diseases such as pemphigus, among other conditions. For cancer, the use of mAb or IgY therapy targeting B-lymphocytes will be valuable for B-cell lymphomas in dogs and cats. In allergic diseases, the use of mAbs to inhibit production of IgE via its promoting cytokines such as interleukins IL-4/IL-13, their cytokine receptors, or IgE itself might be beneficial in dogs and cats with IgE-mediated atopic dermatitis or food allergies. The itch sensation itself could be altered, at least theoretically, by antibodies targeting itch-promoting cytokines such as IL-31, nerve growth factor, thymic stromal lymphopoietin, or neuromediators involved in itch transmission. In arthritis therapeutic mAbs that inhibit pro-inflammatory cytokines (TNF-alpha, IL1, etc.) or their receptors are likely to be of benefit in treating dogs and cats with arthritis. The usefulness of anti-NGF mAbs as an analgesic must be confirmed. In autoimmune diseases, the use of mAbs specific for B-lymphocyte surface proteins could theoretically lead to reduced production of autoantibodies.

Veterinary vaccines have had, and continue to have, a major impact not only on animal health and production but also on human health, through increasing safe food supplies and preventing animal-to-human transmission of infectious diseases. The continued interaction between animals and human researchers and health professionals will be of major importance for adapting new technologies, providing animal models of disease, and confronting new and emerging infectious diseases. One area of research where more information is needed is on factors that limit efficacy of vaccination IFOMA, particularly in calves <1 month old. This is a complex issue. However, research continues to evolve in the area of newborn calf vaccinations.

Passive immunity provided by chicken egg antibodies will gain increasing use in production animals. There is no doubt that chicken Abs can be produced and used, with minor modifications, in similar ways to mammalian Abs. It can also be said that, depending on the circumstances, the use of IgY Abs often has significant advantage over the use of mammalian Abs. However, from a realistic point of view, IgY Abs probably will not be able to completely replace the use of IgG Abs in diagnostic systems in the near future (Schade et al. 2005). Chickens have the potential to be used to complete the spectrum of animals that have been used for Ab production. However, a prerequisite is to make IgY technology more popular and to convince the scientific community of its significant advantages. An interesting possibility for the future is the production of chicken mAbs. These would combine the advantages of mAbs with the advantages of chicken Abs. It is to be expected that studies on the therapeutic or prophylactic use of IgY Abs will be intensified in the future. In particular, because of the increasing resistance of microorganisms to antibiotics, research on all aspects related to the development of specific IgY against pathogenic microorganisms will have to be intensified.

In conclusion, the next decade will see continued development of therapeutic mAbs, IgYs, and colostrum antibodies for production and companion animals in both treatment and prevention. These highly specific molecules are likely to prove beneficial to uniquely target disease mechanisms without the side effects associated with broad-spectrum pharmacotherapy. While vaccines will continue to play a very important role in maintaining the health of animals by active immunity, antibodies that provide passive immunity will be an increasing part of the arsenal available to veterinarians to promote growth in production animals in a safe manner and to maintain health in companion animals.

References

Alabama A&M and Auburn Universities (2018) Vaccinations for the swine herd. http://www.aces.edu/pubs/docs/A/ANR-0902/ANR-0902.pdf

Andersen CJ (2015) Bioactive egg components and inflammation. Nutrients 7(9):7889–7913

BMJ (2017) First antibody therapy in veterinary medicine launched for dogs in the UK. Vet Rec 181:6–7

Brandtzaeg P (2010) The mucosal immune system and its integration with the mammary glands. J Pediatr 156:S8–S15

Bulman-Fleming J, Rosenberg M, Hansen G et al (2014) Treatment of canine B-cell lymphoma with doxorubicin with or without an anti-CD20 monoclonal antibody: an open-label pilot study. In: Proceedings, 34th Annual Veterinary Cancer Society Conference, St. Louis, MO, USA

Buragohain M, Dhale GS, Ghalsasi GR et al (2012) Evaluation of hyperimmune hen egg yolk derived anti-human rotavirus antibodies (AntiHRVIgY) against rotavirus infection. World J Vacc 2:73–84

Capolunghi F, Rosado MM, Sinibaldi M et al (2013) Why do we need IgM memory B cells? Immunol Lett 152(2):114–120

Catapanoab AL, Papadopoulosc N (2013) The safety of therapeutic monoclonal antibodies: implications for cardiovascular disease and targeting the PCSK9 pathway. Atherosclerosis 228:18–28

Chamorro MF, Walz PH, Haines DM et al (2014) Comparison of levels and duration of detection of antibodies to bovine viral diarrhea virus 1, bovine viral diarrhea virus 2, bovine respiratory syncytial virus, bovine herpesvirus 1, and bovine parainfluenza virus 3 in calves fed maternal colostrum or a colostrum-replacement product. Can J Vet Res 78(2):81–88

Chamorro MF, Woolums A, Walz PH (2016) Vaccination of calves against common respiratory viruses in the face of maternally derived antibodies (IFOMA). Anim Health Res Rev 17(2):79–84

Chelack BJ, Morley PS, Haines DM (1993) Evaluation of methods for dehydration of bovine colostrum for total replacement of normal colostrum in calves. Can Vet 34(7):407–412

Chen K, Cerutt A (2011) The Function and regulation of immunoglobulin D. Curr Opin Immunol 23(3):345–352

Chigerwe M, Tyler JW, Summers MK et al (2009) Evaluation of factors affecting serum IgG concentrations in bottle-fed calves. J Am Vet Med Assoc 234:785–789

Clarkson MJ, Faull WB, Kerry JB (1985) Vaccination of cows with clostridial antigens and passive transfer of clostridial antibodies from bovine colostrum to lambs. Vet Rec 116(17):467–469

Combs DK, Bringe AN, Crabb JH et al (1993) Protection of neonatal calves against K99-*E. coli* and corona virus using a colostrum-derived immunoglobulin preparation. Agri-Practice 14(5):13–16

Cook ME, Butz D, Li GM et al (2003) Conjugated linoleic acid enhances immune responses but protects against the collateral damage of immune events. In: Sébédio JL, Christie WW, Adlof R (eds) Advances in conjugated linoleic acid research, vol 2. AOCS Press, Champaign, IL, pp 283–291

Cook S, Bach S, Stevenson S et al (2005) Orally administered anti-*Escherichia coli* O157: H7 chicken egg yolk antibodies reduce fecal shedding of the pathogen by ruminants. Can J Anim Sci 85:291–299

Cui HZ, Zhang JL, Zhang H et al (2012) Study and application of the hyperimmunized yolk antibodies against TGEV and PEDV in piglets. China Anim Husb Vet Med 39:173–175

Devillers N, Le Dividich J, Prunier A (2011) Influence of colostrum intake on piglet survival and immunity. Animal 5(10):1605–1612

Diraviyam T, Zhao B, Wang Y et al (2014) Effect of chicken egg yolk antibodies (IgY) against diarrhea in domesticated animals: a systematic review and meta-analysis. PLoS One 9(5):e9771

DuBourdieu D (2014) Stabilized liquid egg material for extended shelf life. United States Patent 8,828,422

Dunham S, Teel J, Bammert G et al (2014) Evaluation of anti-IL-31 monoclonal antibodies in a model of IL-31-induced pruritus in Beagle dogs. Vet Dermatol 25:403

El-Ghany WA (2011) Comparison between immunoglobulins IgY and the vaccine for prevention of infectious Bursal disease in chickens. Global Vet 6(1):16–24

EMA (2017) EMA/CVMP/ADVENT/307606/2017 Committee for Medicinal Products for Veterinary Use (CVMP). Questions and answers on monoclonal antibodies for veterinary use European Medicines Agency. Accessed 7 Dec 2017

Erb KJ (2007) Allergic disorders and IgE-mediated immune responses: where do we stand? Eur J Immunol 37(5):1170–1173

Fagarasan S, Honjo T (2003) Intestinal IgA synthesis: regulation of front-line body defenses. Nature Rev Immunol 3(1):63–72

Farooq A, Rabbani M, Muhammad K et al (2012) Passive immunization in infectious bursal disease virus infected birds using chemically purified immune yolk immunoglobulins (IgY). Afr J Microbiol Res 6:2993–2998

Fayer R, Andrews C, Ungar BL et al (1989) Efficacy of hyperimmune bovine colostrum for prophylaxis of cryptosporidiosis in neonatal calves. J Parasitol 75(3):393–397

Fenger CK, Tobin T, Casey PJ et al (2016) Enhanced bovine colostrum supplementation shortens the duration of respiratory disease in thoroughbred yearlings. J Equine Vet Sci 42:77–81

Food and Drug Administration (2015) Food and drug administration center for veterinary medicine veterinary feed directive guidance for industry #120

Ford RB, Larson LJ, McClure KD et al (2017) American animal hospital association canine vaccination guidelines. Trends Magaz 27–35.

Gearing DP, Virtue ER, Gearing RP et al (2013) A fully caninised anti-NGF monoclonal antibody for pain relief in dogs. BMC Vet Res 9:226

Germine SS, Ebied MH, Ibrahim FK et al (2011) Field evaluation of egg yolk antibodies in prevention and treatment of enteric colibacillosis in calves. Benha Vet Med J (special issue I):108–114

Gitlin AG, Nussenzweig MC (2015) Immunology: fifty years of B lymphocytes. Nature 517:139–114

Godden SM, Smolenski DJ, Donahue M et al (2012) Heat-treated colostrum and reduced morbidity in preweaned dairy calves: results of a randomized trial and examination of mechanisms of effectiveness. J Dairy Sci 95:4029–4040

Gould HJ, Sutton BJ, Beavil AJ et al (2003) The biology of IgE and the basis of allergic disease. Ann Rev Immunol 21:579–628

Graczyk TK, Cranfield MR, Bostwick EF (1999) Hyperimmune bovine colostrum treatment of moribund Leopard geckos (*Eublepharis macularius*) infected with *Cryptosporidium* spp. Vet Res 30 (4):377–382

Gulliksen SM, Lie KI, Solverod L et al (2008) Risk factors associated with colostrum quality in Norwegian dairy cows. J Dairy Sci 91:704–712

Hamal KR, Burgess SC, Pevzner IY et al (2006) Maternal antibody transfer from dams to their egg yolks, egg whites, and chicks in meat lines of chickens. Poult Sci 85(8):1364–1372

Hey A (2015) History and practice: antibodies in infectious diseases. Microbiol Spectr 3(2):AID-0026-2014

Holmgren J, Czerkinsky C (2005) Mucosal immunity and vaccines. Nat Med 11(4 Suppl):S45–S53

Hurley WL, Theil PK (2011) Perspectives on immunoglobulins in colostrum and milk. Nutrients 3(4):442–474

Ishikawa H, Kanamori Y, Hamada H et al (2005) Development and function of organized gut-associated lymphoid tissues. In: Mestecky J, Lamm M, Strober W, Bienenstock J, McGhee JR, Mayer L (eds) Mucosal immunology, 3rd edn. Academic, Burlington, MA

Li C, Liu Y, Jiang Y, Xu N, Lei J (2017) Immunomodulatory constituents of human breast milk and immunity from bronchiolitis. Ital J Pediatr 43:8

Li-Chan E, Kummer A, Losso JN et al (1994) Survey of immunoglobulin G content and antibody specificity in cow's milk from British Columbia. Food Agric Immunol 6:443–451

Marchalonis JJ, Jensen I, Schluter SF (2002) Structural, antigenic and evolutionary analyses of immunoglobulins and T cell receptors. J Mol Recogn 15(5):260–271

Marcotte H, Hammarström L (2015) Chapter 71 - Passive immunization: toward magic bullets. In: Mucosal immunology, vol 2, 4th edn. Academic, Amsterdam, pp 1403–1434

Mayer B, Doleschall M, Bender B, Bartyik J et al (2005) Expression of the neonatal Fc receptor (FcRn) in the bovine mammary gland. J Dairy Res 72:107–112

Meeusen NT, Walker J, Peters A et al (2007) Current status of veterinary vaccines. Clin Microbiol Rev 20(3):489–510

Meganck V, Hoflack G, Opsomer G (2014) Advances in prevention and therapy of neonatal dairy calf diarrhoea: a systematical review with emphasis on colostrum management and fluid therapy. Acta Vet Scand 56:75

Missouri (2018) Programs for the cow/calf operation. http://extension.missouri.edu/ozark/documents/Vaccination%20Protocol%20Script.pdfVaccination

Morrill KM, Conrad E, Lago A et al (2011) Nation-wide evaluation of quality and composition of colostrum fed to dairy calves in the United States. J Dairy Sci 94(E-Suppl) 1):277 10

Mueller RS, Janda J, Jensen-Jarolim E et al (2016) Allergens in veterinary medicine. Allergy 71(1):27–35

Naciri M, Mancassola R, Répérant J et al (1994) Treatment of experimental ovine cryptosporidiosis with ovine or bovine hyperimmune colostrum. Vet Parasitol 53:173–190

Nagler-Anderson C (2001) Man the barrier! Strategic defences in the intestinal mucosa. Nat Rev Immunol 1:59–67

Niewiesk S (2014) Maternal antibodies: clinical significance, mechanism of interference with immune responses, and possible vaccination strategies. Front Immunol 5:446

Ogilvie G, Proulx D, Van Horn L et al (2014) Treatment of canine B-cell lymphoma with chemotherapy and a canine anti-CD20 monoclonal antibody: a prospective double-blind, randomized, placebo-controlled study. In: Proceedings, 34th Annual Veterinary Cancer Society Conference, St. Louis, MO

Pakkanen R, Aalto J (1997) Growth factors and antimicrobial factors of bovine colostrum. Int Dairy J 7(5):285–297

Pennsylvania State University (2017). Colostrum and replacer. https://extension.psu.edu/colostrum-supplements-and-replacer

Pfister K, Turner K, Currie A et al (1983) IgE production in rat fascioliasis. Parasit Immunol 5(6):587–593

Pommer JL (2010) Sheep antiserum as an antibody supplement in newborn lambs. Sheep Goat Res J 25:45–48

Preud'homme JL, Petit I, Barra A et al (2000) Structural and functional properties of membrane and secreted IgD. Mol Immunol 37:871–887

Quigley JD, Drewry JJ (1998) Nutrient and immunity transfer from cow to calf pre- and post-calving. J Dairy Sci 98(10):2779–2790

Schade R, Sarmiento R, Calzado EJ et al (2005) Chicken egg yolk antibodies (IgY-technology): a review of progress in production and use in research and human and veterinary medicine. Altern Lab Anim 33(2):129–154

Selim SA, Cullor JS, Oelsner IE (1995) Passive immunotherapy in neonatal calves - I. Safety and potency of a J5 *Escherichia coli* hyperimmune plasma in neonatal calves. Vaccine 13(15):1449–1453

Shibata I, Ono M, Mori M (2001) Passive protection against porcine epidemic diarrhea (PED) virus in piglets by colostrum from immunized cows. J Vet Med Sci 63(6):655–658

Snoeck V, Peters IR, Cox E (2006) The IgA system: a comparison of structure and function in different species. Vet Res 37(3):455–467

Stewart-Brown B (2018) Vaccination programs in poultry. http://www.merckvetmanual.com/poultry/nutrition-and-management-poultry/vaccination-programs-in-poultry

Stilwell G, Carvalho RC (2011) Clinical outcome of calves with failure of passive transfer as diagnosed by a commercially available IgG quick test kit. Can Vet J 52(5):524–526

Sty AC, Sangild PT, Skovgaard K et al (2006) Spray dried, pasteurized bovine colostrum protects against gut dysfunction and inflammation in preterm pigs. J Pediatr Gastroenterol Nutr 63(2):280–287

Sugita-Konishi Y, Shibata K, Yun SS, Hara-Kudo Y et al (1996) Immune functions of immunoglobulin Y isolated from egg yolk of hens immunized with various infectious bacteria. Biosci Biotechnol Biochem 60(5):886

Thomas HS (2017, January) Precalving vaccination programs for cows. 46 Herefor World pp 46–47

Trotz-Williams LA, Leslie KE, Peregrine AS (2008) Passive immunity in Ontario dairy calves and investigation of its association with calf management practices. J Dairy Sci 91:3840–3849

Vega C, Bok M, Chacana P, Saif L et al (2011) Egg yolk IgY: protection against rotavirus induced diarrhea and modulatory effect on the systemic and mucosal antibody responses in newborn calves. Vet Immunol Immunopathol 142:156–169

Vega CG, Bok M, Vlasova AN et al (2012) IgY antibodies protect against human rotavirus induced diarrhea in the neonatal gnotobiotic piglet disease model. PloS One 7:e42788

Vighi G, Marcucci F, Sensi L et al (2008) Allergy and the gastrointestinal system. Clin Exp Immunol 153(Suppl 1):3–6

Watanabe N, Bruschi F, Korenaga M (2005) IgE: a question of protective immunity in *Trichinella spiralis* infection. Trends Parasitol 21 (4):175–178

Webster RP, Anderson GI, Gearing DP (2014) Canine brief pain inventory scores for dogs with osteoarthritis before and after administration of a monoclonal antibody against nerve growth factor. Am J Vet Res 75:532–535

Woolley JA, Landon J (1995) Comparison of antibody production to human interleukin-6 (IL-6) by sheep and chickens. J Immunol Methods 178:253–265

Woolums AR (2007) Vaccinating calves: new information on the effects of maternal Iimmunity. The AABP Proc 40:10–17

Yolken RH, Losonsky GA, Vonderfecht S et al (1985) Antibody to human rotavirus in cow's milk. N Engl J Med 312(10):605–610

Part II

Prebiotics, Probiotics, Synbiotics, and Antimicrobials

Prebiotics and Probiotics in Feed and Animal Health

Arturo Anadón, Irma Ares, Maria Rosa Martínez-Larrañaga, and Maria Aŕanzazu Martínez

Abstract

The ban of antimicrobial growth promoters (AGP) has been a challenge for animal nutrition, increasing the need to find alternative methods to control and prevent the colonization of pathogenic bacteria. The elimination of antibacterials in animal nutrition has had adverse consequences on the production, health, and welfare of animals. Much research has been focused on the development of antibiotic alternatives to maintain or improve animal health and performance. Modulation of the gut microbiota with zoo-technical feed additives such as prebiotics and probiotics for host protection to support animal husbandry, including livestock, poultry, and fish farming, is the key to maximize productivity and maintain animal health and welfare. This chapter describes the classes of available prebiotics, probiotics, and synbiotics alternatives to increase productivity and aid performance in several food-producing animals. For farm animals, optimal combinations of various alternatives coupled with good management and husbandry practice, better housing conditions, and improvement of biosecurity measures are essential.

Keywords

Prebiotics · Probiotics · Synbiotics · Feed · Animal health · Animal welfare

1 Introduction

The concept of improving animal health and welfare through enhanced gut health has been known in food animal production for decades; however, only now are the tools available to identify microbes in the intestine associated with improved performance (Ballou et al. 2016). Preserving the integrity of the intestinal barrier is also critical for animal health and welfare. As well as ensuring nutrient absorption, the intestinal barrier is important in protecting the animal immune system (i.e., mucus production, prevention against bacteria and toxins entering the bloodstream). The more important objective of animal husbandry now is to deliver foods safe for human consumption while taking into account animal welfare and respect for the environment (Gaggìa et al. 2010).

Prebiotic and probiotic approaches require using microbial food supplements that benefit the host by improving intestinal microbial balance (Gibson and Roberfroid 1995). Dietary administration of spore-forming bacteria can restore the natural balance of the animal gut microflora and return the gut to its normal nutritional, growth, and health status (Fuller 1989). Researchers have used the term synbiotic to describe the use of prebiotic and probiotic mixtures that may benefit animal or human gastrointestinal (GI) systems (Kolida and Gibson 2011).

2 Gastrointestinal Microbiota and Microbiome

The microbiota is considered a "super-organism" and is an integral part of the gastrointestinal tract (GIT). This concept refers to the close relationship between microbes residing in the GIT and the animal host developed during the long course of evolution (Ley et al. 2008). The GI microbiota is a complex population of microorganisms that are significant in health and disease. Numerous functions benefiting the host are ascribed to the gut microbiota of mammals, such as the digestion and fermentation of carbohydrates, production of vitamins, maintenance of normal intestinal villi function, regulation of immune responses, and protection from pathogenic bacteria. The functions of microbiota include "nutrition" [fermentation of nondigestible substrates (i.e., carbohydrates) to generate short-chain fatty acids (SCFA)],

A. Anadón (✉) · I. Ares · M. R. Martínez-Larrañaga · M. A. Martínez
Universidad Complutense de Madrid, Madrid, Spain
e-mail: anadon@vet.ucm.es

R. C. Gupta et al. (eds.), *Nutraceuticals in Veterinary Medicine*, https://doi.org/10.1007/978-3-030-04624-8_19

absorption of ions, production of amino acids and vitamins K, B_9, B_{12}, "protection" (the barrier effect that prevents invasion by nonnative microbes), and trophic effects on the intestinal epithelium and immune system, that is, the development and homeostasis of local and systemic immunity (Guarner 2007). Moreover, the intestinal bacteria are important in GI health. Resident commensal organisms (normal microflora, indigenous microbiota) promote gut health through the induction of mucus production and enterocyte turnover (Kamada et al. 2013) and also are important in host immunity, nutrient absorption, and metabolism. The resident commensal organisms of the gut flora both protect against invading organisms (e.g., enterotoxigenic *Escherichia coli* strains) within the GIT and are responsible for (1) the synthesis of vitamins; (2) the bioconversion of toxic compounds to nontoxic residues; (3) the stimulation of the immune system; (4) the maintenance of gut peristalsis and intestinal mucosal integrity, and (5) the provision of a barrier against colonization by pathogens. These effects as produced by resident commensal organisms may be mediated through direct competition for nutrients, stimulation of antimicrobial peptide production by the enterocyte, and host immunomodulation (Sancak et al. 2004).

Bacteria also promote self-tolerance by inducing hyporesponsiveness to the resident commensal organisms (Seepersadsingh et al. 2004). These important functions of health can be significantly impaired by bacterial dysbiosis (i.e., altered gut bacterial composition), which occurs when bacteria populations within the GIT become unbalanced. Dysbiosis is likely caused by an altered environment within the GIT, such as changes in pH, motility, oxygen level, and the presence of blood, and has been associated with the pathogenesis of many inflammatory diseases and infections.

The GI microbiota of domestic animals is a dense, large, and complex bacterial community, composed of bacteria, protozoa, fungi, Archaea, and viruses. The microbiota colonizes the gut with metabolic activity that affects the physiology and pathology of the host mucosal immune system. The microbiota located in the GIT of mammals can be considered a "metabolically active organ" whose composition and functions have been characterized for better understanding of the major contribution of the gut microbiota to animal nutrition. The GI microbiota is complex. Bacterial species of the genera *Lactobacillus* and *Bifidobacterium* have been shown to supply beneficial host effects of their metabolic function and end-products. Homeostasis regulation by the microbiota enhances its beneficial components, so it could be possible to treat various intestinal disorders and maintain host well-being (O'Hara and Shanahan 2007). Faecal microbial transplantation thus might be a novel therapy for dysbiosis in veterinary medicine (Redfern et al. 2017).

The GI microbiota promotes the supply, digestion, and fermentation of plant polymers in herbivorous animals and the absorption of nutrients, improves growth performance and prevents pathogen colonization, and maintains normal mucosal immunity. The importance of the GI microbiota and the host–microbe crosstalk is highlighted by the results of studies using germ-free animals.

In chickens, diet and environment affect the GIT microbial status. The microbiota of chickens varies according to factors such as diet, location, and age. Microbial richness and diversity increase with age, with dramatic changes in the microbial community as chickens grow older. Most studies examined the effect of time on the chicken caecum microbiota, as this is the organ with the greatest diversity and abundance in the entire intestinal tract. However, microbial diversity of the chicken microbiota is relatively low compared to the intestinal microbiota of other animals, which is attributed to the rapid transit of food through the digestive system, with short retention times. For example, a typical retention time for a 29-day-old broiler chicken is between 4 and 5 h, compared to humans, where the average is 20 h (Clavijo and Vives Flórez 2018).

Dirty litter and other animal management parameters affect GIT microbial composition both directly by providing a continuous source of bacteria and indirectly by influencing the physical condition and defence of the birds. Animals reared under conditions that prevent bacterial colonization display impaired intestinal immune system development and function (O'Hara and Shanahan 2007). This aspect demonstrates that there is a symbiotic relationship between host and microbiota. The intestinal microbiota is a highly complex milieu of more than 600 species, which may be present at levels up to 10^{11} colony-forming units of bacteria per gram of intestinal contents. Data show that bacterial densities in the ileum and caecum of broiler chickens 1 day after hatching already reach 10^8 and 10^{10} per gram of digesta, respectively. The numbers of microbes reach 10^{11} per gram of caecal digesta and 10^9 per gram of ileal digesta during the first 3 days post hatching and remain relatively stable for the following 30 days (Apajalahti et al. 2004). More than half the 640 species found in chickens represent previously unknown bacterial genera composing a healthy microbiota crucial for the health of the host.

The intestinal environment consists of microbiota, the mucosal immune system, and the gut structure and function, which affect host health and animal productivity. These aspects of the intestinal environment are all influenced by diet; thus, better understanding of the relationships between nutrition, the intestine, and host health is important for optimizing animal production. Because the GIT, particularly the large intestine (i.e., colon and caecum), is the most important site of fermentative activity, the importance of microflora activity (i.e., fermentation) must be clarified in relationship to host health. Although the intestinal contents pass through the human small intestine in only 2–4 h, the large bowel transit time is normally 20–80 h, so there is more

than enough time for the development and activity of the microflora (Williams et al. 2001).

As indicated previously, the intestinal tract, in addition to absorption and digestion, is also the body's largest organ of host defence. This organ represents the largest surface area in contact with the antigens of the external environment, and the dense wall-to-wall of the gut microbiota overlying the mucosa normally accounts for the largest proportion of the antigens presented to the resident immune cells and those stimulating the pattern recognition receptors such as toll-like receptor (TLRs) and NOD-like receptors (NLRs). Part of the intestinal mucosal barrier function is formed by a common mucosal immune system, which provides communication between the different mucosal surfaces of the body (Sekirov et al. 2010). The gut microbiota is intimately involved in numerous aspects of normal host physiology, from nutritional status to behavior and stress response, acting as a microbial organ. The indigenous microflora is host- and location specific, very complex in composition, and generally possesses properties that are beneficial to the host. Therefore, a major concern of antibiotic use is the long-term alteration of the normal healthy gut microbiota and horizontal transfer of resistance genes, which could result in a reservoir of organisms with a multidrug-resistant gene pool (Da Costa et al. 2013). Many environmental factors can affect the composition and function of gut microbiota in livestock animals. Feeding practices, animal diets, farm management, and productivity constraints also influence microbial balance in the GIT and consequently affect feed efficiency, digestive health, and animal welfare (Chaucheyras-Durand and Durand 2010).

In this regard, piglet weaning represents a critical period during which the still immature gut microbiota confronts a radical diet change, leading to increased susceptibility of the young animals to pathogen colonization. Multiple stressors encountered at piglet weaning induce transient anorexia, intestinal inflammation, and unbalanced gut microbiota (Gresse et al. 2017). The circumstances of piglet weaning transition often cause GI infections (e.g., colibacillosis diarrhea), and the potential use of feed supplements to achieve better animal health, welfare, and productivity through manipulation of the GIT microbial ecosystem has gained considerable attention.

Data from the rumen environment suggest it is home to a diverse population of microbes encompassing all three domains of life: Bacteria, Archaea, and Eukarya. Of the three domains of life inhabiting the rumen, the bacteria are predominant (10^{10}–10^{11} cells per gram of rumen content). Viruses are the most abundant biological entities, participating in microbe balance within an ecosystem and facilitating horizontal gene transfer.

Most feed ingredients of plant origin contain considerable amounts of fiber redefined to be soluble and insoluble nondigestible carbohydrates with three or more monomeric units, lignins that are intrinsic and intact in plants, and certain isolated and synthetic nondigestible carbohydrates with three or more monomeric units (Gibson et al. 2017). Insoluble fibers have traditionally been regarded as an inert nutrient diluent with little or no nutritive value in monogastric animal diets; however, they have further functions such as (1) improving gut health, (2) enhancing nutrient digestion, and (3) modulating animal behavior. It is suggested that monogastric animals need fiber because their gut development requires physical stimulation by hard, solid particles of feed (Hetland et al. 2004). So, Choct et al. (1996) demonstrated that addition of soluble non-starch polysaccharides (NSP) to a broiler chicken diet drastically increased volatile fatty acid (VFA) production in the ileum, which was easily reversed when the NSP were depolymerized with an enzyme. The VFA levels in the ileum were negatively correlated with apparent metabolisable energy and starch digestion. The microbiota also degrades polysaccharides that are not digestible by the host, thus increasing the nutritive value of the diet (Choct 2009). The functions of microbiota in pathogen exclusion and the synthesis of vitamins, minerals, and other biologically active compounds are well documented (Patterson and Burkholder 2003).

The link between nutrition and the microbiota is well established. Diet is considered as one of the main drivers in shaping the gut microbiota over the lifetime. The intestinal function and gut microflora of broiler chickens are influenced by cereal grain and microbial enzyme supplementation (Shakouri et al. 2009), dietary fat content, feed form, and NSP-degrading enzymes (Torok et al. 2008). The GIT of chickens harbor microbiomes important in (1) growth and development, including the production of energy-rich SCFA; (2) promotion of GIT villus and crypt morphology; (3) nutrient utilization, including reduction in luminal viscosity and deconstruction of dietary polysaccharides; (4) nutrient absorption; and (5) well-being of their chicken hosts, including detoxification. During the deconstruction of dietary polysaccharides, GIT bacteria produce SCFA. The composition and proportions of these SCFA vary depending on microbial composition, which is to some degree adaptable, and fine-tuned by the composition and structure of the fiber component of the chicken's diet. Acetate is the primary SCFA produced in most GIT environments, including the chicken, followed by propionate and butyrate. The chicken GIT is inhabited by various bacteria, methanogenic archaea, fungi, and viruses (Yeoman et al. 2012). The chicken GIT microbiome produces enzymes enabling the deconstruction of dietary polysaccharides (Beckmann et al. 2006). These enzymes are critical to host nutrition because chickens, similar to most animals, lack the genes for glycoside hydrolase, polysaccharide lyase, and carbohydrate esterase enzymes that are necessary to facilitate this process (Yeoman et al. 2012).

The gut "microbiome" (i.e., the natural intestinal microbial communities of the host, which refers to genetic elements

or the genome of the intestinal microbiota) contains more than 100 times the number of genes in our human genome and confers on us functional features that we have not evolved ourselves (Turnbaugh et al. 2007; Gibson et al. 2017). The GI microbiome is a diverse consortium of bacteria, archaea, fungi, protozoa, and viruses that inhabit the gut of all mammals. The microbiomes exist within greater systems that are organic in nature, including human, animal, plant, and invertebrate) or inorganic, such as soil, water, manufactured products, and the constructed environment. Dysfunctional microbiomes are associated with reduction in animal productivity, such as increases in antimicrobial resistance in livestock and poultry. Studies in humans and other mammals have implicated the microbiome in a range of physiological processes that are vital to host health including energy homeostasis, metabolism, gut epithelial health, immunological activity, and neurobehavioral development. The microbial genome confers metabolic capabilities exceeding those of the host organism alone, making the gut microbiome an active participant in host physiology (Barko et al. 2018).

Supporting an optimal gut microbiome may also prove beneficial in animal science as a means to manage stressful situations and to increase the productivity of farm animals. The microbiome and its genetics are an underestimated influence on animal health and growth. Although there remains a paucity of data about the intestinal microbiome in small animals, recent studies have helped characterize its role in host animal health and associated disease states (Barko et al. 2018). Microbiome characterization has progressed rapidly in recent years as DNA-based profiling technologies have verified and replaced traditional culture-based techniques. Using those newer techniques, it was found that 90% of the bacteria in the chicken GIT represent previously unknown species (Apajalahti et al. 2004).

As with humans, the microbiomes of plants and animals are necessary in plant and animal growth and development. Plants are constantly confronted by both abiotic and biotic stresses that seriously reduce their productivity. Recent evidence shows that a combination of abiotic and biotic stress can have a positive effect on plant performance by reducing susceptibility to biotic stress. Such an interaction between both types of stress suggests crosstalk between their respective signaling pathways. This synergistic or antagonistic crosstalk may include the involvement of phytohormones, transcription factors, kinase cascades, and reactive oxygen species (ROS). In certain cases, such crosstalk can lead to cross-tolerance and enhancement of plant resistance against pathogens (Rejeb et al. 2014).

Animal microbiomes are investigated and manipulated along with modern agricultural practices to increase productivity. The rumen microbiome reportedly contains up to 28,000 different viral genotypes obtained from each environment, with prophage sequences outnumbering potential lytic phages by approximately 2:1: the most abundant bacteriophage and prophage types are associated with members of the dominant rumen bacterial phyla Firmicutes and Proteobacteria (Berg et al. 2012). Viruses have been shown to be a driving factor in the evolution of microbiomes in various environments with important roles in controlling the numbers of microbes in an ecosystem, naturally selecting phage-resistant microbes, and facilitating horizontal gene transfer (Rohwer and Thurber 2009; Parsley et al. 2010).

The microbiome of a mature sow could contain between 10 and 100 trillion organisms. Every organ contains its own specific microbiome (lungs, skin, intestinal tract, etc.) (van Haandel 2016). There are different species of microbiomes and, within the species, there are genetic variations. The microbiome of the pig intestinal tract is dominated by two major groups, Firmicutes and *Bacteroides*, with the composition varying moderately from the beginning to the end of the GIT (Jensen 1998; Niu et al. 2015). The gut microbiome in young pigs is dramatically shaped by the composition of dietary glycans, reflected by the different functional capacities of the microbiome before and after weaning. Before weaning the microbial flora appears to be milk oriented. During the weaning phase the pig and its microbiome are subjected to a drastic period of transition, but during the nursing phase the microbial population is rather stable. After weaning (day 28 and beyond), populations of *Bacteroides* and *Enterobacteriaceae* decline and populations of *Lactobacillaceae*, *Ruminococcaceae*, *Veillonellaceae*, and *Prevotellaceae* increase (Frese et al. 2015). The microbiome adapts its bacterial composition to changes in the living environment or substrates in the diet (Lallès et al. 2007). The microbiome is in continuous symbiosis with its host, containing pathogens and other bacteria that are perfectly in balance in a healthy animal.

Little is known about the acquisition and development of the intestinal microbiome in dogs and cats, limited to a handful of studies in kittens and puppies. To date, only one longitudinal study of developing kittens has been published (Deusch et al. 2015). As in humans, the early faecal microbiome is characterized by a high degree of interindividual variation, and that intraindividual diversity and compositional stability increase with age. Also, similar to humans, the relative abundance of *Lactobacillus* and *Bifidobacterium* decreased with age, whereas *Bacteroides* and bacterial genes associated with the ability to metabolize complex carbon sources increased with age. However, there was no major change in bacterial gene repertoires between weeks 30 and 42 in these kittens (Barko et al. 2018).

Relative to the poultry microbiome and food safety, it should be highlighted that *Salmonella* affects people in many countries each year, giving rise to hospitalizations and deaths. This aspect is addressed by researchers who

studied the development of the chick microbiome from hatch to 28 days. The microbiome of growing chicks develops rapidly from days 1 to 3, and the microbiome is primarily *Enterobacteriaceae*, but Firmicutes increase in abundance and taxonomic diversity starting around day 7. Predicted metagenomic content suggests that, functionally, treatment may stimulate more differences at day 14, despite the strong taxonomic differences at day 28. These studies found that both vaccination and prebiotic use (i.e., microbial nutrients) with the chick diet help reduce the persistence of *Salmonella* in the challenged birds (Ballou et al. 2016). The GI microbiome in poultry differs ecologically from that of mammals in that colonization occurs primarily from the surrounding environment and individuals of the same age are reared in close proximity rather than in direct contact with adults. Colonization of poultry by microbes from environmental sources may have important biosecurity and management implications if human pathogens are transferred from environmental reservoirs through the poultry supply chain to consumers (Oakley et al. 2013). The microbial communities associated with agricultural animals are important for animal health, food safety, and public health. The use of high-throughput sequencing (HTS) to characterize the poultry microbiome across a series of farm-to-fork samples demonstrates the utility of HTS in monitoring the food supply chain and identifying sources of potential zoonoses and interactions among taxa in complex communities (Oakley et al. 2013). Similar approaches with other poultry and livestock may help prevent other food-borne diseases.

Growth performance that may differ between chicken breeds could be associated with the GIT microbiome. However, there may always be variation among individuals, probably because of initial bacterial colonization at posthatch. It was reported that the jejune microbiota was dominated by lactobacilli (more than 99% of jejune sequences) and showed no difference between birds with high and low feed conversion ratios (FCR), whereas the caecal microbial community displayed higher diversity with 24 unclassified bacterial species, significantly differentially more abundant between high- versus low-performing birds (Stanley et al. 2012). Many broiler chickens and microbiota studies contain only data from males or the sex of the broiler chickens was unknown. This sex bias in the literature might influence our understanding of the microbiota development in chickens, and therefore the sex of the chicken should always be reported (Stanley et al. 2012).

The genetic codes of a number of commensal bacteria, including *Lactobacillus acidophilus* and *L. johnsonii* (Pridmore et al. 2004), which produced SCFA, have recently been sequenced; this will facilitate future studies on microbial gene expression and improve our understanding of interactions between the host and the microbiome.

A healthy gut is the key to a healthy animal. An infected gut (i.e., by coccidiosis or by necrotic enteritis) is not a healthy gut, and is not efficient in digesting and transporting nutrients (Choct 2009). Thus, a balanced and diverse microbial composition is essential for optimal digestion and nutrient uptake. The most important tool for good gut health is to provide the best feed possible that meets the nutritional needs for the age category and stage of production.

The brain–gut–microbiota axis comprises an extensive communication network between the brain, the gut, and the microbiota (Wiley et al. 2017). Development of a diverse gut microbiota is crucial for multiple features of behavior and physiology, as well as many fundamental aspects of brain structure and function. Appropriate early-life assembly of the gut microbiota is also believed to influence subsequent emotional and cognitive development. If the composition, diversity, or assembly of the gut microbiota is impaired, this impairment can have a negative impact on host health and lead to disorders. Recent advances in DNA sequencing technology show that changes in the gastrointestinal microbiome are associated with diseases including inflammatory diseases, asthma, obesity, diabetes, cardiovascular disease, immune-mediated conditions, and even potentially neuropsychiatric illnesses including anxiety and depression (Wiley et al. 2017). Microbiomes represent one source of human and animal genetic and metabolic diversity. For example, aging predisposes humans and animals to a natural degeneration in GI function, epithelial barrier integrity, GI microbiota composition, and immune system function (adaptive and innate), elevating the risk of infections. Potentially pathogenic bacteria (i.e., enterobacteria and *Clostridia*) increase with aging, whereas *Bifidobacterium* species, which contribute to the protection of the intestinal tract, decrease. Little is known about the acquisition and development of the intestinal microbiome in dogs and cats. As in humans, the early faecal microbiome is characterized by a high degree of interindividual variation and that intraindividual diversity and compositional stability increase with age (Yatsunenko et al. 2012). Also similar to humans, the relative abundance of *Lactobacillus* and *Bifidobacterium* decreased with age, whereas *Bacteroides* and bacterial genes associated with the ability to metabolize complex carbon sources increased with age (Barko et al. 2018). In chickens, the microbiota varies according to diverse factors such as diet, location, and age. Dramatic changes have been described in the microbial community as chickens grow older. Most of the studies examined the effect of time on the chicken caecum microbiota (i.e., organ with the greatest diversity and abundance in the entire intestinal tract). However, the microbial diversity of the chicken microbiota is relatively low compared to the intestinal microbiota of other animals, which is attributed to the rapid transit of food through the digestive system, with short retention times; for example, a typical retention time for a

29-day-old broiler chicken is between 4 and 5 h, compared to humans, where the average is 20 h.

3 Classes of Alternatives to Antimicrobial Growth Promoters

The ban of antibiotic growth promoters (AGPs) has been a challenge for animal nutrition, increasing the need to find alternative methods to control and prevent the colonization of pathogenic bacteria (Anadón et al. 2006). Although the EU has banned antibiotics that are applied as growth promoters, they are still regularly used for therapeutic reasons. When antibiotics are used, the intestinal microbiota becomes unbalanced, and restrains the future intestinal health of the animal. Better intestinal health will have a positive impact on FCR and uniformity across all species. An ideal alternative should have the same benefits as AGP, ensure optimum animal performance, and increase nutrient availability (Huyghebaert et al. 2011). Considering the proposed mechanism of action of AGPs (microbiome and immunomodulating activities), a practical alternative should possess both these properties in addition to having a positive impact on FCR and growth (Huyghebaert et al. 2011; Seal et al. 2013). Applications of prebiotics and probiotics are needed not only for health- and welfare-promoting properties and performance, but also to displace the application of antibiotics in animal feed. Prebiotics and probiotics are regarded as components of strategies to reduce or even eliminate routine antimicrobial use in animal production and are seen as potential alternatives to in-feed antibiotics. Modulation of the gut microbiota with prebiotics and probiotics as the new feed additives for host-protecting functions to support animal health and welfare is an important issue in animal production.

3.1 Prebiotic Concept: Prebiotic Effects

Prebiotics are products that confer health benefits. Found naturally in many foods, prebiotics are also isolated from plants, or synthesized from lactose or sucrose by enzymatic methods, and promote the selective growth of certain indigenous gut bacteria. A prebiotic was defined by Gibson and Roberfroid (1995) as "a non-digestive food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health." These authors revised this concept and proposed a new prebiotic definition "as a selectively fermented ingredient that allows specific changes; both in the composition and/or activity in the GI microbiota that confers benefits upon host well-being and health" (Gibson et al. 2004; Roberfroid 2007). The latest definition equalizes "prebiotic" and "bifidogenic" and includes in the definition the "prebiotic index" (i.e., gives the absolute increase of the faecal bifidobacteria concentrate per gram of daily consumed prebiotics). As the prebiotic effects, or rather "bifidogenic effects," depend on the type and concentration of the prebiotic and on the *Bifidobacterium* concentration in the intestine of the host, therefore there is not a simple dose–effect relationship. Other investigators have proposed definitions that preferentially emphasized one or more different functional characteristics. FAO (2007) describes prebiotics as "non-viable food components that confer a health benefit on the host associated with modulation of the microbiota." The definition arose from observations that particular dietary fibres bring about a specific modulation of the gut microbiota, particularly increased numbers of *Bifidobacterium* or *Lactobacillus* spp., and that a decrease in potential harmful bacteria is a sufficient criterion for health promotion.

The selectivity for bifidobacteria may be promoted by the ingestion of substances such as fructo-oligosaccharides (FOS) and inulin, *trans*-galactosylated oligosaccharides, and soybean oligosaccharides (Mitsuoka et al. 1987). Recently, an expert consensus document updated the definition of a prebiotic (target-associated microbiota) as a substrate that is selectively utilized by host microorganisms, conferring a health benefit (Gibson et al. 2017). Prebiotics as substrate are nondigestible feed ingredients that influence the microbiota in a manner favourable for host health by stimulating growth or activity of potentially positive microbial flora in the large intestine (Patterson and Burkholder 2003), Thus, a prebiotic is a substrate that is selectively utilized by the host microorganisms conferring a health benefit (Gibson et al. 2017). All prebiotics are fibre, but not all fibres are prebiotics. Beneficial animal health effects must be documented for a substance to be considered a prebiotic. Prebiotics for use by animals in which microbial-focused strategies to maintain health and prevent disease are as relevant as for humans (Gibson et al. 2017).

During the past two decades, prebiotics have been recognized for their ability to manipulate host microbiota for the benefit of the host. Prebiotics include a diversity of non-starch polysaccharides (NSP) or oligosaccharides including mannan-oligosaccharide (MOS), fructans (FOS and inulin), oligofructose, galactans [galacto-oligosaccharide (GOS)], malto-oligosaccharide, lactulose, lactitol, gluco-oligosaccharide, xylo-oligosaccharide, soya-oligosaccharide, isomalto-oligosaccharide (IOS), and pyrodextrins.

Nondigestible in the small intestine, FOS could be utilized only by a few bacterial species, notably *Bifidobacterium*, and including such oligosaccharides in the food increased the count of bifidobacteria in the intestine. It should be stated that is difficult to test the selective stimulation of individual bacterial strains among the more than 400 cultivable and nonculturable bacterial strains in the human gut; for that

reason, the prebiotic effect has been defined as "the selective stimulation of growth and/or activity(ies) of one or a limited number of microbial genus(era)/species in the gut microbiota that confer(s) health benefits to the host" (Roberfroid et al. 2010). The diversity of bacterial species in the gut is one of the most important factors for the establishment of a stable ecosystem in the intestinal tract. Young birds have fewer bacterial species in the intestinal tract than do adult birds, so their gut microflora are more susceptible to disturbances than that of the adults (Mead 1989). A stable flora is essential for an animal to resist infections, particularly in the gut.

Although the concept of prebiotics has been developed over time, to be considered as an effective prebiotic, it is proposed that a candidate prebiotic must fulfill the following criteria, which are to be proven by in vitro and in vivo studies: (1) nondigestibility (i.e., resistance to low pH gastric acid, enzymatic digestion, and intestinal absorption in the upper part of the GIT), (2) good fermentation by the large intestinal microbiota; this can be investigated by measuring breath hydrogen or fecal recovery of the administered carbohydrate after a single prebiotic meal, and (3) selective stimulation of growth and activity of intestinal bacteria (i.e., measuring bacterial counts in faecal samples, or intestinal content, before and during exposure to the test material in batch or multi-chamber fermentation systems) that has associated health-promoting effects (Macfarlane et al. 2006; De Vrese and Schrezenmeir 2008).

Prebiotics are nonabsorbable carbohydrates, such as inulin, galacto-oligosaccharides (GOS), and FOS that promote growth and metabolic activity of presumably beneficial gut bacteria, most notably species of *Bifidobacterium* and *Lactobacillus*. These products may confer health benefits through the production of SCFA, including lactate and butyrate, which may reduce cytokine production within the intestinal mucosa (Sartor 2004).

Prebiotics undergo fermentation by beneficial microflora in the large intestine, providing sources of energy for the microflora. Only the carbohydrates [i.e., inulin and oligofructose (OF), (*trans*-galacto-oligosaccharides (TOS or GOS), or lactulose], which are not digestible but can be fermented by the intestinal flora (Gibson 1999), fulfill this criterion.

The "probiotic approach" adds one or two species to a spectrum of hundreds of species in the gut flora, but the "prebiotic approach" aims at fertilization of the intestinal ecosystem. Functional imitations of the naturally occurring prebiotics GOS and FOS stimulate intestinal growth of bifidobacteria as a marker of probiotics in a dose-dependent manner (Patterson and Burkholder 2003; Gaggìa et al. 2010).

In common terms, prebiotics are "food components" for live microorganisms that are considered beneficial for health and well-being, and it is scientifically accepted that prebiotics are valuable dietary additions for modulating the growth and activity of specific bacterial species in the colon that are considered health supporting (Gibson et al. 2010). An example of feed ingredients for animals is the eubiotic lignocellulose, which influences microflora that it does not digest but traverses to the large intestine where bacteria ferment it (Metzler and Mosenthin 2008).

A different substrate in the large intestine contains a diverse gut microflora. In pig faecal and turkey excreta samples, the amount of volatile fatty acids (VFA) (i.e., acetic acid, propionic acid, butyric acid, total VFA) and lactate can be high when the animals receive lignocellulose A and B. Dry matter digestibility was found to be about 75%, 28%, and <5% for swine/turkey diet, and lignocellulose A and B, respectively. Lignocellulose A resulted in a greater amount of gases, VFA, and lactate compared to product B. Lignocellulose A can be used as an alternative source of fiber to maintain the health and function of the digestive tract (Youssef and Kamphues 2018). The inclusion of lignocellulose in the chicken diet, in particular at a dose of 0.5%, promotes the growth of *Lactobacillus* spp. and *Bifidobacterium* spp., and reduces the number of *Escherichia coli* and *Clostridium* spp. as well as enhancing the concentration of SCFAs (i.e., acetic acid and propionic acids) and lactic acid, which suggests the prebiotic effect of lignocellulose on the broiler chicken GIT, although lignocellulose does not have a substantial effect on the pH of ileal and caecal digesta (Bogusławska-Tryk et al. 2015; Choct 2009; Mancabelli et al. 2016).

Although prebiotics are defined as "a nonviable food component that confers a health benefit on the host associated with modulation of the microbiota" (Pineiro et al. 2008), there are some limitations as to which food components actually count as prebiotics. The compounds need to be resistant to hydrolysis and absorption by the upper GIT so that they can reach the target organisms in the lower GIT. It is desirable that these compounds be substrates more or less only for those microorganisms that one intends to support. It has been argued that only fructo-oligosaccharides and inulin meet these criteria (Roberfroid 2007); however, numerous other compounds have been included in lists of prebiotics such as galacto-oligosaccharides, soy oligosaccharides, xylo-oligosaccharides, pyrodextrins, isomalto-oligosaccharides, lactulose, pectin oligosaccharides, lactosucrose, sugar alcohols, gluco-oligosaccharides, levans, resistant starch, and xylosaccharides.

Prebiotics are nondigestible feed ingredients (i.e., carbohydrates) that selectively promote the development of one or more species of microorganisms in the GIT of humans or animals. Oligosaccharides are the main components: the range is diverse and may be based on any of the hexose monosaccharides, including glucose, fructose, galactose, and mannose (Durst 1996) with a degree of polymerization

between 2 and 20 monosaccharides. Grain legumes are the most common natural sources of oligosaccharides (e.g., raffinose, stachyose, verbascose). "Synthetic" oligosaccharides are derived from the direct polymerization of disaccharides or from the fractionation of both vegetable and microbial cells. Oligosaccharides such as arabinogalactose, arabinoxylan, and rhamnogalacturonan, which are derived from polysaccharides of soybean (with about 3–5% galacto-oligosaccharides), wheat, and fruit, respectively (Van Craeyveld et al. 2009), are generally referred to as nondigestible oligosaccharides.

Some of the nondigestible oligosaccharides currently added to animal feed across different animal species are mannose oligosaccharides (MOS), fructose oligosaccharides (FOS), lactulose and galacto-oligosaccharides (GOS), chito-oligosaccharides (COS), arabinoxylan oligosaccharides (AXOS), xylo-oligosaccharide (XOS), *trans*-galacto-oligosaccharide (TOS), glucan, yeast cell wall, inulin, inactivated yeast bacteria, galactomannan, galactoglucomannan-oligosaccharide-arabinoxylan complex (GGMO-AX), levan, polydextrose, peptidoglycan, chitin, galactomannan-oligosaccharides (GMOS), acidic oligosaccharides (AOS), arabinogalactan, phosphorylated mannans (MAN), arabinoxylan, and mannobiose (Anadón et al. 2016a). These substances influence the intestinal ecosystem by, for instance, improving lactic acid fermentation.

Prebiotics compounds can reduce risk for certain conditions and promote better health; they have a long history of safe use and are known for their health benefits for humans including an increase in the bioavailability of minerals, modulation of the immune system, prevention of GI infections, modification of inflammatory conditions, regulation of metabolic disorders, and reduction of the risk of cancer (Roberfroid et al. 2010). For a dietary substrate to be classed as a prebiotic, at least three criteria are required: (1) the substrate must not be hydrolysed or absorbed in the stomach or small intestine; (2) it must be selective for beneficial commensal bacteria in the large intestine such as the Bifidobacteria; and (3) fermentation of the substrate should introduce beneficial luminal (systemic) effects within the host (Manning and Gibson 2004). The oligosaccharide β-glucans are thought to stimulate performance because of their immunomodulatory effects. Their main action is to enhance phagocytosis and proliferation of monocytes and macrophages (Novak and Vetvicka 2008). As macrophages have a crucial role in immunomodulation, the interaction of glucans with macrophages can have very large effects on the host. Recent reviews have elaborated the action of glucans on immune stimulation (Novak and Vetvicka 2008). Studies with broiler chickens have documented significant health benefits from using immune-modulating β-1,3- or 1,6-glucans (from yeast cell walls obtained from *S. cerevisiae*) as a feed ingredient. However, changes in thymus and liver relative weights and villus morphology of broilers were observed (Morales-López et al. 2009).

Prebiotics are a special form of dietary fiber, are not affected by heat, cold, acid, or time, provide a wide range of health benefits, and beneficially affect the host by selectively stimulating growth, activity, or both, of one or a limited number of bacteria species already resident in the colon (Nagpal and Kaur 2011). Prebiotics are specialized plant fibers which beneficially nourish the good bacteria located in the large bowel or colon. Prebiotics are used to increase bifidobacteria or lactobacilli towards being the numerically predominant genus in the colon, properly improving colonization resistance.

Prebiotic compounds are able to modulate both the luminal and mucosal microbial composition and activities and beneficially regulate host–microbe interactions. Moreover, the changes of gut microbiota composition (especially the number of bifidobacteria) contribute to modulate human metabolic processes associated with obesity and diabetes type 2 (Roberfroid et al. 2010). The prebiotics induce not only changes in the intestinal microbiota and the mucosal surface of the colon but the trans-epithelial transport of the SCFA, stimulating shifts of fluid to and from the lumen; furthermore, the transport of cationic minerals is stimulated by the lowered pH of the lumen. The intraluminal colonic propionate induces the nonneuronal release of acetylcholine synthesized by the epithelial crypt cells to the serosal surface, especially in the distal colon, and this was associated with modifications of the electrical parameters of the mucosa and chloride excretion (Yajima et al. 2011).

The proposed mechanisms of action for prebiotics include blocking receptor sites for pathogen adhesion, immunomodulation, production of antimicrobial compounds on fermentation, and modifying gut morphology (Pourabedin and Zhao 2015). Immunomodulation by prebiotics is thought to result from activation of innate immunity by the interaction of the sugars with certain receptors present on the surface of dendritic cells and macrophages, which can then stimulate production of cytokines, proliferation of lymphocytes, and the activity of natural killer (NK) cells (Saad et al. 2013).

Most prebiotics for the gut require an oral dose of 3 g/day or more to elicit an effect (Roberfroid et al. 2010). Products containing dosages lower than this level should not be called prebiotics, unless such a low dose has been proven to elicit selective effects on the microbiota with concomitant health aspects (Gibson et al. 2017).

3.1.1 Application to Benefit Animals

Prebiotics have been studied and used for companion animals and animal husbandry, including livestock, poultry, and aquaculture. The inherent differences among animal species with regard to the living environment, anatomy and physiology,

dietary composition, and reliance on the gut microbiota for energy must be considered when evaluating the effect of prebiotics on animal health (Stevens and Hume 1998, Gibson et al. 2017). Most prebiotics appear to stimulate acid lactic and bifidogenic bacteria. The functions described for prebiotics are that they attach to pathogens, serve as substrates for fermentation, increase osmosis in the lumen of the intestine, and may also indirectly stimulate the response of macrophages and the production of SCFAs and modulate the immune system (Patel and Goyal 2012).

Poultry

Poultry, which are used primarily for the production of meat or eggs, include land fowl species (for example, chickens, turkeys, quail) and waterfowl species (for example, ducks, geese) which respond to prebiotics although most have a fairly short midgut and hindgut that includes a short, straight colon and twin ceca. Dietary prebiotics, including inulin, yeast cell wall extracts, lactulose, and GOS are usually fed at concentrations up to 0.2% (weight/volume) of diet (Bednarczyk et al. 2016). Prebiotics provide substrates for microbial fermentation in the gut, resulting in the production of SCFA, an energy source for enterocytes. Fermentation of dietary fibers by commensal bacteria in the gut leads, in general, to production of SCFA in the hindgut that can be easily absorbed and contribute to the energy sources for the animal host. Many prebiotics, including fructo-oligosaccharide and mannan-oligosaccharide, increase levels of beneficial bacteria such as *Lactobacillus* and *Bifidobacterium* spp. and decrease levels of pathogens such as *Escherichia coli* (Fukata et al. 1999; Xu et al. 2003; Baurhoo et al. 2007). Terminal restriction fragment length polymorphism (T-RFLP) and denaturing gradient gel electrophoresis analysis have been used to demonstrate that FOS and MOS affect the composition of the bacterial population and *Lactobacillus* species profiles in broiler chickens. These microbial changes did not affect performance, indicating that numerous microbial compositions may facilitate a high level of performance under these conditions (Geier et al. 2009).

Compared to probiotics, the risks of undesirable side effects in the host are lower. Prebiotics are macromolecules that are either derived from plants or synthesized by microorganisms. MOS, derived from the outer cell wall layer of *Saccharomyces cerevisiae*, has been studied extensively as a prebiotic supplement in poultry diets.

Two kinds of prebiotics have been described for use in aviculture. Most of those currently used are nondigestible synthetic oligosaccharides that contain one or more molecules of a sugar, or a combination of simple sugars such as glucose, fructose, xylose, galactose, and mannose. MOS found in the cell walls of yeasts have proved to be most important as they contain compound proteins and glucan (Rehman et al. 2009). The other kind of prebiotic described corresponds to lactose and lactose derivatives such as lactulose and lacto-sucrose (van Immerseel et al. 2002). Lactulose is a nondigestible, synthetic disaccharide that shows prebiotic effect in broiler chickens diets, improving body weight and FCR, increasing villi height, goblet cell numbers, total SCFA concentrations, and *Lactobacillus* counts (Calik and Ergün 2015). Other prebiotics found to have beneficial effects in poultry include lignin, inulin, and palm kernel extract.

Several studies of prebiotics in chickens provide evidence of positive effects for oligosaccharides of mannose or fructose in inhibition of the pathogens *Salmonella* and *E. coli* (Chambers and Gong 2011; Stanley et al. 2014). Conflicting results obtained with or without oligosaccharides that occur naturally in feed ingredients (e.g., the raffinose series oligosaccharides) present an unclear scenario regarding the effect of their inclusion in diets for broiler chickens (Iji and Tivey 1998); however, their nutritional impact cannot be separated from other anti-nutritive components in the diet.

Prebiotics in poultry indicate their usefulness in controlling or reducing the growth of *Clostridium perfringens* implicated in necrotic enteritis. Fucosyllactose, a functional oligosaccharide present in human milk that protects against infection by enteric pathogens, seems to favour coaggragation with pathogens instead of pathogen contaminant being eliminated by the mucosal lining of the poultry intestine (Lee et al. 2012). The addition of various levels of MOS to the broiler chicken diet significantly increased their body weight and improved FCR with increased intestinal villi height, improved immuno-competence in the intestine, altered jejunal gene expression, and influenced intestinal microbiota. FOS, which is derived from plants, has also been shown to possess significant prebiotic effects and improve performance in broiler chickens. Another class of prebiotics includes IOS showing their efficacy in improving weight gain and FCR when fed to broiler chickens (Mookiah et al. 2014). In pigeons, dietary administration of MOS induced changes of gut morphology and lowered the pH of excreta, reflecting a reduced bacterial challenge in the intestine; thus, MOS has potential as a prebiotic strategy in birds (Abd El-Khalek et al. 2012).

A number of characteristics should be taken into consideration when selecting prebiotics for poultry, including resistance to gastric acidic environment, intestinal/pancreatic enzyme hydrolysis, and absorption across intestinal epithelium. The most important characteristic of a standard prebiotic is the ability to selectively enrich beneficial microorganisms associated with health and well-being. Thus, the majority of the beneficial effects of prebiotics are thought to be mediated predominantly through altering the intestinal microbiota (Pourabedin and Zhao 2015). Prebiotics also prevent pathogen colonization either by binding directly or by competitive exclusion by promoting the growth of

beneficial microbes or by stimulating them to produce bacteriocins and lactic acid (Spring et al. 2000). In particular, MOS acts by binding to type 1 fimbriae of enteric pathogens and prevents their adhesion to intestinal epithelial cells (Spring et al. 2000) and acts as adjuvants, and help boost the host immune responses (Ferket et al. 2005). Overall, prebiotics also act by beneficially altering luminal or systemic aspects of the host immune system.

The fermentation of prebiotics by microflora also leads to the production of SCFAs that act as energy sources for intestinal epithelial cells and thus maintain the integrity of the gut lining (Ferket et al. 2005). Several studies have revealed that synbiotic treatment was more efficacious than an individual prebiotic in reducing pathogen transmission and infections in poultry.

Pigs

Different types of chemically defined or undefined dietary compounds are added to the diet of pigs to test their influence on GI microbiota or on the health status improvement during challenge with pathogens. When added to the pig diet, fermentable and nonfermentable fiber appears to have a significant positive impact on growth and gut health: it seems to influence the total digestion and fermentation processes, contributing to a different equilibrium particularly in the large intestine of monogastric animals. Prebiotics such as oligosaccharides of fructose, mannose, and chitin protect piglets against high environmental stressors (for example, antibiotics) and pathogen loads, including faecal *Escherichia coli* shedding, and reduced infection-associated responses to *Salmonella enterica* serovar typhimurium infection or porcine reproductive and respiratory syndrome virus (Liu et al. 2008; Che et al. 2011). Symbiotic applications could be beneficial as significant improvement of growth performance parameters in suckling and in growing pigs was observed (Modesto et al. 2011).

Feeding fiber-rich diets to pregnant sows at the end of their lactation period shows a positive impact as these additives continuously stimulate the GI tract and have a positive influence on the duration of partus (i.e., a shorter duration of partus increases piglet survival rates).

Refined functional carbohydrates (RFCs) are the components harvested from yeast cells (*S. cerevisiae*) using specific enzymes during the manufacturing process to ensure a high level of bioavailability and uniformity. This proprietary enzymatic hydrolysis yields MOS, β-glucans (1,3/1,6), and D-mannose. These compounds are naturally present in all yeast cells but are not readily bioavailable. The method of processing used to refine the yeast cells influences the size and structure of these liberated components, which in turn affect bioavailability and functionality. Research shows that each RFC has a specific mode of action and outcome when fed to various livestock species, including dairy, beef, and poultry. RFCs also have been shown to positively influence the immune response of nursery pigs. RFCs act as a prebiotic by feeding the beneficial bacteria found in the intestine while blocking sites for attachment by pathogens.

Ruminants

Calves are born in a pre-ruminant state and function as non-ruminants until the rumen and other compartments of the stomach fully develop (Quigley et al. 1997). During the first few weeks of life, or longer in the case of veal calves maintained on low-roughage diets (that is, low in fibrous material), prebiotics can be used to increase growth, improve FCR, reduce the incidence and severity of scours (diarrhea), or reduce the incidence of respiratory diseases (Quigley et al. 1997; Ghosh and Mehla 2012; Roodposhti and Dabiri 2012).

The use of prebiotics in cattle has been limited by the ability of ruminants to degrade most prebiotics; however, enhancement in rumen-protective technologies may allow these feed substances to be used in feedlot and dairy cattle, considering also that several classes of nondigestible oligosaccharides are found in the plant cell wall in nature including plants normally used for livestock feeding (Callaway et al. 2008). However, the prebiotics used in pre-weaned calves are cello-oligosaccharides, galactosyl lactose, yeast cell wall extracts, and MOS.

Horses

Horses are large non-ruminant herbivores that rely heavily on microbial fermentation for energy, with more than half of their maintenance energy requirement coming from microbial fermentation occurring in their enlarged caecum and colon (Stevens and Hume 1998). As their typical diet is high in roughage and feedstuffs that are consumed throughout the day, prebiotic interventions might improve the effectiveness of fermentation (Morgan et al. 2007; Respondek et al. 2011). Commonly used prebiotics have stimulatory effects on lactic acid bacteria (i.e., *Lactobacillus*, *Bifidobacterium*, *Enterococcus*). Many of these bacterial strains have been used successfully as equine probiotics. These indigestible prebiotics serve as a substrate for lactic acid bacteria (LAB), potentially improving the microbiota of the large intestine. There have been a limited number of equine digestibility studies using prebiotics as digestive aids. Similar to studies using probiotics, the results are variable. When *S. cerevisiae* fermentation products were supplemented in conjunction with low-quality forage, the apparent digestibility of dry matter, crude protein, and neutral detergent fiber was greater,

indicating prebiotic supplementation is more effective when high-starch, high-fiber, or low-quality forage diets are fed.

Starch intake after supplementation the diet of the horse with short-chain fructo-oligosaccharides (scFOS) (Respondek et al. 2011) or MOS have beneficial effects in preventing digestive disorders associated with both prebiotics. Furthermore, use of scFOS in horses reduced disruptions in colonic microbial populations after an abrupt change in diet and altered faecal VFA concentrations towards propionate and butyrate (Coverdale 2016).

Dogs and Cats

Dogs and cats evolved as Carnivora, eating diets high in protein and fat but low in fibre. They are non-ruminants with short, simple GIT that have little capacity to ferment nondigestible substances, which action occurs predominantly in the colon (Stevens and Hume 1998). Nevertheless, some health benefits have been achieved with prebiotic administration such as reduced infections, improved insulin sensitivity, and better faecal consistency (Respondek et al. 2008; Verbrugghe et al. 2009). Most studies have investigated the effects of dietary supplementation with prebiotics on the bacterial flora in healthy dogs and cats. FOS supplemented at 0.75% dry matter produced qualitative and quantitative changes in the faecal flora of healthy cats (Sparkes et al. 1998a, b).

Compared with samples from cats fed a basal diet, increased numbers of lactobacilli and *Bacteroides* species and decreased numbers of *E. coli* were associated with the FOS diet. However, bacteriological examination of the duodenal juice in these same cats showed wide variation in the composition of the duodenal flora, across sampling periods, which was not affected by FOS supplementation. In a separate trial, healthy cats fed a diet containing short-chain GOS and FOS had greater faecal *Bifidobacterium* species populations and butyrate concentrations versus the control (Kanakupt et al. 2011). Further, healthy Beagle dogs fed a 1% FOS diet over a 3-month trial showed inconsistent faecal excretion of species of *Lactobacillus* and *Bifidobacterium* (Willard et al. 2000). There is a single report evaluating the effects of GOS on the faecal microbiota in healthy cats and cats with inflammatory bowel disease (IBD) (Biagi et al. 2013). Using a randomized, double-blinded, cross-over feeding trial, oligonucleotide probes targeting specific bacterial populations showed no significant differences in the faecal microbiota of IBD cats and healthy cats fed the same diet. Overall, interanimal variation was moderately high whereas a trend of increased *Bifidobacterium* species levels was observed with GOS supplementation.

Farmed Aquatic Species

Farmed aquatic species include finfish and shellfish. Although anatomy varies among carnivorous (e.g., turbot), omnivorous (e.g., catfish), and herbivorous (e.g., sturgeon) species, all fish have a fairly simple and short GIT. The short length and simple structure (lack of special adaptations) of the fish gut results in the rapid transit of digested material, limiting the time available for microbial or prebiotic activity. The prebiotics are indigestible fibers that increase beneficial gut resident commensal bacteria, resulting in improvement of the host health. Prebiotics are found to stimulate the growth of species of intestinal bacteria in aquatic species. Effective prebiotic doses in aquatic host species are in the range of 1% to 3% (weight/volume) of diet (Li et al. 2007; Hoseinifar et al. 2013, 2014). The beneficial effects of prebiotics result from the by-products generated from their fermentation by gut commensal bacteria. Prebiotics such as FOS, MOS, inulin, or β-glucan are called immunosaccharides. These additives directly enhance innate immune responses including phagocytic activation, neutrophil activation, activation of the alternative complement system, and increased lysozyme activity, among others, in farmed aquatic fish (Table 1). Immunosaccharides directly activate the innate immune system by interacting with pattern recognition receptors (PRR) expressed on innate immune cells (Kyu Song et al. 2014). They can also associate with microbe-associated molecular patterns (MAMPs) to activate innate immune cells. Proper immune responses are important not only for combating pathogens but also for appropriate weight gain. Many studies have indicated that immunosaccharides are beneficial to both finfish and shellfish (see Table 1).

The prebiotic mannan-oligosaccharide improves growth and enhances digestive enzymes such as protease and amylase (Xu et al. 2009).

4 Probiotics

Prebiotic and probiotic approaches both demand the use of microbial food supplements that beneficially affect the host by improving its intestinal microbial balance (Gibson and Roberfroid 1995). Probiotics are another feed additive that

Table 1 Prebiotics as immunostimulants in aquaculture

Oligosaccharides	Polysaccharides
• Fructo-oligosaccharides (FOS) • Mannan-oligosaccharide (MOS) • Mannan-oligosaccharide (MOS) + β-glucan • Galacto-oligosaccharide (GOS) • Arabinoxylan-oligosaccharide (AXOS)	• Inulin • β-Glucan • Chitin/chitosan

is gaining acceptance as a potential alternative to antibiotics to improve production efficacy in livestock, poultry and aquaculture. Probiotics have been defined as "mono- or mixed cultures of living microorganisms which beneficially affect the host by improving the properties of the indigenous microbiota" (Fuller 1992). Probiotics are defined as "live microbial feed additive that beneficially affects the host animal by improving its intestinal balance; probiotics, sometimes used interchangeably with the term direct-feed microbial (DFM), used in the US for products that are given to animals. Microorganisms used in animal feed in the European Union (EU) are mainly bacterial strains of gram-positive bacteria belonging to the types *B. licheniformis*, *B. subtilis*, *Enterococcus* (*E. faecium*), *Lactobacillus* (*L. acidophilus*, *L. casei*, *L. farciminis*, *L. plantarum*, *L. rhamnosus*), *Pediococcus* (*P. acidilactici*), and *Streptococcus* (*S. infantarius*); some other probiotics are microscopic fungi such as strains of yeast belonging to *Saccharomyces cerevisiae* and *Kluyveromyces* (Anadón et al. 2006). *Bacillus* and *Lactobacillus* bacteria differ in many characteristics, and *Bacillus* and the yeasts are not usual components of the gut microflora. Although most of the species and genera are apparently safe, particularly lactobacilli and bifidobacteria, certain microorganisms may be problematic, particularly the enterococci, which may harbour transmissible antibiotic-resistant determinants and bacilli, especially those belonging to the *Bacillus cereus* group that are known to produce enterotoxins and an emetic toxin (Anadón et al. 2006). For example ***Bacillus cereus* var. *toyoi* NCIMB 40112/CNCM I-1012) as a feed additive was withdrawn in the EU in 2015 for all animal species authorized previously**.

To date, reported performance enhancement in domestic animals has primarily been obtained through the application of one specific species or a mixture of probiotic strains within a species (Williams et al. 2001). For humans, specific microbial strains could have an important role in colonization resistance in the intestinal, respiratory, and urogenital tracts, cholesterol metabolism, inhibition of carcinogenesis by stimulating the immune system and lactose metabolism, absorption of calcium, and synthesis of vitamins (Anadón et al. 2016). For farm animals, the most important claims are growth promotion, improved FCR, health control such as prevention of intestinal disturbances (especially in young animals), pre-digestion of anti-nutritional factors (e.g., trypsin inhibitors, phytic acid, glucosinolates) (Havenaar et al. 1992), and welfare promotion.

Livestock probiotics commonly feature various strains of *Bacillus*, *Lactobacillus*, *Enterococcus*, and *Saccharomyces* yeast. There is still much to learn about their interactions with pathogens, but it is understood that certain strains of *Bacillus* have been proven to decrease growth of certain species of pathogenic bacteria including *E. coli*, *Clostridium*, *Streptococcus*, and *Salmonella*. Probiotics help prevent and control GI pathogens or improve the performance and productivity of livestock animals through various mechanisms. The selection of suitable probiotic strains is absolutely essential because such strains must not carry antibiotic resistance genes. Other genes that should, of course, be absent in probiotic strains are those that code for the production of toxins or compounds that can interfere in any way with an animal's well-being and productivity (Anadón et al. 2016; Joerger and Ganguly 2017).

A few genetically modified strains have been tested with the aim of improving animal performance. For example, *Lactococcus lactis* was engineered to express the epidermal growth factor EGF-LL in an effort to boost the performance of early-weaned piglets (Bedford et al. 2012), and the yeast *Pichia pastoris* was modified by the introduction of the *Clostridium perfringens* alpha toxin gene in an attempt to induce immunity against *C. perfringens* in broiler chickens (Gil de los Santos et al. 2012).

A suitable probiotic organism should be able to resist processing and storage, survive in the gastric acidic environment, adhere to the epithelium or mucus in the intestines, produce antimicrobial compounds, and modulate immune responses. However, not all probiotic strains exhibit all these properties and the most suitable probiotic strains or their combinations that will achieve maximum beneficial effect should be selected. Protection of probiotic organisms during their passage through the upper alimentary tract, such as a microencapsulation, should be considered to ensure viability and colonization in the intestine (Anadón et al. 2016b).

Probiotics or active microbials can help modulate the microflora, and slow-release butyrates can have a positive effect on intestinal integrity. Butyrate seems to have a positive influence on epithelial cells, leading to better epithelial cell proliferation and differentiation. Apart from better water and nutrient absorption, this leads to an improved barrier and pathogen control and seems positively to influence the immune system (Eeckhaut et al. 2008).

Probiotics are live organisms that, when ingested in sufficient quantities, transfer a health benefit to the host. Common probiotics include members of the *Lactobacillus* and *Bifidobacterium* genera and organisms such as *Saccharomyces cerevisiae*. Probiotic activity could be related to genera, species, or strains. The efficacy of single-strain and multi-strain probiotics for livestock has been investigated: the beneficial properties include removal or competitive exclusion of pathogens, enhanced immune system development and responsiveness, and production of beneficial compounds and metabolic by-products (Patterson and Burkholder 2003).

For probiotics, two main mechanisms of action have been suggested, summarized as follows: (1) nutritional effect, characterized by reduction of metabolic reactions that

produce toxic substances, stimulation of indigenous enzymes, and production of vitamins and antimicrobial substances; and (2) health or sanitary effects, distinguished by increase in colonization resistance, competition for gut-surface adhesion, and stimulation of the immune response (Guillot 2003); the latter effect acting as 'bio-regulators of the gut microflora' and reinforcing the host natural defences. In this latter mechanism there is an increase in cell-mediated immune response, TLR signalling, antibody production, and decrease of cellular apoptosis, among others (Khan et al. 2016). Host intestinal epithelial cells and dendritic cells have certain receptors [e.g., TLRs, nucleotide-binding oligomerization domain (NOD) proteins] activated by probiotic MAMPs such as fimbriae, flagellae, lipopolysaccharide, lipoteichoic acid, and peptidoglycan. Activation of these receptors leads to induction of signal transduction pathways in the host cell for transcription of genes coding for chemokines and cytokines, which can subsequently stimulate host systemic and mucosal immunity (Hardy et al. 2013). Interleukin 12 (IL-12), a proinflammatory cytokine, and interleukin (IL-10), an antiinflammatory cytokine, are of particular interest with respect to probiotics. Immunostimulatory probiotics induce IL-12 proliferation, which in turn increases the potency of NK cells and induces T-helper pathways. Immunoregulatory probiotics induce IL-10 proliferation, which then induces the T-regulatory pathway (Yaqoob 2014). Probiotics have also been known to alter the gut epithelial architecture. The mucus layer, composed of a class of glycoproteins known as mucins, forms the first line of host defence along with the gut epithelium. Studies have shown that certain probiotic species increase the expression of mucin 2 (MUC-2) and mucin 3 (MUC-3) genes, which code for the synthesis of mucins by goblet cells. Increased mucus production in the gut prevents the adherence and subsequent colonization of the intestinal epithelium by pathogenic bacteria (Hardy et al. 2013). Probiotics can also maintain the integrity of epithelial tight junctions by upregulating genes that code for junction proteins, which are responsible for tight junction signalling, as well as the restoration of mucosal integrity (Syngai et al. 2016). It was reported that *Lactobacillus rhamnosus GG* produced soluble proteins which protect the intestinal epithelial tight junctions and the barrier function from hydrogen peroxide-induced disruption by the activation of protein kinase C isoforms and the mitogen-activated protein kinase (MAPK)-dependent mechanism (Seth et al. 2008).

The delivery of certain living microorganisms during food animal production, with a variety of microorganisms used being LAB, various *Bacillus* species, and the yeast *Saccharomyces cerevisiae*, have been particularly used in the pig industry. The establishment of a beneficial LAB population at birth may lead to healthier animals: this may be most readily achieved by treating sows, which provides an amplification step and floods the neonatal pig environment with desirable bacterial strains (Kenny et al. 2011). Dietary supplementation with DFMs are used to promote health in livestock and poultry resulted in energy repartitioning to the immune system and an increase in antibody production independent of changes in whole-body metabolism or growth performance (Qiu et al. 2012).

Dietary feeding of probiotic-supplemented feed reduced intestinal inflammatory cytokine expression and enhanced growth performance in poultry (Higgins et al. 2011). Moreover, *Bacillus subtilis* strain PB6, provided as a powder preparation, may have preclinical antiinflammatory effects in an acute mice model, reducing symptoms of inflammatory bowel disease that are dependent on immunomodulatory responses (Foligné et al. 2012).

Probiotics would therefore have a role in the balance of gut microflora, increasing resistance to pathogenic agents, both through a strengthening of the intestinal barrier and by stimulating the immune system directly.

Competitive Exclusion Probiotics operate by "competitive exclusion," meaning that when adequate populations of probiotic bacteria are present, they reduce the ability of pathogenic bacteria to get out of control and overwhelm the host. The available probiotics can be classified into (1) 'colonising' species, such as *Lactobacillus* and *Enterococcus* spp., and (2) free-flowing 'noncolonizing' species, such as *Bacillus* spp. (spores) and *S. cerevisiae*. Competitive exclusion describes the treatment of day-old chicks with an undefined microbiota derived from adult animals, resulting in colonization resistance against pathogenic microorganisms (Huyghebaert et al. 2011).

Current feed additive products designed for microbiotal manipulation of food animals with live microorganisms (or with products directly derived from the culture of these organisms) fall into two categories. The predominant group (first category) attempts to improve or maintain animal health status under the conditions encountered in modern animal husbandry practices without making specific claims to target pathogens that are of concern to human health. The second category (smaller group) claims to establish or modify intestinal microbiota that have a direct measurable effect on pathogens of concern to humans, such as *Salmonella enterica* and *Clostridium jejuni*. The two categories are not mutually exclusive because healthier animals are expected to be less susceptible to colonization with certain human pathogens or to carry fewer of these pathogens. Similarly, the microbiota changes designed to inhibit food-borne pathogen colonization can also improve overall animal health and lead to gains in body weight. The number of probiotic products put on the market that claim to be directed against pathogens or are "competitive exclusion" products is exceedingly small compared to products that claim to improve FCR, growth, immune system function, or resistance to stressful events (Joerger and Ganguly 2017). The ecological definition of

"competitive exclusion" states that two species competing for the same resources cannot coexist stably. Therefore, one of the competitors will always dominate the other, leading to an evolutionary modification, a shift to another niche, or extinction. The intestinal microbiota competes with the colonizing pathogenic bacteria and can reduce the adhesion and colonization of pathogens in the intestine. This reduction might be a consequence of different mechanisms, perhaps the physical occupation of space, competition for resources in a given niche, or direct physical or chemical confrontation with the potential colonizer (Chaucheyras-Durand and Durand 2010; Clavijo and Vives Flórez 2018). In birds, competitive exclusion is the process by which favourable bacteria exclude bacteria that may be detrimental to the animal or that are of public health interest, such as *Salmonella* spp. The exclusion of *Salmonella* implies preventing the establishment of harmful bacteria in the gut. The aim is to provide, early in a bird's life, good bacteria having optimal ability to establish and maintain themselves in the gut environment. Thus, administering bacterial mixtures of *Salmonella* spp. from faecal or caecal sources of broiler chickens was more protective than administering single bacterial isolates or a combination of only a few isolates (Kerr et al. 2013). In practice, it is mainly used as a prophylactic measure aimed at increasing the resistance of chicks and poults to *Salmonella* infection. It does imply that the young birds being treated are *Salmonella* free, because the good bacteria are not likely to be able to displace *Salmonella* if it has had the opportunity to become established first in the gut. To achieve this, is imperative to administer the treatment immediately posthatch, before the chicks or poults can be exposed to *Salmonella* spp. The main mode of action of "competitive exclusion" is the establishment of a physical barrier (good bacteria culture attaching to the gut wall) between the intestinal wall and the lumen of the gut. Establishment of favourable bacteria increases the production of VFA and lactate, which lower the gut pH. The lower pH and high VFA concentration produces a hostile environment for unwanted bacteria, such as *Salmonella* spp. and *E. coli* (Lutful Kabir 2010).

4.1 Probiotic Application in Different Animal Species

The International Scientific Association for Probiotics and Prebiotics has defined probiotics "as a mixture of live microorganisms which when administered in adequate amounts confer a health benefit on the host" (Smith 2014). Before explaining the mechanisms and benefits provided by these microorganisms, it is important to specify why, if a microorganism is to be considered a probiotic, it should meet a range of requirements: (1) not pathogenic; (2) can adhere to epithelial cells; (3) can colonize and reproduce itself in the host; (4) able to survive passage through the GIT; (5) is resistant to gastric acidity and bile content; (6) produces metabolites that inhibit or kill pathogenic bacteria; and (7) has undergone trials in vitro and in vivo that demonstrate its benefits. Finally, a probiotic should remain viable under processing, production, and storage conditions (Kabir 2009). The following benefits are expected from administering probiotics (Syngai et al. 2016): (1) stimulation of the development of beneficial microbiota; (2) reduction and prevention of colonization by enteric pathogens; (3) modulation of immunological activity; (4) stimulation of epithelial health; (5) increased digestive capacity; and (6) aid in maturation of intestinal tissue. Probiotics can influence the immune system both directly and indirectly. Direct influence is exerted by different species of *Lactobacillus* that increase cytokine and antibody levels (Haghighi et al. 2006; Brisbin et al. 2011).

Poultry

Numerous probiotic strains have been tested for use for poultry to improve performance, prevent pathogenic colonization, and improve immunity. Probiotics such as *Lactobacillus johnsonii*, *Bacillus subtilis,* and a multi-strain *Lactobacillus johnsonii* FI9785 modified the intestinal microbiota by reducing levels of pathogenic bacteria such as *Salmonella enteritidis* and *Clostridium perfringens* in neonatal broiler chicks (La Ragione et al. 2004; Higgins et al. 2008). Also, a multi-strain probiotic significantly increased the numbers of lactobacilli and bifidobacteria in the caeca of broiler chickens (Mountzouris et al. 2007).

Probiotics for poultry may contain one or more strains of microorganisms and may be given either alone or in combination with other additives in feed or water. The use of probiotics in broiler chickens to control *Salmonella* spp. was effective when hatched chicks were fed a suspension of the intestinal contents of adult chickens (Nurmi and Rantala 1973). However, this first proposed use of "probiotic" proved to have serious limitations, principally the potential transfer of diseases along with the beneficial microorganisms. For this reason, subsequent research has focused on developing defined probiotics capable of being cultivated and administered as pure cultures (Smith 2014). A range of probiotics has been developed, obtained in various ways and for which dosage and time in the cycle in which they are administered also varies. Novel application strategies such as spraying the probiotic on chicks or embryonated eggs are also studied, and potential methods such as in ovo application are being evaluated (Cox and Dalloul 2015).

A variety of bacteria species (*Bacillus*, *Bifidobacterium*, *Enterococcus*, *Lactobacillus*, *Streptococcus*, and *Lactococcus* spp.) and yeast species (*Saccharomyces* spp.) have been tested and used as probiotics in poultry. The majority of the conducted research was specifically aimed at investigating the effects of probiotics in reducing the numbers of pathogenic microorganisms in the GIT. However,

a considerable amount of research also examined the effects of probiotics on improving growth and performance in poultry without apparent disease. Supplementation of diets with a single strain of *Lactobacillus* sp. (*L. casei*, *L. fermentum*, *L. bulgaricus*, *L. reuteri*) was shown to improve body weight and feed efficiency in broiler chickens. Similar results were shown when broiler chickens were given multiple strains of *Lactobacillus* sp. Probiotics based on *Bacillus* sp. (*B. coagulans*, *B. subtilis*, *B. licheniformis*, and *B. amyloliquefaciens*) were also successfully employed in poultry diets and shown to have growth-promoting effects.

Different trial studies have shown that chickens treated with probiotics produce a greater number of antibodies in response to a given antigen (Brisbin et al. 2010). Probiotics may also have indirect effects, promoting the growth of other bacteria. For example, *Lactobacillus agilis* and *Lactobacillus salivarus* can stimulate butyrate-producing microbiota and reestablish microbiota balance (Meimandipour et al. 2009). Another benefit of probiotics is competing with pathogenic microorganisms such as *Salmonella*, *Enterobacter sakasaki*, and *Clostridium difficile*, which have a high capacity of adhesion to the intestinal mucosa (Collado et al. 2005).

Strains of probiotics that help to reduce these levels of adhesion include bacteria of the genera *Bifidobacterium* (Collado et al. 2005) and *Lactobacillus* (Servin and Coconnier 2003). However, this ability is highly dependent on the source of the microorganism, as bacteria from the intestines of chickens show a greater capacity to adhere to the mucosa and, therefore, to displace pathogenic microorganisms (Collado et al. 2005).

The inhibitory effects of probiotic bacteria on undesirable microorganisms might result from the production of metabolites such as hydrogen peroxide (H_2O_2), diacetyl, bacteriocins, and organic acids. A purified bacteriocin produced by *Lactobacillus salivarius* NRRL B-30,514 was used to treat chickens, causing a clear reduction in the numbers of *C. jejuni* in their intestines (Stern et al. 2006). Other compounds that assist in the exclusion of human pathogenic microorganisms are organic acids such as lactic, acetic, or propionic acid, which diminish pH levels in the intestine and reduce the speed of pathogen multiplication (Blajman et al. 2015).

The effectiveness of probiotics depends on several factors, such as the composition of the mixed rations, the time when they are administered, and the origin of the microorganisms. It seems that the effectiveness of probiotic cultures is greater when they contain a larger number of genera (Chambers and Gong 2011). Similarly, origin affects effectiveness, as strains that come directly from chicken intestines are more effective than those from other sources. Additionally, the probiotic composition may be beneficial for one breed of chicken but not for others. Another factor affecting the effectiveness of probiotics is the time point at which they are administered. When probiotics are administered at an early stage of the cycle they will have positive effects only up to week 6, showing greater diversity and abundance of *Lactobacillus* and a significant reduction in the presence of chicken pathogens compared to the control (Nakphaichit et al. 2011). It has also been suggested that the administration of probiotics has a greater effect on pathogenic microorganisms following a change in diet or after antibiotic therapy (Zulkifli et al. 2000).

Lactobacillus is the most commonly used probiotic; its reported benefits include increased weight gain, improved feed utilization effectiveness, and reduction in mortality (Zulkifli et al. 2000; Kalavathy et al. 2003; Timmerman et al. 2006). The probiotic model has been used widely in broiler chickens for the control of *Salmonella*, and it has been reported that employment of these cultures led to reductions in colonization by this pathogen, an effect that is also correlated with increased weight gain and improved conversion of feed into body mass (Chambers and Gong 2011).

Pigs

Weaning as currently practised is one of the most critical periods for pigs, being characterized by a drop in food consumption, leading to severe anorexia, increased susceptibility to digestive disorders, growth delays, and microbial infections. The change in food substrate also leads to significant changes in the functionality of the intestine. *Saccharomyces cerevisae* yeasts, their cell walls or extracted fractions (mannan-oligosaccharides, β-glucans), seem to constitute positive alternatives. Their use in porcine diets can contribute to improving growth performance, stimulating the immune system, maintaining the balance of digestive microflora, and preventing bacterial adhesion to intestinal epithelial cells. Yeasts or yeast products might be potential alternatives to antibiotic growth promoters for swine.

The effect of including the yeast *S. cerevisiae* or its cell wall fraction in diets for weanling piglets for growth performance, nutrient utilization, and some morphological and immunological parameters has been evaluated. Two diets were supplemented with 1 g/kg of live yeast or yeast cell walls for an experiment lasting 5 weeks. Overall, increases in weight gain and in final body weight were observed, and the feed:gain ratio tended to improve with yeast diets. The inclusion of yeasts or yeast cell walls reduced the number of intraepithelial lymphocytes, and increased VFA production and the percentage of acetate, having beneficial effects on the productive performance of piglets after weaning (Lizardo et al. 2008).

Effects of *S. cerevisiae* [strain CNCM I-4407, 10(10) cfu/g] has been studied on postweaning diarrhea, immune response, and growth performance in weaned piglets orally challenged with enterotoxigenic *Escherichia coli* strain O149:K88. The live yeast was fed to sows and their piglets

in the late gestation, suckling, and postweaning periods. Sows were fed a basal diet without or with supplementation (i.e., 1 g/kg of live yeast) from day 94 of gestation and during lactation until weaning of piglets (day 28). Suckling piglets of the supplemented sows were orally treated with 1 g live yeast in porridge carrier three times a week until weaning. Weaned piglets were fed a basal starter diet without or with supplementation (i.e., 5 g of live yeast/kg feed for 2 weeks). Significantly lower daily diarrhea scores, duration of diarrhea, and shedding of pathogenic *E. coli* bacteria in faeces were detected in the supplemented piglets. Administration of live yeast significantly increased IgA levels in piglet serum. Evidence indicates that decreased infection-related stress and decreased severity of diarrhea in yeast-fed weaned piglets positively affected their growth capacity in the postweaning period. Thus, dietary supplementation with live yeast *S. cerevisiae* to sows and piglets in late gestation, suckling, and postweaning periods can be useful in the reduction of the duration and severity of postweaning diarrhea caused by *E. coli*. Decreased infection-related stress and severity of diarrhea in yeast-fed weaned piglets can positively affect growth performance in the pre-weaning period. The results from this study suggest that live yeast *S. cerevisiae* (strain CNCM I-4407) could be an alternative for prevention and treatment of postweaning diarrhea. In addition, *S. cerevisiae* can decrease inflammatory responses induced by F4+ enterotoxigenic *E. coli* in porcine intestinal epithelial cells.

Enterotoxigenic *E. coli* are pathogenic gram-negative bacteria that infect several species of farm animals, including pigs. Enterotoxigenic *E. coli* infection and enterotoxic secretion can induce intestinal inflammation and diarrhea, resulting in reduced growth rate, increased mortality, and economic loss (Fairbrother et al. 2005). Probiotic yeasts may provide protection against intestinal inflammation induced by enteric pathogens. In piglets, infection with F4+ enterotoxigenic *E. coli* causes inflammation, diarrhea, and intestinal damage. The yeast strains *S. cerevisiae* (strain CNCM I-3856) and *S. cerevisiae* var. *boulardii* (strain CNCM I-3799) were investigated for decreased expression of pro-inflammatory cytokines and chemokines in intestinal epithelial IPI-2I cells cultured with F4+ enterotoxigenic *E. coli*. Results showed that viable *S. cerevisiae* inhibited ETEC-induced TNF-α gene expression whereas *Saccharomyces boulardii* did not. In contrast, killed *S. cerevisiae* failed to inhibit the expression of pro-inflammatory genes: this inhibition was dependent on secreted soluble factors. *S. cerevisiae* culture supernatant decreased the TNF-α, IL-1α, IL-6, IL-8, CXCL2, and CCL20 enterotoxigenic *E. coli*-induced mRNA. Furthermore, the *S. cerevisiae* culture supernatant filtrated fraction at 10 kDa displayed the same effects, except for TNF-α.

Inclusion of *Lactobacillus sobrius* in pig diets may be significantly effective in the reduction of *E. coli* F4 colonization and may improve the weight gain of infected piglets (Konstantinov et al. 2008). Also, a multi-strain probiotic containing *L. acidophilus*, *L. bulgaricus, B. subtilis*, and *S. cerevisiae* significantly increased ileal and colonic bifidobacteria levels and decreased the levels of colonic coliforms.

Ruminants

The yeast *S. cerevisiae* has been used as an alternative to antimicrobial feed additives in ruminants for more than 15 years. Production responses showed improved live weight gain in beef cattle and increased milk yield and fat production in dairy cows. However, responses were highly variable and apparently influenced by diet composition and animal physiological stage. The yeast is generally available in two different DFM forms: yeast culture products and live yeast cell products. Ruminant animals, including cattle, sheep, and goats, principally depend on microbial degradation of their feed rather than on direct enzyme degradation, as in most non-ruminants. The enlarged foregut of ruminant livestock (reticulorumen) allows a large and diverse microbial population to gain access to feedstuff before the products of this fermentation and the microbial cells enter the absorptive regions of the GIT (Russell 2002).

The rumen is a symbiosis pathway between the ruminant host and microbes. It is known that microorganisms are involved in the animal host in supplying protein, vitamins, and short-chain organic acids. In the cattle rumen, for example, live yeast can improve milk yield and weight gain by microbial activity stimulation, although this stimulation might depend on certain microbial species. Cattle feed supplemented by live yeast *S. cerevisiae* Sc47 (0.5 or 5 g/day) modifies bacterial diversity and population and changes in the fermentation pattern and physicochemical parameters in the rumen which can modify microbiota composition. In this study the improvement of zootechnical parameters goes together with a shift in the mannin fibrolytic group (i.e., *Fibrobacter* and *Ruminococcus*) (Pinloche et al. 2013).

Early-lactation high-producing dairy cows have a nutrition strategy with the objective to provide adequate energy and rumen-undegraded protein to support high requirements in regard to milk production increase; most of the time, cows are in negative energy balance (Julien et al. 2015). Therefore, it should be important to use live yeast as a feed additive for the dietary rumen-degradable protein level, increasing the feed energy with a certain amount of grain (Julien et al. 2015).

Inclusion of live yeast stabilizes the rumen environment through higher pH values and enhances fibre digestion (Campanile et al. 2008). These key results certainly explain why the impact of live yeast supplementation has been studied primarily on energetic metabolism, in relationship to the dietary forage:concentrates ratio (Lascano et al. 2009). The

consensus was that the effects of live yeast were enhanced when animals consumed a highly concentrated diet or during an abrupt dietary transition (Chaucheyras-Durand et al. 2008). However, some studies have considered the potential effect of live yeast on ruminal ammonia nitrogen metabolism, more specifically on protein degradation or microbial proteosynthesis. In studying the interaction between live yeast and dietary rumen-degradable protein level, Julien et al. (2015) concluded that the rumen-degradable protein content of diet-fed lactating dairy cows could directly impact the acidogenic capacity of the diet: tanned soybean meal was less acidogenic than soybean meal when used as the main protein source. The positive effect of live yeast on ruminal pH in cows receiving a highly acidogenic diet and therefore suffering from subacute ruminal acidosis (SARA) is already known, as live yeast could modulate dietary N digestion in early-lactating dairy cows whose diet had an inadequate rumen-degradable protein content. In fact, live yeast seemed to have a post-ruminal effect on N digestibility even more pronounced than the quantity of bypass N, that is, with sources of protected dietary protein. In both cases, live yeast used as a dietary feed additive permits a better utilization of diet in dairy cows (Julien et al. 2015), and, moreover, increased ruminal total VFA.

Heat stress negatively affects the productivity and longevity of dairy cows. Heat stress has reduced intake and increased reliance on glucose, so feeding strategies capable of improving diet digestibility are plausible for improving post-rumen nutrient flow and performance. Advances in management such as cooling systems and nutrition strategies may attenuate the negative effects of heat stress, but the economic loss from reduced milk production, reproductive efficiency, and animal health during warm seasons is a major issue for the dairy industry worldwide (St. Pierre et al. 2003). The effect of live yeast (*S. cerevisiae*) on digestion and performance of lactating cows during the warm summer months of southeastern Brazil was evaluated (Salvati et al. 2015) using treatments with *S. cerevisiae* equivalent to 25×10^{10} cfu of live cells and 5×10^{10} cfu of dead cells top-dressed to the diet in the morning. A trend was observed for increased plasma glucose with yeast (62.9 vs. 57.3 mg/dl), lowered respiratory frequency (48 vs. 56 breaths/min), and increased plasma niacin content (1.31 vs. 1.22 μg/ml), although the cows had similar rectal temperature. Ruminal lactate and butyrate as proportions of ruminal organic acids were reduced by yeast. Plasma urea nitrogen was increased by yeast over 24 h. Yeast treatment produced a higher blood pH compared with the control, 7.34, and 7.31, respectively. Yeast supplementation improved the lactation performance of dairy cows under heat stress, although this improvement apparently involved regulation of body homeothermia rather than improved digestibility (Salvati et al. 2015).

In high-yielding dairy cows, live yeast *S. cerevisiae* differs from sodium bicarbonate to stabilize ruminal pH (Marden et al. 2008). Early-lactating Holstein cows were supplemented with 150 g/day of sodium bicarbonate or 5 g/day of live yeast during a 21-day experimental period. Total VFA, acetate, and propionate were greater with both additives, but butyrate remained constant; and mean total lactate concentrations decreased 67% with *S. cerevisiae*. The conclusion was *S. cerevisiae* prevented accumulation of lactate and allowed better fiber digestion, whereas sodium bicarbonate seemed to act only as an exogenous buffer.

Moreover, a bio-energetic–redox approach to the effect of *S. cerevisiae* on ruminal pH during induced SARA in dairy cows was described by Marden et al. (2013). The capacity of *S. cerevisiae* at 4 g/cow/day in optimizing ruminal pH was evaluated to understand its mode of action during induced acidosis in the cows. The beneficial effects of live yeast on concentrations of VFA and proportion of propionate in ruminal fluid were higher compared to the control diet. The proportion of butyrate decreased, from 15.8% to 14.2% total VFA, and lactate concentration decreased by 55% on average. Stabilization of ruminal pH (>6) is the outcome of *S. cerevisiae* ability to scavenge oxygen after feeding a high-starch diet. Live yeast seems to act on the reducing power of the ruminal milieu by decreasing oxygen partial pressure and thereby enhancing the activity of anaerobic bacteria.

Horses

Intensive management practices in the horse industry present a unique challenge to the microbiome of the large intestine. Common management practices such as high-concentrate diets, low forage quality, meal feeding, and confinement housing have an impact on intestinal function, specifically large intestinal fermentation. The microbiome of the equine large intestine is a complex and diverse ecosystem, and disruption of microbiota and their environment can lead to increased incidence of GI disorder. In horses, whose targeted digestive compartment is the caecum-colon, probiotic distribution appears particularly relevant in case of stress (e.g., transportation) or during distribution of a high-concentrate diet (Coverdale 2016). Research concerning the use of probiotics in horses to improve hindgut fermentation and diet digestibility has been limited. Most studies used live yeast culture (*S. cerevisiae*) supplemented to a variety of diets. Despite a lack of evidence for colonization with the supplemented strain of *S. cerevisiae*, improvements in cell wall digestibility were evident regardless of diet. In particular, when added to high-starch diets, *S. cerevisiae* supplementation appears to mitigate some of the disruptions, such as reduced fiber digestibility, that occur in the hindgut. Maintenance of fiber digestion is of particular interest when horses consume high-concentrate diets for the purpose of athletic

performance or maximum production (lactation, growth, etc.).

Probiotics, or direct-fed microbials, have been widely used in horses for treatment and prevention of GI disease. Introduction of these live, beneficial microorganisms orally into the intestinal tract has yielded variable results. However, it is difficult to compare data because of variations in choice of organism, dosage, and basal diet. Although there are still many unanswered questions about the mode of action of successful probiotics, evidence indicates competitive inhibition and enhanced immunity. A variety of microbial species have been tested in the horse as probiotics, such as *Lactobacillus* spp., *Enterococcus* spp., *Bifidobacterium* spp., and *Saccharomyces* spp. (Coverdale 2016). Diets containing *Lactobacillus acidophilus* had limited effects either on reducing the risk of acidosis associated with feeding high-starch concentrates to horses or on nutrient digestibility. Live yeasts have been demonstrated to elicit an increase in fibre digestibility in the colon and to modulate the balance of hindgut bacterial communities, with a decreased risk of lactic acidosis (Jouany et al. 2008). Use of these products has resulted in improved fibre digestibility in horses offered both high-starch and high-fibre diets.

Rabbits

Intensive breeding of rabbits can alter the environment, causing physiological stress, and increasing the frequency of enteric diseases, subsequently causing high mortality and decreased reproductive and productive performance of rabbit does (Combes et al. 2013). Application of probiotics as dietary supplements could control enteric diseases. Thus, some probiotics exert a barrier effect against pathogenic microorganisms by preventing their development and colonization within the digestive tract (Vanderpool et al. 2008). The most frequently examined microorganism related to probiotics has been *S. cerevisiae* yeast, known to improve growth performance in cross-breed rabbits. The effect of live yeast supplementation in the diet of rabbit does on their mortality and reproductive performance and the performance of their progeny was studied in two groups differing in diet during two reproductive cycles. Natural mating was performed 11 days after kindling and kits were weaned at 28 days of age. The addition of 1 g *S. cerevisiae*/kg of diet enhanced fertility and reduced mortality of rabbit does, while improving the viability rate of kits at birth; no difference was observed during the second lactation. However, diet supplemented with the tested probiotic had no effect on other reproductive performance traits in rabbit does (Belhassen et al. 2016).

Dogs and Cats

Lactobacillus spp. and *Enterococcus* spp. were studied as probiotics for dogs and *Bifidobacterium* spp. for cats.

Aquaculture

Probiotics intended for aquatic usage must take into account the relationship an aquatic organism has with its direct environment. Gram-negative facultative anaerobic bacteria are dominant in fish and shellfish digestive tracts; however, the intestinal microbiota may often change with the intrusion of microbes from water and food (Chaucheyras-Durand and Durand 2010). Thus, a large number of probiotics developed in aquaculture probably are bacteria directly originating from the aquatic environment. However, most probiotics commonly used in aquaculture are prokaryotic bacteria or yeast such as *Lactobacillus* spp., *Pediococcus* spp., *Bacillus* spp., *Vibrio* spp., and *S. cerevisiae*. Yeast species have also been used as probiotics and for delivery of enzymes in animal feeds. A number of eukaryotic microorganisms are able to survive passage through the acidic conditions and bile salts of the GIT to the intestine. Because these microorganisms may be beneficial for host health, feed utilization, and growth performance, they could also be used as alternative probiotics. Probiotics can target fish eggs and larvae, fish juveniles and adults, crustaceans, bivalve mollusks, and also live food such as rotifers, *Artemia*, or unicellular algae (Verschuere et al. 2000). Growth-promoting effects through better feed utilization and digestion, as well as biological control of pathogen colonization, are the most important expected benefits of probiotic applications (Chaucheyras-Durand and Durand 2010). Disease outbreaks caused by *Vibrio* spp. or *Aeromonas* spp. have been recognized as a significant constraint on aquaculture production (Verschuere et al. 2000), particularly in the shrimp subsector, where vibriosis is currently one of the main diseases identified (Castex et al. 2008). Some probiotics have been shown to protect rainbow trout against skin infections caused by *Aeromonas bestiarum* and a eukaryotic pathogen, *Ichthyophthirius multifiliis* (Pieters et al. 2008).

The effects of dietary probiotic *S. cerevisiae* microencapsulated with guar gum in the striped catfish (*Pangasianodon hypophthalmus*) for a 120-day culture period demonstrated that *S. cerevisiae*-supplemented diets significantly improved growth performance, including growth rate and FCR. *S. cerevisiae* had no effects on hematological parameters and blood chemistry but increased the humoral immune parameters including total immunoglobulin, lysozyme, and alternative complement activities (Boonanuntanasarn et al. 2018).

In Asian sea bass, the mixture of *L. casei* M15, *L. plantarum* D8, *L. pentosus* BD6, *L. fermentum* LW2, *Enterococcus faecium* 10–10, *B. subtilis* E20, and *S. cerevisiae* P13 improved either growth performance or disease resistance. A diet containing 10^9 cfu (kg diet)$^{-1}$ probiotic mixture is recommended to improve the growth and health status of Asian sea bass (Lin et al. 2017).

The effects of dietary substitution of fishmeal with live yeast, *S. cerevisiae*, and increasing water temperature on the

diversity and composition of gut microbiota of rainbow trout were described. The trout were reared in water temperatures of either 11 °C (cold) or 18 °C (warm) for 6 weeks. Feeding live yeast mainly increased yeast load in the trout gut, whereas increased water temperature significantly altered the bacterial diversity and abundance of the gut microbiota. Live yeast can replace 40% of fishmeal without disrupting bacteria communities in the gut of rainbow trout, although increased water temperature from seasonal fluctuations or climate change may cause a gut dysbiosis that jeopardizes farmed fish health (Huyben et al. 2018).

Honey Bee

Honey bees (*Apis millifera*), as pollinators in agriculture, have a critical role in global food production. Worldwide, 75% of the crops traded on the global market depend on pollinators to some degree. Bees are often the most important crop pollinators and honey bees are the pollinators most widely used. Studies show that a diversity of pollinators can improve crop yield or fruit quality. Restoring and maintaining pollinator diversity is thus very important for agriculture as well as for natural vegetation. Recently, honey bee populations in the US, Canada, and Europe have suffered unexplained annual losses from a phenomenon known as "colony collapse disorder." Several members of the *Apis mellifera* microbiota (*Acetobacteriaceae*, *Bifidobacterium*, *Lactobacillus*, *Simunsiella*) produce SCFA such as lactic and acetic acids as waste products during the metabolism of carbohydrates (Vasquez et al. 2012). Honey bees possess an abundant, diverse, and ancient LAB microbiota in their honey crop with beneficial effects for bee health, defending them against microbial threats. This microbiota will become central to studies on honey bee health, including colony collapse disorder, and act as an exemplar case of insect–microbe symbiosis. Honey bee species plus related apid bees show one of the largest collections of novel species from the genera *Lactobacillus* and *Bifidobacterium* ever discovered within a single insect, suggesting a long (>80 million years) history of association. Bee-associated microbiota highlight *Lactobacillus kunkeei* as the dominant LAB member. Prophylactic practices that enhance LAB, or supplementary feeding of LAB, may serve in integrated approaches to sustainable pollinator service provision (Vasquez et al. 2012).

SCFA can be absorbed through the rectal wall in insects, and the majority of the pollen and bacterial biomass within an adult *A. mellifera* is contained inside the rectum (Bradley 2008). Overwintering *Apis* may obtain additional nutrition from these rectal bacteria, as consumed food is stored for longer periods of time within the rectum during winter months (Lindström et al. 2008). The probiotics *Lactobacillus* and *Bifidobacterium* have evolved in synergy with bees and are important in defending their host.

5 Synbiotics

A synbiotic is a combination of one or more probiotics and prebiotics. Prebiotics may enhance the survival of probiotic strains, as well as stimulating activity of the host endogenous bacteria. Synbiotics are additives that combine the use of probiotics and prebiotics such that they act synergistically (Alloui et al. 2013). The use of synbiotics was based on the concept that a mixture of probiotics and prebiotics beneficially affect the host by improving the survival and implantation of probiotic organisms and by selectively promoting the growth or metabolism of beneficial bacteria in the intestinal tract (Gibson and Roberfroid 1995). It was suggested that clinical effects vary from modest to significant from a single strain of probiotics < multi-strain probiotics < or < single-strain/single-fiber synbiotics < multi-strain/multi-fiber synbiotics. A combination of a prebiotic and probiotic, termed a synbiotic, is thought to exert synergistic effects to maintain gut health. Specifically, the probiotic fraction is thought to promote the growth of pathogenic bacteria. Moreover, synbiotics help to reduce the concentration of undesirable metabolites, including nitrosamines, to inactivate carcinogens, and to prevent constipation and diarrhoea of diverse aetiology in human beings (Bengmark and Martindale 2005). Compared with the use of individual components, synbiotics seem to modulate beneficially the composition of the gut microbiota by increasing beneficial bacteria (i.e., lactobacilli and bifidobacteria) and reducing other less desirable bacteria (i.e., coliforms, enterococci) (Modesto et al. 2011).

Poultry

Few research trials have been conducted to demonstrate the effects of synbiotics on broiler chicken performance. Supplementation of diets with a synbiotic product compared with basal diets supplemented with probiotic (homofermentative and a heterofermentative *Lactobacillus* sp.) was shown to significantly improve body weight, average daily gain, feed efficiency, and carcass yield percentage of synbiotic products compared with controls or probiotic-fed broiler chickens (Awad et al. 2009). Synbiotics were also shown to beneficially alter their intestinal microbiota composition and increase villi height and crypt depth in the intestinal mucosa. The increase in the villus height and villus height:crypt depth ratio was associated with improvement of growth performance for both synbiotics and probiotics (Awad et al. 2009). Significant increase in weight gain and a decrease in the FCR was reported when birds were fed diets with a combination of IOS and a multi-strain probiotic (consisting of 11 strains of *Lactobacillus* spp.). A combination of these dietary additives as a synbiotic on caecal bacterial populations and concentrations of caecal volatile fatty acids

and nonvolatile fatty acids of broiler chickens were also evaluated (Mookiah et al. 2014).

Dogs and Cats

There are sparse data on the use of synbiotics in dogs and cats. In one study, the effect of a multispecies symbiotic on the faecal microbiota was investigated in healthy dogs and cats (Garcia-Mazcorro et al. 2011). The symbiotic (containing 5×10^9 colony-forming units of a mixture of seven probiotic strains and a blend of FOS + arabinogalactans) was fed daily for 21 days, with changes in faecal microbiota analysed by culture-independent analyses targeting 16S rRNA bacterial genes (e.g., 454-pyrosequencing). Synbiotic ingestion led to increased abundance of some probiotic species in the faeces; however, no significant changes in bacterial species composition were identified.

Aquaculture

One example of synbiotics in aquaculture is the combination of prebiotic oligosaccharides and probiotic bacteria. An evaluation of the acute-phase response in rainbow trout (*Oncorhynchus mykiss*) fed functional diets supplemented with pre- and probiotics (i.e., mannan-oligosaccharides and *S. cerevisiae*, respectively) and challenged by either *Vibrio anguillarum* or chronic stress via maintenance under high stocking densities suggests that both supplements have high immunostimulatory potentials for farmed fish. In juvenile rainbow trout (*Oncorhynchus mykiss*) fed functional diets supplemented with either pre- or probiotics (0.6% mannan-oligosaccharides and 0.5% *S. cerevisiae*, respectively) or the mixture of both shows a dynamic shift of the microbiome composition and the microbiome modulation dynamics by functional diets based on mannan-oligosaccharides (Goncalves and Gallardo-Escarate 2017).

In Nile tilapia (*Oreochromis niloticus*), encapsulated and freeze-dried *S. cerevisiae* JCM 7255 improved intestinal structure and growth performance. Intraepithelial lymphocytes in the proximal intestine were significantly greater than in the control, and reduced cumulative mortality after the oral streptococcal challenge was also seen (Pinpimai et al. 2015).

In juvenile pacu (*Piaractus mesopotamicus*) stressed and experimentally infected with *Aeromonas hydrophila*, the efficacy of a commercial product (Glucan-MOS®) derived from the yeast *S. cerevisiae*, containing two combined products, β-1,3- or 1,6-glucans and mannans, fed during 30 days, in periods before intensive management, improved growth and innate immunity. The supplementation of 0.1% Glucan-MOS® improved weight gain, feed conversion, and the protein efficiency ratio compared to a control diet. The 0.2% and 0.4% Glucan-MOS® diets were sufficient to increase the respiratory burst of leukocytes and lysozyme activity, the number of thrombocytes, neutrophils, and monocytes in the blood after stressful handling and bacterial challenge, and minimized stress response as shown by decreased cortisol and glucose levels when compared to the control. The 30-day period was sufficient to stimulate growth performance, improve nutrient utilization, minimize stress response, and modulate innate immunity responses (Pereira Soares et al. 2018).

6 Concluding Remarks and Future Directions

There is great potential for the use of prebiotics and probiotics as alternatives to antibiotics to improve performance and reduce pathogenic load in the intestines of animals. The intestinal microbiota is complex and it is not clear how bacteria provide benefit to the host. Modulation of the intestinal bacteria towards a "healthy" community by specialty carbohydrates supporting beneficial bacteria (so-called prebiotics) or by feeding live bacteria (so-called probiotics) is currently undergoing active research. Microbial community analysis has become more accurate, providing reliable data that bacteria in the GIT can be modulated in a number of ways. Although the mechanisms by which antibiotics enhance health and productivity have not been fully elucidated, new research tools, for example, metagenomics and other genome-enabled technologies, may provide new ways to elucidate the ecology of the gut microbiome, host–pathogen interactions, immune development, nutrition, and health. Careful consideration must be given when selecting combinations of prebiotics and probiotics to be used as synbiotics, and research trials should be conducted according to the guidance approved by the regulatory authorities to demonstrate their synergistic effect compared with the use of either product alone and depending on the intended use, and finally according to the quality, efficacy, and safety requirements. The inclusion of specific prebiotics will not be of any benefit without the presence of the targeted, beneficial bacteria products and will not succeed if the environment into which they are introduced is unfavourable. The growth enhancement and health improvement of domestic animals achieved by promoting the growth of certain microbes in the GIT with prebiotics or probiotics is a beneficial and rational strategy, but their use in some production systems such as aquaculture is just beginning. Limited research concerning the use of probiotics in horses to improve hindgut fermentation and diet digestibility also produced contrasting results. Probiotic bacteria have a positive effect on GI function on different species. Yeast species have also been used as probiotics, and for delivery of enzymes in animal feeds; development of genetically engineered yeast and bacterial cells expressing new

substances as antibacterials may have potential as probiotics (Biliouris et al. 2012). In many of these studies it is unclear how much of the positive response obtained with probiotics can or should be considered in the context of their effect on preventing health problems of improving health and welfare and how much to their direct effect on diet utilization. These approaches have been utilized in production systems of food animals for promoting health, but assessing their effectiveness and mechanisms of action is needed. The target of such nutraceutical products is to improve GI health by selecting for beneficial microflora and suppressing known intestinal and food-borne pathogens. If the growth requirements of the bacteria differ, it is possible in theory to shift the microbial community from harmful to nonharmful bacteria by changing the diet and consequently the gut dynamics. Specific species can be selected for resistant feed components, which escape digestion by the host but are readily available for the metabolic machinery of the target microbes. Direct-fed microbials (probiotics) are targeted to improve GI health, but these are likely to be effective only if their growth requirements are fulfilled. In fact, a synbiotic product, which contains both a probiotic strain and a prebiotic favouring the growth of that strain, may be a good solution in many cases.

Acknowledgments This work was supported by Project S2013/ABI-2728 (ALIBIRD-CM Program) from Comunidad de Madrid, and by Project Ref. RTA2015-00010-C03-03 from Ministerio de Economía, Industria y Competitividad, Spain.

References

Abd El-Khalek E, Kalmar ID, De Vroey M et al (2012) Indirect evidence for microbiota reduction through dietary mannanoligosaccharides in the pigeon, an avian species without functional caeca. J Anim Physiol Anim Nutr 96:1084–1090

Alloui MN, Szczurek W, Świątkiewicz S (2013) The usefulness of prebiotics and probiotics in modern poultry nutrition: a review. Ann Anim Sci 13:17–32

Anadón A, Martínez-Larrañaga MR, Martínez MA (2006) Probiotics for animal nutrition in the European Union. Regulation and safety assessment. Regul Toxicol Pharmacol 45:91–95

Anadón A, Martínez-Larrañaga MR, Arés I, Martínez MA (2016) Chapter 1. Prebiotics and probiotics: an assessment of their safety and health benefits. In: Ross Watson R, Preedy VR (eds) Probiotics, prebiotics, and synbiotics. Bioactive foods in promoting health: probiotics and prebiotics. Academic, San Diego, CA, pp 3–23

Anadón A, Martínez-Larrañaga MR, Aresi MMA (2016a) Chapter 54. Prebiotics: safety and toxicity considerations. In: Gupta RC (ed) Nutraceuticals: efficacy, safety and toxicity. Academic, Amsterdam, pp 757–775

Anadón A, Martínez-Larrañaga MR, Aresi MMA (2016b) Chapter 55. Probiotics: safety and toxicity considerations. In: Gupta RC (ed) Nutraceuticals: efficacy, safety and toxicity. Academic, Amsterdam, pp 777–853

Apajalahti J, Kettunen A, Graham H (2004) Characteristics of the gastrointestinal microbial communities, with special reference to the chicken. Worlds Poult Sci J 60:223–232

Awad WA, Ghareeb K, Abdel-Raheem S et al (2009) Effects of dietary inclusion of probiotic and synbiotic on growth performance, organ weights, and intestinal histomorphology of broiler chickens. Poult Sci 88:49–55

Ballou AL, Rizwana AA, Mendoza MA et al (2016) Development of the chick microbiome: how early exposure influences future microbial diversity. Front Vet Sci 3:2

Barko PC, McMichael MA, Swanson KS et al (2018) The gastrointestinal microbiome: a review. J Vet Intern Med 32:9–25

Baurhoo B, Letellier A, Zhao X et al (2007) Cecal populations of lactobacilli and bifidobacteria and *Escherichia coli* populations after in vivo *Escherichia coli* challenge in birds fed diets with purified lignin or mannanoligosaccharides. Poult Sci 86 (12):2509–2516

Beckmann L, Simon O, Vahjen W (2006) Isolation and identification of mixed linked β-glucan degrading bacteria in the intestine of broiler chickens and partial characterization of respective 1, 3-1, 4-β-glucanase activities. J Basic Microbiol 46(3):175–185

Bedford A, Li Z, Li M et al (2012) Epidermal growth factor-expressing *Lactococcus lactis* enhances growth performance of early-weaned pigs fed diets devoid of blood plasma. J Anim Sci 90:4–6

Bednarczyk M, Stadnicka K, Kozlowska I et al (2016) Influence of different prebiotics and mode of their administration on broiler chicken performance. Animal 10:1271–1279

Belhassen T, Simon E, Potel A et al (2016) Effect of diet supplementation with live yeast (*Saccharomyces cerevisiae*) on performance of rabbit does and their progenies. World Rabbit Sci 24:77–82

Bengmark S, Martindale R (2005) Prebiotics and synbiotics in clinical medicine. Nutr Clin Pract 20:244–261

Berg ME, Miller ME, Yeoman CJ et al (2012) Phage–bacteria relationships and CRISPR elements revealed by a metagenomic survey of the rumen microbiome. Environ Microbiol 14(1):207–227

Biagi G, Cipollini I, Bonaldo A et al (2013) Effect of feeding a selected combination of galacto-oligosaccharides and a strain of *Bifidobacterium pseudocatenulatum* on the intestinal microbiota of cats. Am J Vet Res 74:90–95

Biliouris K, Babson D, Schmidt-Dannert C et al (2012) Stochastic simulations of a synthetic bacteria-yeast ecosystem. BMC Syst Biol 6(1):58

Blajman JE, Zbrun MV, Astesana DM et al (2015) Probióticos en pollos parrilleros: una estrategia para los modelos productivos intensivos? Rev Argent Microbiol 47(4):360–367

Bogusławska-Tryk M, Szymeczko R, Piotrowska A, Burlikowska K, Śliżewska K (2015) Ileal and cecal microbial population and short-chain fatty acid profile in broiler chickens fed diets supplemented with lignocellulose. Pak Vet J 35(2):212–216

Boonanuntanasarn S, Ditthab K, Jangprai A et al (2018) Effects of microencapsulated *Saccharomyces cerevisiae* on growth, hematological indices, blood chemical, and immune parameters and intestinal morphology in striped catfish, *Pangasianodon hypophthalmus*. Probiotics Antimicrob Proteins. https://doi.org/10.1007/s12602-018-9404-0

Bradley TJ (2008) Active transport in insect recta. J Exp Biol 211 (Pt 6):835–836

Brisbin JT, Gong J, Parvizi P et al (2010) Effects of lactobacilli on cytokine expression by chicken spleen and cecal tonsil cells. Clin Vaccine Immunol 17(9):1337–1343

Brisbin JT, Gong J, Orouji S et al (2011) Oral treatment of chickens with lactobacilli influences elicitation of immune responses. Clin Vaccine Immunol 18(9):1447–1455

Calik A, Ergün A (2015) Effect of lactulose supplementation on growth performance, intestinal histomorphology, cecal microbial population, and short-chain fatty acid composition of broiler chickens. Poult Sci 94(9):2173–2182

Callaway TR, Edrington TS, Anderson RC et al (2008) Gastrointestinal microbial ecology and the safety of our food supply as related to *Salmonella*. J Anim Sci 86:163–178

Campanile KA, Zicarelli F, Vecchio D et al (2008) Effects of *Saccharomyces cerevisiae* on *in vivo* organic matter digestibility and milk yield in buffalo cows. Livest Sci 114:358–361

Castex M, Chim L, Pham D et al (2008) Probiotic *P. acidilactici* application in shrimp *Litopenaeus stylirostris* culture subject to vibriosis in New Caledonia. Aquaculture 275:183–193

Chambers JR, Gong J (2011) The intestinal microbiota and its modulation for *Salmonella* control in chickens. Food Res Int 44 (10):3149–3159

Chaucheyras-Durand F, Durand H (2010) Probiotics in animal nutrition and health. Benef Microbes 1(1):3–9

Chaucheyras-Durand F, Walker ND, Bach A (2008) Effects of active dry yeasts on the rumen microbial ecosystem: past, present and future. Anim Feed Sci Technol 145:5–26

Che TM, Johnson RW, Kelley KW et al (2011) Mannan oligosaccharide improves immune responses and growth efficiency of nursery pigs experimentally infected with porcine reproductive and respiratory syndrome virus. J Anim Sci 89:2592–2602

Choct M (2009) Managing gut health through nutrition. Br Poult Sci 50 (1):9–15

Choct M, Hughes RJ, Wang J et al (1996) Increased small intestinal fermentation is partly responsible for the antinutritive activity of non-starch polysaccharides in chickens. Br Poult Sci 37:609–621

Clavijo V, Vives Flórez MJ (2018) The gastrointestinal microbiome and its association with the control of pathogens in broiler chicken production: a review. Poult Sci 0:1–16

Collado MC, Gueimonde M, Hernandez M et al (2005) Adhesion of selected *Bifidobacterium* strains to human intestinal mucus and the role of adhesion in enteropathogen exclusion. J Food Prot 68 (12):2672–2678

Combes S, Fortun-Lamothe L, Cauquil L, Gidenne T (2013) Engineering the rabbit digestive ecosystem to improve digestive health and efficacy. Animal 7:1429–1439

Coverdale JA (2016) Horse species symposium: can the microbiome of the horse be altered to improve digestion? J Anim Sci 94:2275–2281

Cox CM, Dalloul RA (2015) Immunomodulatory role of probiotics in poultry and potential in ovo application. Benef Microbes 6(1):45–52

Da Costa PM, Loureiro L, Matis AJF (2013) Transfer of multidrug-resistant bacteria between intermingled ecological niches: the interface between humans, animals and the environment. Int J Environ Res Public Health 10:278–294

De Vrese M, Schrezenmeir J (2008) Probiotics, prebiotics, and synbiotics. Adv Biochem Eng Biotechnol 111:1–66

Deusch O, O'Flynn C, Colyer A et al (2015) A longitudinal study of the feline faecal microbiome identifies changes into early adulthood irrespective of sexual development. PLoS One 10:e0144881

Durst L (1996) Inclusion of fructo- and galacto-oligosaccharides in broiler diets. Arch Geflugelkd 60:160–164

Eeckhaut V, Van Immerseel F, Teirlynck E et al (2008) *Butyricicoccus pullicaecorum* gen. nov., sp. nov., an anaerobic, butyrate-producing bacterium isolated from the caecal content of a broiler chicken. Int J Syst Evol Microbiol 58(12):2799–2802

Fairbrother JM, Nadeau É, Gyles CL (2005) *Escherichia coli* in postweaning diarrhea in pigs: an update on bacterial types, pathogenesis, and prevention strategies. Anim Health Res Rev 6(1):17–39

FAO (2007) Technical meeting report. FAO Technical Meeting on Prebiotics, Food Quality and Standards Service (AGNS), 15–16 Sept 2007

Ferket PR, Santos AA Jr, Oviedo-Rondon EO (2005) Dietary factors that affect gut health and pathogen colonization. In: Proceedings of 32nd annual Carolina poultry nutrition conference, Research Triangle Park, NC, p 22

Foligné B, Peys E, Vandenkerckhove J et al (2012) Spores from two distinct colony types of the strain *Bacillus subtilis* PB6 substantiate anti-inflammatory probiotic effects in mice. Clin Nutr 31:987–994

Frese SA, Parker K, Calvert CC, Mills DA (2015) Diet shapes the gut microbiome of pigs during nursing and weaning. Microbiome 3:28. https://doi.org/10.1186/s40168-015-0091-8

Fukata T, Sasai K, Miyamoto T et al (1999) Inhibitory effects of competitive exclusion and fructooligosaccharide, singly and in combination, on *Salmonella* colonization of chicks. J Food Prot 62 (3):229–233

Fuller R (1989) Probiotics in man and animals. J Appl Bacteriol 66:365–378

Fuller R (1992) The effect of probiotics on the gut micro-ecology of farm animals. In: The lactic acid bacteria, vol 1. Springer, Boston, MA, pp 171–192

Gaggìa F, Mattarelli P, Biavati B (2010) Probiotics and prebiotics in animal feeding for safe food production. Int J Food Microbiol 141: S15–S28

Garcia-Mazcorro JF, Lanerie DJ, Dowd SE et al (2011) Effect of a multi-species synbiotic formulation on fecal bacterial microbiota of healthy cats and dogs as evaluated by pyrosequencing. FEMS Microbiol Ecol 78(3):542–554

Geier MS, Torok VA, Allison GE et al (2009) Indigestible carbohydrates alter the intestinal microbiota but do not influence the performance of broiler chickens. J Appl Microbiol 106 (5):1540–1548

Ghosh S, Mehla RK (2012) Influence of dietary supplementation of prebiotics (mannanoligosaccharide) on the performance of crossbred calves. Trop Anim Health Prod 44:617–622

Gibson GR (1999) Dietary modulation of the human gut microflora using the prebiotics oligofructose and inulin. J Nutr 129(7):1438S–1441S

Gibson GR, Roberfroid MB (1995) Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. J Nutr 125:1401–1412

Gibson GR, Probert HM, Van Loo J et al (2004) Dietary modulation of the human colonic microbiota: updating the concept of prebiotics. Nutr Res Rev 17:259–275

Gibson GR, Scott KP, Rastall RA et al (2010) Dietary prebiotics: current status and new definition. Food Sci Technol Bull Funct Foods 7 (1):1–19

Gibson GR, Hutkins R, Sanders ME et al (2017) The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. Nat Rev Gastroenterol Hepatol 14:491–502

Gil de los Santos JR, Storch OB, Fernandes CG et al (2012) Evaluation in broilers of the probiotic properties of *Pichia pastoris* and a recombinant *P. pastoris* containing the *Clostridium perfringens* alpha toxin gene. Vet Microbiol 156:448–451

Goncalves AT, Gallardo-Escarate C (2017) Microbiome dynamic modulation through functional diets based on pre- and probiotics (mannan-oligosaccharides and *Saccharomyces cerevisiae*) in juvenile rainbow trout (*Oncorhynchus mykiss*). J Appl Microbiol 122:1333–1347

Gresse R, Chaucheyras-Durand F, Fleury MA et al (2017) Gut microbiota dysbiosis in postweaning piglets: understanding the keys to health. Trends Microbiol 25(10):851–873

Guarner F (2007) Prebiotics in inflammatory bowel diseases. Br J Nutr 98(Suppl 1):S85–S89

Guillot JF (2003) Probiotic feed additives. J Vet Pharmacol Ther 26 (Suppl 1):52–55

Haghighi HR, Gong J, Gyles CL et al (2006) Probiotics stimulate production of natural antibodies in chickens. Clin Vaccine Immunol 13(9):975–980

Hardy GA, Sieg S, Rodriguez B et al (2013) Interferon-α is the primary plasma type-I IFN in HIV-1 infection and correlates with immune activation and disease markers. PLoS One 8(2):e56527

Havenaar R, Ten Brink B, Huis JH (1992) Selection of strains for probiotic use. In: Probiotics. Springer, Dordrecht, pp 209–224

Hetland H, Svihus B, Choct M (2004) Role of insoluble non-starch polysaccharides in poultry nutrition. Worlds Poult Sci J 60:415–422
Higgins SE, Higgins JP, Wolfenden AD et al (2008) Evaluation of a *Lactobacillus*-based probiotic culture for the reduction of *Salmonella enteritidis* in neonatal broiler chicks. Poult Sci 87:27–31
Higgins SE, Wolfenden AD, Tellez G et al (2011) Transcriptional profiling of cecal gene expression in probiotic- and *Salmonella*-challenged neonatal chicks. Poult Sci 90:901–913
Hoseinifar SH, Khalili M, Rostami HK et al (2013) Dietary galactooligosaccharide affects intestinal microbiota, stress resistance, and performance of Caspian roach (*Rutilus rutilus*) fry. Fish Shellfish Immunol 35:1416–1420
Hoseinifar SH, Soleimani N, Ringø E (2014) Effects of dietary fructooligosaccharide supplementation on the growth performance, haemato-immunological parameters, gut microbiota and stress resistance of common carp (*Cyprinus carpio*) fry. Br J Nutr 112:1296–1302
Huyben D, Sun L, Moccia R et al (2018) Dietary live yeast and increased water temperature influence the gut microbiota of rainbow trout. J Appl Microbiol 124:1377–1392
Huyghebaert G, Ducatelle R, Van Immerseel F (2011) An update on alternatives to antimicrobial growth promoters for broilers. Vet J 187:182–188
Iji PA, Tivey DR (1998) Natural and synthetic oligosaccharides in broiler chicken diets. Worlds Poult Sci J 54(2):129–143
Jensen BB (1998) The impact of feed additives on the microbial ecology of the gut in young pigs. J Anim Sci 7:45–64
Joerger RD, Ganguly A (2017) Current status of the preharvest application of pro- and prebiotics to farm animals to enhance the microbial safety of animal products. Microbiol Spectr 5(1). https://doi.org/10.1128/microbiolspec.PFS-0012-2016
Jouany JP, Gobert J, Medina B et al (2008) Effect of live yeast culture supplementation on apparent digestibility and rate of passage in horses fed a high-fiber or high-starch diet. J Anim Sci 86(2):339–347
Julien C, Marden JP, Auclair E et al (2015) Interaction between live yeast and dietary rumen degradable protein level: effects on diet utilization in early-lactating dairy cows. Agric Sci 6:1–13
Kabir SM (2009) The role of probiotics in the poultry industry. Int J Mol Sci 10(8):3351–3546
Kalavathy R, Abdullah N, Jalaludin S et al (2003) Effects of *Lactobacillus* cultures on growth performance, abdominal fat deposition, serum lipids and weight of organs of broiler chickens. Br Poult Sci 44(1):139–144
Kamada N, Seo SU, Chen GY, Nuñez G (2013) Role of the gut microbiota in immunity and inflammatory disease. Nat Rev Immunol 13:321–335
Kanakupt K, Vester Boler BM, Dunsford BR et al (2011) Effects of shortchain fructooligosaccharides and galactooligosaccharides, individually and in combination, on nutrient digestibility, fecal fermentative metabolite concentrations, and large bowel microbial ecology of healthy adult cats. J Anim Sci 89:1376–1384
Kenny M, Smidt H, Mengheri E et al (2011) Probiotics – do they have a role in the pig industry? Animal 5:462–470
Kerr AK, Farrar AM, Waddell LA et al (2013) A systematic review-meta-analysis and meta-regression on the effect of selected competitive exclusion products on *Salmonella* spp. prevalence and concentration in broiler chickens. Prev Vet Med 111:112–125
Khan N, Vidyarthi A, Pahari S et al (2016) Signaling through NOD-2 and TLR-4 bolsters the T cell priming capability of dendritic cells by inducing autophagy. Sci Rep 6:19084
Kolida S, Gibson GR (2011) Synbiotics in health and disease. Annu Rev Food Sci Technol 2:373–393
Konstantinov SR, Smidt H, De Vos WM et al (2008) S layer protein A of *Lactobacillus acidophilus* NCFM regulates immature dendritic cell and T cell functions. Proc Natl Acad Sci USA 105 (49):19474–19479
Kyu Song S, Ram Beck B, Kim D et al (2014) Prebiotics as immunostimulants in aquaculture: a review. Fish Shellfish Immunol 40:40–48
La Ragione RM, Narbad A, Gasson MJ et al (2004) *In vivo* characterization of *Lactobacillus johnsonii* FI9785 for use as a defined competitive exclusión agent against bacterial pathogens in poultry. Lett Appl Microbiol 38:197–205
Lallès J-P, Bosi P, Smidt H, Stokes C (2007) Weaning – a challenge to gut physiologists. Livestock Sci 108(1–3):82
Lascano GJ, Zanton GI, Heinrichs AJ (2009) Concentrate levels and *Saccharomyces cerevisiae* affect rumen fluid-associated bacteria numbers in dairy heifers. Livest Sci 126:189–194
Lee W-H, Pathanibud P, Quarterman J et al (2012) Whole cell biosynthesis of a functional oligosaccharide, 2′-fucosyllactone, using engineered *Escherichia coli*. Microb Cell Factories 11:48. https://doi.org/10.1186/1475-2859-11-48
Ley RE, Hamady M, Lozupone C et al (2008) Evolution of mammals and their gut microbes. Science 320:1647–1651
Li P, Burr GS, Gatlin DM et al (2007) Dietary supplementation of shortchain fructooligosaccharides influences gastrointestinal microbiota composition and immunity characteristics of Pacific white shrimp. *Litopenaeus vannamei*, cultured in a recirculating system. J Nutr 137:2763–2768
Lin H-L, Shiu Y-L, Chiu C-S et al (2017) Screening probiotic candidates for a mixture of probiotics to enhance the growth performance, immunity, and disease resistance of Asian seabass, *Lates calcarifer* (Bloch), against *Aeromonas hydrophila*. Fish Shellfish Immunol 60:474–482
Lindström A, Korpela S, Fries L (2008) Horizontal transmission of *Paenibacillus larvae* spores between honey bee (*Apis mellifera*) colonies through robbing. Apidologie 39:515–522
Liu P, Piao XS, Kim SW et al (2008) Effects of chito-oligosaccharide supplementation on the growth performance, nutrient digestibility, intestinal morphology, and fecal shedding of *Escherichia coli* and *Lactobacillus* in weaning pigs. J Anim Sci 86:2609–2618
Lizardo R, Nofrarias M, Guinvarch J et al (2008) Influence de l'incorporation de levures *Saccharomyces cerevisiae* ou de leur parois dans l'aliment sur la digestión et les performances zootechniques des porcelets en post-sevrage. J Recher Porc 40:183–190
Lutful Kabir SM (2010) Avian colibacillosis and salmonellosis: a closer look at epidemiology, pathogenesis, diagnosis, control and public health concerns. Int J Environ Res Public Health 7 (1):89–114
Macfarlane SMGT, Macfarlane GT, Cummings JT (2006) Prebiotics in the gastrointestinal tract. Aliment Pharmacol Ther 24(5):701–714
Mancabelli L, Ferrario C, Milani C (2016) Insights into the biodiversity of the gut microbiota of broiler chickens. Environ Microbiol 18 (12):4727–4738
Manning TS, Gibson GR (2004) Microbial-gut interactions in health and diasease. Prebiotics. Best Pract Res Clin Gastroenterol 18 (2):287–298
Marden JP, Julien C, Monteils V et al (2008) How does live yeast differ from sodium bicarbonate to stabilize ruminal pH in high-yielding dairy cows? J Dairy Sci 91(9):3528–3535
Marden J-P, Bayourthe C, Auclair E et al (2013) A bioenergetic-redox approach to the effect of live yeast on ruminal pH during induced acidosis in dairy cow. Am J Anal Chem 4:60–68
Mead GC (1989) Microbes of the avian cecum: types present and substrates utilized. J Exp Zool Suppl 3:48–54
Meimandipour A, Shuhaimi M, Hair-Bejo M et al (2009) In vitro fermentation of broiler cecal content: the role of lactobacilli and pH value on the composition of microbiota and end products fermentation. J Appl Microbiol 49(4):415–420
Metzler BU, Mosenthin R (2008) A review of intreractions between dietary fiber and the gastrointestinal microbiota and their

consequences on intestinal phosporous metabolism in growing pigs. Asian Aust J Anim Sci 21(4):603–615
Mitsuoka T, Hidaka H, Eida T (1987) Effect of fructo-oligosaccharides on intestinal microflora. Nahrung 31(5–6):427–436
Modesto M, Steanini I, D'Aimmo MR et al (2011) Strategies to augment non-immune system based defence mechanisms against gastrointestinal diseases in pigs. NJAS – Wageningen J Life Sci 58 (3–4):149–156
Mookiah S, Sieo CC, Ramasamy K et al (2014) Effects of dietary prebiotics, probiotic and synbiotics on performance, caecal bacterial populations and caecal fermentation concentrations of broiler chickens. J Sci Food Agric 94:341–348
Morales-López R, Auclair E, Garcia F et al (2009) Use of yeast cell walls; β-1, 3/1, 6-glucans; and mannoproteins in broiler chicken diets. Poult Sci 88:601–607
Morgan LM, Coverdale JA, Froetschel MA et al (2007) Effect of yeast culture supplementation on digestibility of varying forage quality in mature horses. J Equine Vet Sci 27:260–265
Mountzouris KC, Tsistsikos P, Kalamara E et al (2007) Evaluation of the efficacy of a probiotic containing *Lactobacillus*, *Bifidobacterium*, *Enterococcus*, and *Pediococcus* strains in promoting broiler performance and modulting cecal microflora composition and metabolic activities. Poult Sci 86:309–317
Nagpal R, Kaur A (2011) Synbiotic effects of various prebiotics on in vitro activities of probiotic lactobacilli. Ecol Food Nutr 50 (1):63–68
Nakphaichit M, Thanomwongwattana S, Phraephaisarn C et al (2011) The effect of including *Lactobacillus reuteri* KUB-AC5 during post-hatch feeding on the growth and ileum microbiota of broiler chickens. Poult Sci 90(12):2753–2765
Niu Q, Li P, Hao S, Zhang Y, Kim SW, Li H, Ma X, Gao S, He L, Wu WJ, Huang X, Hua J, Zhou B, Huang R (2015) Dynamic distribution of the gut microbiota and the relationship with apparent crude fiber digestibility and growth stages in pigs. Sci Rep 5:9938. https://doi.org/10.1038/srep09938
Novak M, Vetvicka V (2008) Beta-glucans, history, and the present: immunomodulatory aspects and mechanisms of action. J Immunotoxicol 5:47–57
Nurmi E, Rantala M (1973) New aspects of *Salmonella* infection in broiler production. Nature (Lond) 241(5386):210–211
O'Hara AM, Shanahan F (2007) Gut microbiota: mining for therapeutic potential. Clin Gastroenterol Hepatol 5:274–284
Oakley BB, Morales CA, Line J et al (2013) The poultry-associated microbiome: network analysis and farm-to-fork characterizations. PLoS One 8(2):e57190
Parsley LC, Consuegra EJ, Thomas SJ et al (2010) Census of the viral metagenome within an activated sludge microbial assemblage. Appl Environ Microbiol 76:2673–2677
Patel S, Goyal A (2012) The current trends and future perspectives of prebiotics research: a review. 3 Biotech 2(1):1–15
Patterson JA, Burkholder KM (2003) Applications of prebiotics and probiotics in poultry production. Poult Sci 82:627–631
Pereira Soares M, Cristina Oliveira F et al (2018) Glucan-MOS® improved growth and innate immunity in pacu stressed and experimentally infected with *Aeromonas hydrophila*. Fish Shellfish Immunol 73:133–140
Pieters N, Brunt J, Austin B et al (2008) Efficacy of in-feed probiotics against *Aeromonas bestiarum* and *Ichthyophthirius multifiliis* skin infections in rainbow trout (*Oncorhynchus mykiss*, Walbaum). J Appl Microbiol 105(3):723–732
Pineiro M, Asp N-G, Reid G et al (2008) FAO technical meeting on prebiotics. J Clin Gastroenterol 42(Suppl 3 Pt 2):S156–S159
Pinloche E, McEwan N, Marden J-P et al (2013) The effects of a probiotic yeast on the bacterial diversity and population structure in the rumen of cattle. PLoS One 8(7):e67824
Pinpimai K, Rodkhum C, Chansue N et al (2015) The study on the candidate probiotic properties of encapsulated yeast, *Saccharomyces cerevisiae* JCM7255, in Nile tilapia (*Oreochromis niloticus*). Res Vet Sci 102:103–111
Pourabedin M, Zhao X (2015) Prebiotics and gut microbiota in chickens. FEMS Microbiol Lett 362:fnv122
Pridmore RD, Berger B, Desiere F et al (2004) The genome sequence of the probiotic intestinal bacterium *Lactobacillus johnsonni* NCC 533. Proc Natl Acad Sci USA 101:2512–2517
Qiu R, Croom J, Ali RA et al (2012) Direct fed microbial supplementation repartitions host energy to the immune system. J Anim Sci 90:2639–2651
Quigley JD, Drewry JJ, Murray LM et al (1997) Body weight gain, feed efficiency, and fecal scores of dairy calves in response to galactosyllactose or antibiotics in milk replacers. J Dairy Sci 80:1751–1754
Redfern A, Suchodolski J, Jergens A (2017) Role of the gastrointestinal microbiota in small animal health and disease. Vet Rec 181(14):370
Rehman H, Vahjen W, Kohl-Parisini A et al (2009) Influence of fermentable carbohydrates on the intestinal bacteria and enteropathogens in broilers. Worlds Poult Sci J 65(1):75–90
Rejeb KB, Abdelly C, Savouré A (2014) How reactive oxygen species and proline face stress together. Plant Physiol Biochem 80:278–284
Respondek F, Swanson KS, Belsito KR et al (2008) Short-chain fructooligosaccharides influence insulin sensitivity and gene expression of fat tissue in obese dogs. J Nutr 138:1712–1718
Respondek F, Myers K, Smith TL et al (2011) Dietary supplementation with short-chain fructooligosaccharides improves insulin sensitivity in obese horses. J Anim Sci 89:77–83
Roberfroid M (2007) Prebiotics: the concept revisited. J Nutr 137:830–837
Roberfroid M, Gibson GR, Hoyles L et al (2010) Prebiotic effects: metabolic and health benefits. Br J Nutr 104(Suppl 2):S1–S63
Rohwer F, Thurber RV (2009) Viruses manipulate the marine environment. Nature 459:207–212
Roodposhti PM, Dabiri N (2012) Effects of probiotic and prebiotic on average daily gain, fecal shedding of *Escherichia coli*, and immune system status in newborn female calves. Asian-Australas J Anim Sci 25:1255–1261
Russell JB (2002) Rumen microbiology and its role in ruminant nutrition. Cornell University, Ithaca, NY
Saad N, Delattre C, Urdaci M et al (2013) An overview of the last advances in probiotic and prebiotic field. LWT – Food Sci Technol 50:1–16
Salvati GGS, Morais Junior NN, Melo ACS et al (2015) Response of lactating cows to live yeast supplementation during summer. J Dairy Sci 98(6):4062–4073
Sancak AA, Rutgers HC, Hart CA et al (2004) Prevalence of enteropathic *Escherichia coli* in dogs with acute and chronic diarrhoea. Vet Rec 154:101–106
Sartor RB (2004) Therapeutic manipulation of the enteric microflora in inflammatory bowel diseases: antibiotics, probiotics, and prebiotics. Gastroenterology 126:1620–1633
Seal BS, Lillehoj HS, Donovan DM et al (2013) Alternatives to antibiotics: a symposium on the challenges and solutions for animal production. Anim Health Res Rev 14(1):78–87
Seepersadsingh N, Adesiyun AA, Seebaransingh R (2004) Prevalence and antimicrobial resistance of *Salmonella* spp. in non-diarrhoeic dogs in Trinidad. J Vet Med B Infect Dis Vet Public Health 51:337–342
Sekirov I, Russell SL, Antunes CM, Finlay BB (2010) Gut microbiota in health and disease. Physiol Rev 90:859–904
Servin AL, Coconnier MH (2003) Adhesion of probiotic strains to the intestinal mucosa and interaction with pathogens. Best Pract Res Clin Gastroenterol 17(5):741–754
Seth A, Yan F, Polk DB et al (2008) Probiotics ameliorate the hydrogen peroxide-induced epithelial barrier disruption by a PKC-and MAP

kinase-dependent mechanism. Am J Physiol Gastrointest Liver Physiol 294:1060–1069
Shakouri MD, Iji PA, Mikkelsen LL et al (2009) Intestinal function and gut microflora of broiler chickens as influenced by cereal grains and microbial enzyme supplementation. J Anim Physiol Anim Nutr 93:647–658
Smith JM (2014) A review of avian probiotics. J Avian Med Surg 28 (2):87–94
Sparkes AH, Papasouliotis K, Sunvold G et al (1998a) Bacterial flora in the duodenum of healthy cats, and effect of dietary supplementation with fructo-oligosaccharides. Am J Vet Res 59:431–435
Sparkes AH, Papasouliotis K, Sunvold G et al (1998b) Effect of dietary supplementation with fructo-oligosaccharides on fecal flora of healthy cats. Am J Vet Res 59:436–440
Spring P, Wenk C, Dawson KA et al (2000) The effect of dietary mannonoligosaccharides on cecal parameters and the concentrations of enteric bacteria in the ceca of *Salmonella*-challenged broiler chicks. Poult Sci 79:205–211
St. Pierre NR, Cobanov B, Schnitkey G (2003) Economic losses from heat stress by US livestock industries. J Dairy Sci 86(E Suppl):E52–E77
Stanley D, Denman SE, Hughes RJ et al (2012) Intestinal microbiota associated with differential feed conversion efficiency in chickens. Appl Microbiol Biotechnol 96:1361–1369
Stanley D, Hughes RJ, Moore RJ (2014) Microbiota of the chicken gastrointestinal tract: influence on health, productivity and disease. Appl Microbiol Biotechnol 98(10):4301–4310
Stern NJ, Svetoch EA, Eruslanov BV et al (2006) Isolation of a *Lactobacillus salivarius* strain and purification of its bacteriocin, which is inhibitory to *Campylobacter jejuni* in the chicken gastrointestinal system. Antimicrob Agents Chemother 50(9):3111–3116
Stevens CE, Hume ID (1998) Contributions of microbes in vertebrate gastrointestinal tract to production and conservation of nutrients. Physiol Rev 78:393–427
Syngai GG, Gopi R, Bharali R et al (2016) Probiotics: the versatile functional food ingredients. J Food Sci Technol 53:921–933
Timmerman HM, Veldman A, Van den Elsen E et al (2006) Mortality and growth performance of broilers given drinking water supplemented with chicken-specific probiotics. Poult Sci 85 (8):1383–1388
Torok VA, Ophel-Keller K, Loo M et al (2008) Application of methods for identifying broiler chicken gut bacterial species linked with increased energy metabolism. Appl Environ Microbiol 74 (3):783–791
Turnbaugh PJ, Ley RE, Hamady M et al (2007) Human microbiome project. Nature 449:804–810
Van Craeyveld V, Holopainen U, Selinheimo E et al (2009) Extensive dry ball milling of wheat and rye bran leads to *in situ* production of arabinoxylan oligosaccharides through nanoscale fragmentation. J Agric Food Chem 57:8467–8473
Van Haandel B (2016) Microbiome: its effect on health and growth. All About Feed 24(7):6–7
Van Immerseel F, Cauwerts K, Devriese LA et al (2002) Feed additives to control *Salmonella* in poultry. Worlds Poult Sci J 58(4):501–513
Vanderpool C, Yan F, Polk DB (2008) Mechanisms of probiotic action: implications for therapeutic applications in inflammatory bowel diseases. Inflamm Bowel Dis 14:1585–1596
Vasquez A, Forsgren E, Fries I et al (2012) Symbionts as major modulators of insect health: lactic acid bacteria in honeybee. PLoS One 7:e33188
Verbrugghe A, Hesta M, Gommeren K et al (2009) Oligofructose and inulin modulate glucose and amino acid metabolism through propionate production in normal-weight and obese cats. J Nutr 102:694–702
Verschuere L, Rombaut G, Sorgeloos P et al (2000) Probiotic bacteria as biological control agents in aquaculture. Microbiol Mol Biol Rev 64:655–671
Wiley NC, Dinan TG, Ross P et al (2017) The microbiota-gut-brain axis as a key regulator of neural function and the stress response: implications for human and animal health. J Anim Sci 95:3225–3246
Willard MD, Simpson RB, Cohen ND et al (2000) Effects of dietary fructooligosaccharide on selected bacterial populations in feces of dogs. Am J Vet Res 61:820–825
Williams BA, Verstegen MWA, Tamminga S (2001) Fermentation in the large intestine of single-stomached animals and its relationship to animal health. Nutr Res Rev 14:207–228
Xu ZR, Hu CH, Xia MS et al (2003) Effects of dietary fructooligosaccharide on digestive enzyme activities, intestinal microflora and morphology of male broilers. Poult Sci 82(6):1030–1036
Xu Q, Chao Y, Wan Q (2009) Health benefit application of functional oligosaccharides. Carbohydr Polym 77:435–441
Yajima T, Inoue R, Yajima M et al (2011) The G-protein in colesterol-rich membrane microdomains mediates mucosal sensing of short-chain fatty acids and secretory response in rat colon. Acta Physiol (Oxf) 203:381–389
Yaqoob P (2014) Ageing, immunity and influenza: a role for probiotics? Proc Nutr Soc 73(2):309–317
Yatsunenko T, Rey FE, Manary MJ et al (2012) Human gut microbiome viewed across age and geography. Nature 486:222–228
Yeoman CJ, Chia N, Jeraldo P et al (2012) The microbiome of the chicken gastrointestinal tract. Anim Health Res Rev 13(1):89–99
Youssef IMI, Kamphues J (2018) Fermentation of lignocellulose ingredients in vivo and in vitro via using fecal and caecal inoculums of monogastric animals (swine/turkeys). BJBAS 7:407–413
Zulkifli I, Abdullah N, Azrin NM et al (2000) Growth performance and immune response of two commercial broiler strains fed diets containing *Lactobacillus* cultures and oxytetracycline under heat stress conditions. Br Poult Sci 41(5):593–597

Synbiotics in Animal Health and Production

Jitendra K. Malik, Atul Prakash, Anil K. Srivastava, and Ramesh C. Gupta

Abstract

This chapter describes the current knowledge on the effects of synbiotics on the health and performance of farm and companion animals. The mechanism of action, beneficial effects, and demonstrated study results proving the efficacy of synbiotics in poultry, cattle, pigs, and companion animals are presented. Synbiotics are now being considered important tools to help maintaining animals in good health and improving growth performance. The majority of studies dealing with synbiotics have been conducted in poultry, but increasing research has been focused on other animal species. The administration of synbiotics in animals is favorable for the beneficial intestinal microbiota which play several and significant roles including production of various nutrients for their host, prevention of infections caused by intestinal pathogens, and modulation of immunological response. Thus, modification of the microorganisms present in the gastrointestinal tract in order to attain, restore, and maintain positive balance in the ecosystem and activity of the intestinal microbiota is essential for the improvement of health and performance of animals. Synbiotics comprising of both most appropriate probiotic microorganisms and synergistic prebiotics may be still more effective and beneficial for animal health and production.

Keywords

Synbiotic · Animal · Gut microbiota · Health · Performance

1 Introduction

Synbiotics are generally considered as nutritional supplements combining probiotic and prebiotic in a form of synergism. Thus, a synbiotic product can enhance the isolated beneficial effects of probiotic and prebiotic. Probiotics are defined as live strains of strictly selected microorganisms which, when administered in adequate amounts, confer a health benefit on the host (FAO 2002). Probiotics are nonpathogenic microorganisms which favor the growth of beneficial intestinal bacteria over that of harmful ones. Prebiotics are nondigestible food ingredients that beneficially affect the health of host by selectively stimulating the growth and/or activity of a selected group of microorganisms living in the colon. An important basis for using a synbiotic is that a true probiotic uses the prebiotic as a food source that facilitates in extending the survival of probiotic microorganisms in the digestive system which would not be possible without otherwise. Without the necessary food source for the probiotic, it will have a greater intolerance for oxygen, low pH, and temperature. By harnessing both the advantages of the prebiotics and probiotics into synergy, the number of good microorganisms increases many folds in the digestive system (Sekhon and Jairath 2010; Malik et al. 2016). Thus, a suitable combination of both probiotic and prebiotic in a single product should ensure a better effect, compared to their alone activity. In addition to the improved survival of beneficial microorganisms added to food or feed, synbiotics also stimulate proliferation of specific native bacterial strains present in the gastrointestinal tract (Gourbeyre et al. 2011). The health effects of synbiotics may be associated with the individual combination of a probiotic and prebiotic. Taking into account a large number of possible

J. K. Malik (✉)
Division of Pharmacology and Toxicology, Indian Veterinary Research Institute, Dehradun, Uttarakhand, India

A. Prakash
Department of Pharmacology and Toxicology, College of Veterinary Science and Animal Husbandry, UP Pandit Deen Dayal Upadhyay Pashu Chikitsa Vigyan Vishwavidyalaya Evam Go-Anusandhan Sansthan, Mathura, UP, India

A. K. Srivastava
Agricultural Scientists Recruitment Board, Indian Council of Agricultural Research, New Delhi, India

R. C. Gupta
Department of Toxicology, Breathitt Veterinary Center, Murray State University, Hopkinsville, KY, USA

R. C. Gupta et al. (eds.), *Nutraceuticals in Veterinary Medicine*, https://doi.org/10.1007/978-3-030-04624-8_20

combinations, the application of synbiotics for the modulation of gut microbiota in humans and animals appears promising (Scavuzzi et al. 2014; Kearney and Gibbons 2018; Markowiak and Śliżewska 2018).

Some of the widely used probiotic microorganisms include *Lactobacillus rhamnosus*, *Lactobacillus reuteri*, bifidobacteria, and certain strains of *Lactobacillus casei*; *Lactobacillus acidophilus* group; *Bacillus coagulans*; *Escherichia coli* strain Nissle 1917; certain enterococci, particularly *Enterococcus faecium* SF68; and the yeast *Saccharomyces boulardii*. Lactic acid bacteria (LAB) including species of *Lactobacillus* serve both as agents of food fermentation and potentially impart health benefits (Pandey et al. 2015; Malik et al. 2016). Probiotic microorganisms contained in feed supplements for use in animals include *Lactobacillus* sp. (*L. brevis*, *L. casei*, *L. crispatus*, *L. farciminis*, *L. fermentum*, *L. murinus*, *L. gallinarum*, *L. paracasei*, *L. pentosus*, *L. plantarum*, *L. reuteri*, *L. rhamnosus*, *L. salivarius*), *Bifidobacterium* sp. (*B. animalis*, *B. longum*, *B. pseudolongum*, *B. thermophilum*), *Enterococcus faecalis*, *Enterococcus faecium*, *Lactococcus lactis*, *Leuconostoc citreum*, *Leuconostoc lactis*, *Leuconostoc mesenteroides*, *Pediococcus acidilactici*, *Pediococcus pentosaceus*, *Streptococcus infantarius*, *Streptococcus salivarius*, *Streptococcus thermophilus*, *Sporolactobacillus inulinus*, *Bacillus cereus*, *Bacillus licheniformis*, *Bacillus subtilis*, *Propionibacterium freudenreichii*, *Saccharomyces cerevisiae* (*boulardi*), *Saccharomyces pastorianus*, *Kluyveromyces fragilis*, *Kluyveromyces marxianus*, *Aspergillus oryzae*, and *Aspergillus niger* (EFSA 2013a, b, 2017; Markowiak and Śliżewska 2018). With more advanced and focused research efforts, new genera and strains of probiotic microorganisms are continuously emerging.

Some of the sources of prebiotics include soybeans, inulin sources (like Jerusalem artichoke, chicory roots, etc.), raw oats, unrefined wheat, unrefined barley, yacon, nondigestible carbohydrates, and in particular nondigestible oligosaccharides. Commonly known prebiotics include fructooligosaccharides (FOS), galactooligosaccharides (GOS), galactooligosaccharides/ transgalactosylated-oligosaccharides (GOS/TOS), inulin, isomaltooligosaccharides (IMO), lactulose, pyrodextrins, and soy oligosaccharides (SOS). The emergent prebiotics are genti-oligosaccharides, glucooligosaccharides, lactosucrose, levans, pectic oligosaccharides, resistant starch, sugar alcohols, and xylooligosaccharides (XOS) (Anadon et al. 2010; Pandey et al. 2015). Prebiotics most commonly used in livestock nutrition are FOS, GOS, inulin, IMO, XOS, mannan oligosaccharide (MOS), lacticol, lactulose, and cereal fiber (Malik et al. 2016; Olveira and González-Molero 2016; Markowiak and Śliżewska 2018). Fermentation of FOS in the colon increases the numbers of bifidobacteria in the colon as well as absorption of calcium and fecal weight. In addition, it leads to decrease in gastrointestinal transit time. The increase in colonic bifidobacteria produces compounds to inhibit potential pathogens, by lowering blood ammonia levels and by producing vitamins and digestive enzymes (Malik et al. 2016).

In view of the decline in the use of antibiotic growth promoters in the animal industry, there is increasing awareness for search and exploiting potential of antibiotic alternatives. Among various alternative agents, probiotics, prebiotics, and synbiotics ought to have beneficial effects to maintain or improve animal health and performance. The significant increase of short-chain fatty acids (SCFAs), ketones, carbon disulfide, and methyl acetate following the intake of synbiotics has been suggested for their potential health-promoting effects (Vitali et al. 2010). In the last few years, studies on synbiotics have started to emerge, with the main focus being their applications in enhancing health and preventing diseases, improvement of other production indices such as growth rate, absorption of nutrients, and quality of meat, milk, and eggs. Synbiotics lead to improvement of survival of the probiotic microbiota during the passage through the upper intestinal tract. With more efficient implantation in the colon as well as a stimulating effect of the growth of probiotics and ubiquitous bacteria, synbiotics contribute to maintain the intestinal homeostasis and deliver specific health benefits. Compared with probiotics and prebiotics, synbiotics are the least investigated substances with respect to animal health and performance. This chapter describes the current knowledge on the effects of synbiotics on the health and performance of poultry, cattle, pigs, and companion animals. The mode of action, beneficial effects, and demonstrated study results proving the efficacy of synbiotics in farm and companion animals are presented.

2 Synbiotics for Animals

Intestinal microorganisms are known to play a significant role in the immunological, physiological, nutritional, and protective functions of the host and can be influenced by the type of food. The use of synbiotics as alternative additives for livestock and poultry feed is likely to produce greater health effects and offer more additive benefits in growth performance, feed conversion ratio, hematological, and biochemical parameters than the individual use of probiotic and prebiotic of these additives. Furthermore, synbiotics could increase the digestibility and availability of many nutrient elements such as vitamins, mineral elements, and proteins (Uyeno et al. 2015; Hamasalim 2016; Malik et al. 2016; Markowiak and Śliżewska 2018).

Synbiotic is designed not only to present beneficial microorganisms populations but also to promote proliferation of autochthonous-specific strains in the intestinal tract. Although studies on the effects of synbiotics on livestock health and performance are yet limited, it is worth mentioning that the health effects will likely depend on the synbiotic

combination and synbiotics seem promising for the modulation of the gut microbiota composition (Scavuzzi et al. 2014).

2.1 Synbiotics for Poultry

Although few research trials have been conducted to demonstrate the effects of synbiotics, the findings of in vivo studies are promising, and recent developments and applications of synbiotics have focused on assessing the beneficial effects of synbiotics on poultry health and performance (Gadde et al. 2017). The effects of some synbiotics investigated on growth response, intestinal microbial ecosystem, immune functions, and other indices of broiler chickens are presented in Table 1.

The combination of probiotics and prebiotics could improve the survival and persistence of the health-promoting microorganisms in the gut of birds because of availability of its specific substrate for fermentation (Yang et al. 2009; Adil and Magray 2012). Synbiotics may have a significant effect on absorption and utilization of feed, daily body weight gain, and quality of meat and eggs. The clinical benefits of synbiotics include inhibiting proliferation of pathogenic bacteria, protecting the intestinal barrier, modulating immune function, and combating diarrhea in chickens. Mohnl et al. (2007) demonstrated that the synbiotic product increased body weight and feed conversion ratio and reduced feed intake and mortality in comparison to controls. The synbiotic had a comparable growth-stimulating potential as antibiotic growth promoter avilamycin in broiler chickens and might be a promising alternative to the use of antibiotic growth promoters in broiler production. A synbiotic product containing *Lactobacillus* sp. with the addition of lactose was shown to improve the body weight gain and feed conversion ratio in *Salmonella*-challenged turkey poults (Vicente et al. 2007). Similarly, dietary inclusion of *Bacillus subtilis* plus FOS improved the average daily growth and the feed conversion ratio, as well as reduced the incidence of diarrhea and mortality of broiler chickens in comparison to birds treated with a tetracycline antibiotic aureomycin. The combination of *Bacillus subtilis* and FOS produced much better improvement on cecal micro-ecosystem in broilers than their alone administration. Also the combination had better effects on reducing diarrhea rate and promoting growth than they were given individually. The synbiotic had selective effects on increasing cecal concentration of beneficial bacteria such as *Lactobacillus* and decreasing concentration of harmful bacteria such as *Escherichia coli* and *Salmonella*, whereas treatment with aureomycin had nonselective effects on cecal microflora which inhibited all bacteria (Li et al. 2008).

Dietary inclusion of synbiotic containing a combination of *Bifidobacterium lactis* bacteria and GOS for 40 days resulted in a significant increase of *Bifidobacterium* and *Lactobacillus* count and in population of total anaerobic bacteria in the fecal microbiota of the broiler chickens (Jung et al. 2008). McReynolds et al. (2009) evaluated the effects of probiotic (*Enterococcus faecium*, *Pediococcus acidilactici*, *Bifidobacterium animalis*, *Lactobacillus reuteri*) in association with a prebiotic containing, but not limited to, FOS and essential oil plant extracts on chicks given an immunosuppressant vaccine, inoculated with *Clostridium perfringens* and in dietary conditions favorable to necrotic enteritis development. Both probiotic and prebiotic resulted in reduction of *Clostridium perfringens* counts, a decrease in the intestinal lesions, and lower mortality. It was suggested that the synbiotic could be used as potential alternatives to help control *Clostridium perfringens* and necrotic enteritis. Erdoğan et al. (2010) showed that addition of synbiotics to the diet produced a decrease of cecal coliform organism counts, which might be due to the positive effects of probiotics and prebiotics on gut microbial ecology.

The positive impact of administration of synbiotics on the performance and ileal microbiota of chickens has also been substantiated in recent studies. The application of *Saccharomyces cerevisiae* with MOS significantly increased the weight gain as well as population of LAB and yeast in the ileal and cecal content of broiler chicks. The population of *Escherichia coli* was significantly decreased in the ileum and cecum. Dietary inclusion of *Saccharomyces cerevisiae* without MOS also produced these effects, but the effects were more pronounced following combined administration (Koc et al. 2010). Since MOS is not enzymatically digested in the small intestine, bacteria bound to MOS likely exit the intestine without attaching to the epithelium (Spring et al. 2000). The enhancement of intestinal morphology and nutrient absorption following the administration of synbiotics appears to contribute to the improved performance of broiler chickens (Awad et al. 2008; Hassanpour et al. 2013). Tayeri et al. (2018) compared the effects of antibiotics, probiotics, prebiotics, and synbiotics on the performance and carcass characteristics of broilers. The birds fed with synbiotic had greater relative gizzard and spleen weights and lighter kidneys and also had thinner walls of the caudal gut segments. Dietary supplementation of the synbiotic also reduced the feed conversion ratio, compared with the control and antibiotic flavomycin treatment. Thus, there were significant performance and health benefits of using probiotics, prebiotics, and synbiotics for broilers, rather than antibiotics.

Feed restriction for long period may induce a great stress on birds. Synbiotics have been shown to ameliorate the negative effects of feed restriction on broiler performance. Accordingly, the effects of synbiotics with and without feed restriction have been investigated on performance, hematological indices, and carcass characteristics of broiler chickens. Diets supplemented with *Bacillus licheniformis* and *Bacillus subtilis* plus MOS, with and without feed restriction, improved broiler performance, without affecting blood

Table 1 Effects of some synbiotics on health and growth performance of poultry

Synbiotic	Main outcome	References
Lactobacillus (*L.*) *acidophilus*, *Enterococcus faecium*, *L. plantarum*, *Pediococcus acidilactici* plus MOS	Reduced the mortality from necrotic enteritis caused by *Clostridium perfringens*	Hofacre et al. (2003)
Enterococcus faecium plus FOS	Prevented necrotic enteritis caused by *Clostridium perfringens* as expressed by reduction of signs, mortalities, lesions, and intestinal count and improved performance indices	El-Ghany (2010)
Biomin®IMBO[a]	Improved body weight gain and feed conversion ratio and protective effect against coccidiosis	Ghasemi et al. (2010)
L. acidophilus, *L. casei*, *L. plantarum*, *L. bulgaricus*, *Bifidobacterium* (*B.*) *bifidum*, *Streptococcus thermophilus*, *Streptococcus faecium*, *Torulopsis* sp., *Aspergillus oryzae* plus inulin	Increased weight gain and ameliorated toxic effects of aflatoxins	Hashem and Mohamed (2009)
Lactobacillus, *Bifidobacterium* plus oligosaccharides derived from yeast cell wall, and other ingredients	Improved the antibody response to Newcastle disease virus and infectious bronchitis virus vaccines	El-Sissi and Mohamed (2011)
Saccharomyces cerevisiae plus MOS	Increased weight gain, reduced the number of *Escherichia coli* in the small intestinal and cecal digesta, and higher villus height in the duodenum, jejunum, and ileum	Abdel-Raheem et al. (2012)
B. longum subsp. *longum* PCB133 plus GOS	Reduced *Campylobacter jejuni* concentration in poultry feces	Baffoni et al. (2012)
Biomin®IMBO[a]	Increased the numbers of lactobacilli and reduced *Escherichia coli* and total coliform populations in the intestine	Dibaji et al. (2014)
Multi-strain probiotic consisting of 11 *Lactobacillus* strains plus IMO	Increased the cecal populations of lactobacilli and bifidobacteria and decreased the cecal *Escherichia coli* and improved the performance and increased the cecal volatile fatty acids	Mookiah et al. (2014)
Biomin®IMBO[a]	Enhanced the humoral immune responses following vaccination against Newcastle disease, infectious bronchitis, and infectious bursal disease	Talebi et al. (2015)
L. acidophilus, *L. casei*, *Streptococcus faecium*, *Bacillus subtilis* plus yeast-derived carbohydrates	Exhibited a balanced T-helper (Th)-1/Th-2 response locally and a more Th-2-dependent response systemically	Yitbarek et al. (2015)
Biomin®IMBO[a]	Improved body weight gain, feed conversion, intestinal morphology, and intestinal microbial ecology and the highest antibody response to Newcastle disease vaccine	Al-Sultan et al. (2016)
L. acidophilus, *L. casei*, *Streptococcus faecium*, *Bacillus subtilis*, *Saccharomyces cerevisiae* plus yeast-derived carbohydrates	Improved humoral immunity by increasing IgG concentration in serum and modulated the adaptive antibody-mediated immune response against infectious bronchitis virus	Alizadeh et al. (2017)
B. longum PCB133 plus XOS	Reduced the cecal *Campylobacter jejuni* and *Campylobacter* sp. upon lifelong administration of the synbiotic in broiler chickens infected with *Campylobacter jejuni* strain M1	Baffoni et al. (2017)
L. reuteri, *Enterococcus faecium*, *B. animalis*, *Pediococcus acidilactici* plus FOS	Increased body weight, enhanced performance, and protected against *Salmonella enterica* Enteritidis infection	Luoma et al. (2017)
L. casei, *L. acidophilus*, *B. thermophilum*, *Enterococcus faecium* plus MOS	Improved specific growth rate, growth efficiency, energy efficiency ratio, protein efficiency ratio and decreased serum cholesterol concentration	Ashayerizadeh et al. (2011)
L. acidophilus, *B. thermophilus*, *B. longum*, *Streptococcus faecium* plus prebiotics	Increased serum overall total antioxidant capacity, paraoxonase and ceruloplasmin activity and decreased serum total oxidant status and homocysteine concentrations	Anwar et al. (2012)
Bacillus subtilis, *Bacillus licheniformis*, *Clostridium butyricum* plus yeast cell wall, and XOS	Increased average daily gain and breast yield, decreased feed/gain ratio and abdominal fat, and lowered malondialdehyde content in the thigh muscle, resulting in the production of meat with a favorable quality and oxidative stability	Cheng et al. (2017)
L. acidophilus, *L. casei*, *B. bifidum*, *Streptococcus faecium*, *Aspergillus oryzae* plus IMO	Decreased serum levels of total cholesterol at 36 weeks of age, low-density lipoprotein cholesterol, alanine aminotransferase and alkaline phosphatase, heterophil percentage, and heterophil to lymphocyte ratio and increased lymphocyte percentage at 36 and 52 weeks of age	Tang et al. (2017)
L. acidophilus, *L. casei*, *B. bifidum*, *Streptococcus faecium*, *Aspergillus oryzae* plus IMO	Decreased the egg yolk cholesterol and total saturated fatty acids and increased total unsaturated fatty acids, total omega	Tang et al. (2015)

(continued)

Table 1 (continued)

Synbiotic	Main outcome	References
	6, and polyunsaturated fatty acids including linoleic and alpha-linolenic acid levels in eggs	
Biomin®IMBO[a]	Improved egg weight, egg production, and egg mass; higher values of shell thickness, Haugh unit, and shell percentage; increased feed conversion ratio; and decreased serum cholesterol	Abdel-Wareth (2016)
Biomin®IMBO[a]	Improved growth performance, intestinal morphology, and nutrient absorption; increased villus height/crypt depth ratio and villus height in ileum; and reduction in ileal crypt depth	Awad et al. (2008)
Biomin®IMBO[a]	Increased average daily body weight gain, carcass yield percentage, feed conversion rate and villus height/crypt depth ratio, and villus height in both duodenum and ileum	Awad et al. (2009)
L. acidophilus, *Enterococcus* ssp. plus MOS	Improved weight gain and feed/gain ratio until 21 days old	Murarolli et al. (2014)
Biomin®IMBO[a]	Increased the consumption of metabolizable energy and crude protein and higher production index, live weight, and feed efficiency	Aziz Mousavi et al. (2015)
L. plantarum, *L. delbrueckii* subsp. *bulgaricus*, *L. acidophilus*, *L. rhamnosus*, *B. bifidum*, *Streptococcus salivarius* subsp. *thermophilus*, *Enterococcus faecium* plus MOS	Increased ileal villus height, crypt depth, and villus surface area	Sohail et al. (2012)
L. plantarum, *L. acidophilus*, *L. bulgaricus*, *L. rhamnosus*, *B. bifidum*, *Streptococcus thermophilus*, *Enterococcus faecium*, *Aspergillus oryzae*, *Candida pintolopesii* plus MOS	Improved mean body weight gain and feed efficiency and ameliorated the effects of cyclic heat stress on relative weights of the spleen, bursa, intestine, and ceca	Sohail et al. (2013)
Bacillus subtilis plus XOS and MOS	Increased daily weight gain; feed efficiency; villus height; and villus/crypt ratio in duodenum, jejunum, and ileum; intestinal mucosa secretory IgA content; and antioxidant capabilities	Min et al. (2016)
Saccharomyces cerevisiae, *Enterococcus faecium*, *Bacillus subtilis*, *Bacillus licheniformis* plus β-glucans, MOS and FOS	Increased body weight, decreased mortality adjusted feed conversion ratio, and necrotic enteritis-associated mortality with no decrease in the severity of intestinal lesion scores in broiler chickens challenged with *Clostridium perfringens*	Krueger et al. (2017)

[a]Biomin®IMBO (*Enterococcus faecium* plus prebiotic derived from chicory and immune-modulating substances derived from sea algae)

parameters and carcass yield. Feed restriction and addition of the synbiotic resulted in a decreased visible fat of the carcass. The use of synbiotic was recommended as an anti-stress factor in feed restriction and to increase weight, improve feed conversion rate, and reduce feed cost of production (Abdel-Hafeez et al. 2017).

The addition of synbiotic to broiler diets not only improved the growth performance but also had a positive impact on blood lipid profile and meat quality (Ghasemi et al. 2016). The synbiotic was shown to improve overall body weight gain or feed conversion ratio and decreased serum cholesterol as well as low-density lipoprotein cholesterol concentrations. The 2-thiobarbituric acid-reactive substances content in thigh meat after 30 days of storage at 4 °C was linearly decreased as the synbiotic inclusion concentrations in the diets increased. Dietary synbiotic also decreased the proportion of monounsaturated fatty acids and increased n-6 polyunsaturated fatty acid concentration in thigh meat, whereas the fatty acid profile of breast meat was not affected by synbiotic supplementation.

In an interesting study, Calik et al. (2017) evaluated the effect of intra-amniotic administration of synbiotic comprising of *Enterococcus faecium* and inulin and its continued supplementation in the diet on the performance, intestinal epithelium integrity, and cecal microflora of broiler chickens. The administration of an intra-amniotic synbiotic to embryonated eggs on day 17 of incubation had no effect on the hatchability or hatching weight of the birds. However, it resulted in a positive effect on villus height and goblet and proliferating cell nuclear antigen-positive cell counts. Intra-amniotic synbiotic injection followed by dietary supplementation with the synbiotic was found to significantly increase *Lactobacillus* colonization and decrease coliform population in the broiler cecum. Furthermore, cecal butyric acid concentration increased proportionally to the cecal *Lactobacillus* count with dietary supplementation of the synbiotic.

In addition to the improved growth performance, synbiotic supplementation was also shown to enhance the general immune function of broiler chickens. Chen et al. (2018) investigated the effects of dietary synbiotic supplementation as an alternative to antibiotics on growth performance,

intestinal morphology, immunity, and oxidative status of broilers. Supplemental synbiotic increased the average daily gain and gain:feed ratio of broilers, as well as the relative weight of the thymus and the secretory immunoglobulin A level in the jejunum and ileum. Dietary synbiotic inclusion promoted the ratio of ileal villus height to crypt depth and reduced the ileal malondialdehyde accumulation of broilers. The synbiotic-induced effects were comparable with that of dietary inclusion of the antibiotic chlortetracycline. It was suggested that dietary synbiotic supplementation may be used as an alternative to the antibiotic. Similarly, Naghi Shokri et al. (2017) reported that chickens fed diet supplemented with synbiotic exhibited better feed conversion ratios at the 14–28 day period and higher average daily gain and duodenal villus height/crypt depth ratio at 42 days than those fed the control diet. Supplemental synbiotic produced a marked increase in the serum antibody titer against infectious bursal disease and infectious bronchitis vaccines.

On the contrary, some of the trials conducted with dietary inclusion of synbiotics did not show that broiler performance was affected. The oral administration of GOS alone or in combination with a *Bifidobacterium lactis*-based probiotic had no significant effect on growth, feed consumption, and feed conversion ratio in broilers (Jung et al. 2008). Dietary inclusion of a combination of probiotic (*Bacillus licheniformis*, *Bacillus subtilis*) and a MOS derived from the cell walls of the yeast (*Saccharomyces cerevisiae*) had no significant effect on body weight gain, feed intake, carcass weight, and carcass yield, but feed conversion ratio was significantly improved (Midilli et al. 2008). Similarly, feeding of diet supplemented with synbiotic did not influence feed intake, feed conversion efficiency, body weight gain, and small intestine weight of broilers (Erdoğan et al. 2010). Dietary supplementation with a combination of commercial probiotic (PrimaLac) and prebiotic (TechnoMos) was found to have no significant effect on daily feed intake, daily body weight gain, feed conversion ratio, carcass traits, intestinal morphology, and bacterial populations of the ileum of broiler chickens (Salehimanesh et al. 2016). The morphometric parameters of the different intestinal wall layers of broiler chickens were not influenced by feeding a combination of probiotic product containing anaerobic bacteria, lactose-fermenting enterobacteria, *Enterococcus* sp. and *Lactobacillus acidophilus*, and MOS. Also the synbiotic did not improve intestinal integrity and broiler performance (Fernandes et al. 2014). Mookiah et al. (2014) reported that synbiotic consisting of 11 *Lactobacillus* strains plus IMO did not show a twofold synergistic effect on the performance, cecal bacterial populations and concentrations of cecal volatile fatty acids (VFA), and non-VFA of broiler chickens compared with those of probiotic or prebiotic alone. The differences in the effects of synbiotics on growth performance and other indices may be attributable to variations in the strain of probiotics and/or type of prebiotics selected, methods of preparation, the dietary supplementation level, and duration of use, diet composition, bird age, and hygiene condition. Also in several cases, the environmental and the stress status of the birds are not reported or considered, as the experimental settings are often too far from farm conditions (Gaggia et al. 2010; Wang et al. 2018).

Calcium ions deficit in bones leads to the deterioration of skeleton structure and reduction of bone strength. The necessity of using bioactive foods and ingredients in feed stimulating the assimilability of minerals in poultry is particularly important when the birds grow intensely and is very heavy (Kwiatkowska et al. 2017). Although prebiotics, probiotics, and synbiotics have been shown to modulate mineral absorption and bone mineral content, synbiotics are the least investigated substances with respect to a bone-health-promoting potential. Studies performed in animals and humans have revealed positive outcomes of prebiotics on mineral absorption and metabolism and bone composition and architecture. This effect of dietary prebiotics is promoted by high dietary calcium content up to a threshold level and an optimum amount and composition of supplemented prebiotics. The underlying mechanisms include increased solubility of minerals due to increased bacterial production of SCFAs, an enlargement of the absorption surface by promoting proliferation of enterocytes mediated by bacterial fermentation products, and increased expression of calcium-binding proteins. With the increased expression of enterocytes as well as calcium-binding proteins, more minerals are absorbed, the health of the gut is improved, and the body is enabled to maintain stronger bone structure with the supply of calcium (Scholz-Ahrens et al. 2007). On the other hand, the probiotic microorganisms produce phytase enzyme that is used to free up more minerals from phytate bondage for absorption. The probiotic microorganisms are also able to hydrolyze glycoside bonds in nutrients that otherwise cannot be broken down by the intestines; as a result both the probiotic bacteria and the host animal benefit with the increased availability of minerals (Parvaneh et al. 2014). Probiotics have an independent effect on facilitating mineral absorption, and synbiotics can induce additional effects. The increase in calcium absorption levels has been reported to coincide with an increase in bone density values (Weaver 2015).

Feeding of diet containing *Bacillus subtilis* and/or inulin increased the content of ash, calcium, and density of the tibia of Lohmann white laying hens. In addition, the supplementation also increased egg production and egg weight and improved the quality of eggshells, including thickness and content of calcium and decreased eggshell deformations. The improvements in performance and eggshell quality following dietary inclusion of the synbiotic were found to be directly related to the colonization of beneficial microflora along with an increase in the villi-crypts absorptive area. The positive

effects of the synbiotic on eggshell quality indices can probably be attributable to the stimulation of mineral availability (Abdelqader et al. 2013). A favorable effect of synbiotic on the accumulation of calcium has also been demonstrated in Japanese quails, in which the content of calcium and phosphorus in the tibia following the use of a commercial synbiotic (Biomin®IMBO) was dependent on the sex of the bird, and significant results were evident in males only (Vahdatpour et al. 2014). The beneficial effects of the use of dietary synbiotic in the nutrition of Hy-Line W-36 layers on eggshell characteristics have been reported by Cesari et al. (2014). Prebiotic (skim milk powder containing 54% lactose) added to a diet containing *Lactobacillus acidophilus* D2/CSL resulted in significant improvements in egg quality characteristics, in terms of specific gravity, shell thickness, Haugh unit, and percentage of shell. Such positive effects could be due to the increased production of SCFAs in the intestine of hens fed with the combination of probiotic bacteria and lactose.

The beneficial microorganisms inhabit the gastrointestinal tract of chickens instantly after hatching, and this development occurs through contact with maternal feces. But in artificial hatching, despite the use of feed additives, the settlement of the intestinal beneficial microorganisms is delayed. Hence, administration of the given substance directly into the egg has been used, and the results are promising (Maiorano et al. 2012; Sławińska et al. 2014a, b). Compared to dietary prebiotic administration, in ovo injection increases the population of beneficial microflora on the day of hatch and leads to a high and stable level of bifidobacteria throughout the broiler chickens growing period (Villaluenga et al. 2004). Furthermore, very low doses of the substances are effective when injected in ovo.

In ovo administration of selected synbiotics is a promising approach for enhancing the chicken immune system as it combines merits of probiotics and prebiotics and by early administration into the embryo and supports development of their immune organs. In ovo administration of *Lactococcus lactis* IBB SL1 plus raffinose family oligosaccharides (RFO), *Lactococcus lactis* IBB SC1 plus RFO, or *Lactobacillus acidophilus* and *Streptococcus faecium* plus lactose into the developing chicken embryo provided stimulus for the immune system stimulation of the growing chickens, but its efficiency depended on chicken genotype. *Lactococcus lactis* probiotics survived in the chicken guts throughout their lifespan (Sławińska et al. 2014a). In ovo administration of synbiotics has also been shown to activate the immune system in adult chickens. Sławińska et al. (2014b) investigated the effects of in ovo administration of *Lactococcus lactis* subsp. *lactis* IBB SL1 plus RFO, *Lactococcus lactis* subsp. *cremoris* IBB SC1 plus RFO, and *Lactobacillus acidophilus* and *Streptococcus faecium* plus lactose on immune-related gene expression in adult chickens. The intestinal tissues had downregulation of expression for the cytokines interleukin (IL) IL-4, IL-6, IL-12p40, IL-18, interferon (IFN)-β, and IFN-γ and 1 chemokine (IL-8) in the cecal tonsils of synbiotic-treated chickens. In spleen which is the peripheral part of the immune system, the expression of IL-4 and IL-6 was upregulated, suggesting that in ovo administration of synbiotics activates the immune system in adult chickens. In ovo administration of *Lactococcus lactis* subsp. *lactis* 2955 plus inulin was associated with downregulation of immune-related gene expression in the cecal tonsils and spleen of broiler chickens. The magnitude of that downregulation increased with age and was most likely caused by stabilization of the gastrointestinal microbiota (Płowiec et al. 2015).

Synbiotics (*Lactococcus lactis* subsp. *lactis* IBB SL1 plus inulin and *Lactococcus lactis* subsp. *cremoris* IBB SC1 plus transgalactooligosaccharide [TGOS]) injected in ovo into the air cell on the 12th day embryonic development have been demonstrated to increase the total activity of pancreatic enzymes amylase and trypsin, whereas lipase activity was increased by *Lactococcus lactis* subsp. *cremoris* IBB SC1 plus TGOS in the growing chickens. The most pronounced changes were observed at the end of the investigated rearing period on day 34. The in ovo injected synbiotics caused no deterioration in the posthatching condition of the chicken liver, as determined by measurement of the activity of marker enzymes serum alanine aminotransferase and serum aspartate aminotransferase. Although treatment with the synbiotics did not change the feed conversion ratio, but *Lactococcus lactis* subsp. *lactis* IBB SL1 plus inulin significantly increased final body weight of chickens (Pruszynska-Oszmalek et al. 2015).

In ovo administration of synbiotics may also be an effective method to increase body weight, improve the SCFAs cecal profile, and increase the villus length: crypt depth ratio in the jejunal mucosa (Miśta et al. 2017). These effects were more pronounced following administration of *Lactococcus lactis* subsp. *lactis* IBB SL1 plus inulin compared to another synbiotic *Lactococcus lactis* subsp. *cremoris* IBB SC1 plus TGOS. In addition, in ovo administration of these two synbiotics at day 12 incubation has been shown to modulate the central and peripheral lymphatic organ development in broilers. *Lactococcus lactis* subsp. *cremoris* IBB SC1 plus TGOS decreased the cortex/medulla ratio in the thymus and slowed the development of the cortex in bursal follicles on day 21 posthatching, with consequent impacts on the primary lymphatic organs. The synbiotic treatment also stimulated formation of germinal centers in the spleens of 21- and 35-day-old chickens, indicating enhanced B-cell proliferation in secondary lymphatic organs. It produced an age-dependent increase in the spleen/bursa of Fabricius ratio (Madej et al. 2015). In another study, *Lactococcus lactis* subsp. *lactis* IBB SL1 plus inulin as well as *Lactococcus lactis* subsp. *cremoris* IBB SC1 plus TGOS delivered *in ovo* were shown to have a

stimulatory effect on the gut-associated lymphoid tissue development after hatch (Madej and Bednarczyk 2016).

The biological effects of synbiotics depend exclusively on careful selection of bioactive probiotic and prebiotic. Dunislawska et al. (2017) presented a workflow associated with in vitro selection of synbiotics and its consequences for the downstream animal study, including abundance of intestinal microbial communities, performance parameters, and molecular responses of the immune system. Based on in vitro findings on hatchability and growth curve, they designed two synbiotics, namely, *Lactobacillus salivarius* IBB3154 plus GOS and *Lactobacillus plantarum* IBB3036 plus RFO. Both synbiotics delivered in ovo had beneficial effects on the overall status of broiler chickens characterized by low mortality and high production parameters, and microbial populations of *Lactobacillus* sp. and *Enterococcus* sp. were higher in the ileum. Of the two synbiotics, *Lactobacillus salivarius* IBB3154 plus GOS caused significant upregulation of IL-6, IL-18, IL-1β, IFN-γ, and IFN-β in the spleen on day 21 and IL-1β on day 7, whereas IL-12, IL-8, and IL-1β on day 42 and IFN-β on day 14 were downregulated in cecal tonsils and were more potent in establishing a beneficial shift in the microbiota composition in the gastrointestinal tract.

In a recent study, Kolodziejski et al. (2018) demonstrated that incretins are involved in the action of synbiotics or that they may even be their target in poultry. In ovo injection of two different synbiotics *Lactobacillus salivarius* IBB3154 plus GOS and *Lactobacillus plantarum* IBB3036 plus RFO led to a lowering of the level of incretins glucagon-like peptide 1 (GLP-1) and gastric inhibitory polypeptide or glucose-dependent insulinotropic peptide (GIP) in blood serum of broiler chickens. Both synbiotics downregulated GLP-1 and GIP mRNA expression in the duodenum and GLP-1 receptors in the pancreas. Furthermore, *Lactobacillus plantarum* IBB3036 plus RFO increased trypsin and lipase activities in the duodenum content, simultaneously decreasing amylase activity, which is likely to promote digestion of proteins and lipids. The in ovo delivery of these synbiotics was also found to significantly affect gut structure which should contribute to improvement in nutrient absorption by the gut (Sobolewska et al. 2017).

2.2 Synbiotics for Ruminants

The current use of probiotics in young preruminants generally targets the lower intestine and represents an interesting means to stabilize the gastrointestinal microorganisms and reduce the risk of colonization of pathogens and ultimately support the beneficial effects including balancing the gut microbiota as well as in animal nutrition and health. On the other hand, prebiotics have been shown to have specific beneficial effects in calves including blocking of colonization of pathogens in the gut, reduction in the incidence and severity of enteric disease, and prevention of adhesion of *Enterobacteriaceae*, particularly *Escherichia coli* and *Salmonella*, to the intestinal epithelium and may ultimately improve growth performance. However, the beneficial health effects with both probiotics and prebiotics seem to be minimal when calves are generally healthy. Probiotics in adult ruminants mainly improve fiber digestion by rumen microorganisms and have positive effects on various digestive processes, particularly cellulolysis and the synthesis of microbial proteins (Heinrichs et al. 2009; Uyeno et al. 2015). Application of synbiotics may assist in decreasing acidosis and stabilizing rumen pH, improving immune function, interfering with pathogen infections, and consequently improve animal health and production efficiency.

As compared to probiotics and prebiotics, studies on synbiotics on the cattle health and performance have received only minimal attention. The efficacy and suitability of the use of synbiotics have been generally assessed on the basis of weight gain, feed intake, and utilization and on status of health (Radzikowski 2017; Markowiak and Śliżewska 2018). In preruminant calves, *Enterococcus faecium* plus lactulose produced beneficial effects on the growth performance. The ileal villus height, the depth of the crypts in the cecum, and the surface area of lymph follicles from Peyer's patches were decreased by the synbiotic treatment (Fleige et al. 2007). Addition of combination of *Streptococcus faecium* and MOS to dairy calf diets was shown to improve fecal consistency and to reduce the fecal score of calves without reducing in the number of scour episodes (Morrison et al. 2010). Preruminant calves fed milk containing synbiotic consisting of probiotic (multi-strain probiotic containing seven bacteria strains and two yeast strains) and prebiotic (*Saccharomyces cerevisiae* cell wall polysaccharides) resulted in an increase in average daily weight gain and a decrease in the number of pathogenic *Escherichia coli* bacteria in feces of calves. However, application of the synbiotic had no significant effect on white blood cell count, plasma IgG1 level, and cell-mediated immune response (Roodposhti and Dabiri 2012). Similar results have been demonstrated by Marcondes et al. (2016) in Holstein Californian calves following symbiotic complex supplementation. The synbiotic-fed calves attained higher average daily weight gain and exhibited increased digestibility of dry matter and neutral detergent fiber and improved animal health. In addition, a combination of commercial probiotics and prebiotics has also been shown to eliminate morbidity and mortality losses associated with Shiga toxin-producing *Escherichia coli* infections in dairy calves (Baines et al. 2013).

Supplementation of feed with a synbiotic product consisting of *Lactobacillus casei* subsp. *casei* and dextran increased the resistance of Holstein dairy cows to adverse

environmental conditions such as high temperature and humidity. The synbiotic improved the milk production and total amounts of fat, protein, and solid nonfat, as a result of a positive change in the bovine intestinal microbiota and a greater resistance to infectious diseases. The cows evaluated also showed a reduction in the number of somatic cells and a decrease in the incidence of mastitis. (Yasuda et al. 2007).

2.3 Synbiotics for Pigs

Studies on the effects of synbiotics on pig health and performance are scarce. The effects of some synbiotics investigated on pig health and performance are presented in Table 2. Postweaning diarrhea is one of the major concerns related to gut health of nursery pigs and is often caused by infection of enterotoxigenic *Escherichia coli* (Sun and Kim 2017). A few studies have been performed to determine the potential usefulness of synbiotics to prevent postweaning diarrhea in pigs. Nemcová et al. (1999) demonstrated the synergistic beneficial effect of *Lactobacillus paracasei* plus FOS on the fecal microbiota of weanling piglets. Animals fed with synbiotic exhibited an increase of total anaerobic and aerobic count and increased number of *Lactobacillus* and *Bifidobacterium*, whereas the bacterial count of *Enterobacteriaceae* and *Clostridium* was decreased in the feces of the weanling pigs. In a later study, Nemcová et al. (2007) reported that the administration of combination of *Lactobacillus plantarum*, maltodextrin, and FOS increased acetic acid concentrations in the ileum and colon and was found to be most effective in inhibiting the counts of *Escherichia coli* O8:K88 adhering to the intestinal mucosa of the jejunum and colon of conventional piglets.

Piglets fed diet containing 0.5% synbiotics (mixture of 0.2% oligofructose plus 0.3% probiotics) exhibited higher average daily weight gain and had a significantly decreased number of total coliform bacteria in the colon, whereas the population of bifidobacteria was significantly increased in the ileum and colon (Shim et al. 2005). Lee et al. (2009) in a 16-day study assessed the effect of synbiotics on growth performance, nutrient digestibility, emission of harmful gases, and fecal microbial population in weaning pigs. Supplementation with the synbiotic product containing a combination of a probiotic originating from anaerobic microflora and a prebiotic (MOS, lactose, sodium acetate, and ammonium citrate) resulted in improved digestion of nutrients, decreased emission of harmful gases, and enteropathogenic bacteria in early-weaning pigs. Growing pigs fed diet supplemented with prebiotics, and 0.2% probiotics from anaerobic bacteria for 15 days showed higher dry matter and crude protein digestibility, decreased fecal ammonia and amine gas emissions, and increased fecal acetate gas emission. Although the growth performance was not affected, fecal *Escherichia coli* population was lower in pigs fed the synbiotic (Chu et al. 2011).

Krause et al. (2010) demonstrated the efficacy of synbiotic consisting of a 50:50 mixture of probiotic *Escherichia coli* strains UM-2 and UM-7 and prebiotic raw potato starch

Table 2 Effects of some synbiotics on health and growth performance of pigs

Synbiotic	Main outcome	References
Lactobacillus (*L.*) *paracasei* plus FOS	Increased fecal counts of *Lactobacillus* sp., *Bifidobacterium* sp., total anaerobes, and total aerobes and significantly decreased *Clostridium* and *Enterobacterium* counts	Bomba et al. (2002)
L. salivarius 1B 4/11 plus lactitol	Positive synergistic effect of the combination as compared with the use of individual components in modulating the cecal microflora in vitro and improved feed efficiency when fed to weaned pigs	Piva et al. (2005)
L. acidophilus ATCC 4962 plus FOS, inulin, and mannitol	Decreased plasma triacylglycerol, total cholesterol, and low-density lipoprotein cholesterol and reduced deformation of erythrocytes in hypercholesterolemic pigs	Liong et al. (2007)
Bacillus subtilis, *L. casei*, *Pichia anomala* plus oligosaccharides	Improved apparent nutrient digestibility, decreased diarrhea rates, and increased fecal counts of LAB and fecal lipase activity	Fan et al. (2015)
Enterococcus faecium, *L. salivarius*, *L. reuteri*, *Bifidobacterium thermophilum* plus inulin	Beneficial effect on the gut microbiota; decreased relative abundance of *Escherichia* in the ileum, cecum, and colon; and increased bifidobacterial numbers in the ileum	Sattler et al. (2015)
L. plantarum—Biocenol™ LP96 (CCM 7512), *L. fermentum*—Biocenol™ LF99 (CCM 7514) plus flaxseed	Decreased lactate dehydrogenase leakage in the blood serum and tissue extracts and likely improvement in the immune status and the integrity of jejunum mucosa during infection	Andrejčáková et al. (2016)
Enterococcus faecium NCIMB 11181 plus lactulose	Decreased *Proteobacteria* abundances, increased the average population of *Lactobacillaceae*, and large decreases in the proportions of *Enterobacteriaceae* in feces	Chae et al. (2016)
L. plantarum—Biocenol™ LP96 (CCM 7512), *L. fermentum*—Biocenol™ LF99 (CCM 7514) plus flaxseed	Positive effects on the blood serum levels of total lipids, the ratio of n-3 polyunsaturated fatty acids (PUFAs)/n-6 PUFAs, and gut health and adaptation process after weaning	Sopková et al. (2017)

(RPS) in young pigs challenged with enterotoxigenic *Escherichia coli* (ETEC) K88. The synbiotic produced a beneficial effect on piglet growth performance and resulted in a reduction of diarrhea and increased microbial diversity in the gut. It has been suggested that microcin produced by probiotic *Escherichia coli* can limit the growth of competitors in an inflamed intestine, including commensal *Escherichia coli* and adherent-invasive *Escherichia coli* (Krause et al. 2010; Sassone-Corsi et al. 2016; Liao and Nyachoti 2017). The prebiotic effect of RPS may be attributable to the starch granules which are much larger than those of cereal grains and consequently reach the distal small intestines and colon, where they modify fermentation. In another study, the potential of *Lactobacillus plantarum* JC1 (B2028), lactulose, and their combination to control postweaning colibacillosis in pigs was evaluated using an ETEC K88 oral challenge (Guerra-Ordaz et al. 2014). Lactulose, a nondigestible and synthetic disaccharide, not only improved the average daily gain but also increased lactobacilli, the percentage of butyric acid in the colon, and the ileum villous height and decreased the pig major acute-phase protein in serum. On the other hand, the probiotic increased the numbers of *Lactobacillus plantarum* bacteria in the ileum and colon and the total lactobacilli in the colon and showed a trend to reduce diarrhea. The concentrations of ammonia in ileal and colonic digesta were decreased, whereas the villous height and number of ileal goblet cells increased. The positive effects of *Lactobacillus plantarum* and lactulose were combined in the synbiotic treatment, resulting in a complementary synbiotic having a potential to control postweaning colibacillosis in piglets.

The Göttingen minipig seems to be a suitable model for gut microbiota research in pigs. Tanner et al. (2015) investigated the impact of *Bifidobacterium thermophilum* RBL67 (RBL67) alone and combined with FOS on the gut microbiota of Göttingen minipigs. During the treatments and at the time of killing of animals, RBL67 was consistently detected in feces, cecum, and colon after feeding RBL67 and synbiotic diets. At the time of killing of animals, significantly higher *Bifidobacterium* numbers in the cecum and colon of synbiotic-fed minipigs were measured compared with RBL67, suggesting that the synbiotic combination represents a valuable strategy to increase probiotic bacteria levels and survival in gastrointestinal tracts for feed and food applications.

2.4 Synbiotics for Companion Animals

The prebiotic component within a synbiotic product should support the growth of the probiotic and enhance its proliferation within the gastrointestinal tract. Obviously, prebiotic additives have to be chosen depending on the probiotic strain. Although combinations of prebiotics with dietary probiotics have not been adequately explored in the domestic canine and feline species, such information might have implications for the design and production of synbiotic formulations for companion animals. Ogué-Bon et al. (2010) conducted in vitro tests to evaluate the synergistic potential of prebiotics FOS, GOS, and inulin and probiotic strains *Bifidobacterium bifidum* 02450B, *Bifidobacterium longum* 05, *Lactobacillus plantarum* 115400B, *Lactobacillus acidophilus* 14150B, *Lactobacillus acidophilus*, and *Lactobacillus rhamnosus*. It was shown that the synbiotic combination GOS plus *Bifidobacterium bifidum* 02450B induced greater modulation of canine fecal microbiota compared with GOS alone. In another in vitro study, the association between commercially available probiotic strains (*Lactobacillus plantarum* 115400B, *Lactobacillus acidophilus* 14150B, *Lactobacillus acidophilus*, *Lactobacillus rhamnosus*, *Bifidobacterium longum* 05, and *Bifidobacterium bifidum* 02450B) and commercially available fiber blends (rice bran, citrus pectin, barley, and maize starch) was investigated. Rice bran was shown to be capable of increasing SCFAs production and stimulating the growth of probiotic strains. Since rice bran is commonly used as a fiber supplement in the pet food industry and could therefore add a prebiotic effect to the known dietary effects tied to the use of this type of fiber, which include increasing the fecal mass and providing a laxative action. Rice bran on its own had the same effect on the fecal counts of bifidobacteria and lactobacilli and concentrations of SCFAs as the various synbiotic combinations, thus indicating lack of synergistic effect between the probiotic strains and fiber source used (Ogue-Bon et al. 2011; Pinna and Biagi 2014).

In a recent study, Salavati Schmitz and Allenspach (2017) tested the growth properties of *Enterococcus faecium* NCIMB 10415 E1707, *Enterococcus faecium* NCIMB 30183, *Bifidobacterium longum* NCIMB 30182, and *Bifidobacterium infantis* NCIMB 30181, with the addition of FOS, MOS, and FOS plus gum Arabic in vitro. The *Enterococcus faecium* NCIMB 10415 E1707 which is most commonly used probiotic strain for small animals was not influenced by any of the prebiotics used. The growth of bifidobacteria was enhanced with commonly used prebiotic oligosaccharides, whereas the addition of gum Arabic had a stronger effect on growth acceleration than FOS alone, suggesting that bifidobacteria might be interesting probiotic candidates in small animals.

Besides in vitro studies, the effects of *Lactobacillus acidophilus* NCFM, FOS, or their combination have been investigated on concentrations of gut microbial populations, fermentative end products, and nutrient digestibilities in healthy adult dogs. *Lactobacillus acidophilus* plus FOS containing synbiotic was proved to be beneficial in healthy dogs. Supplementation of FOS alone enhanced gut microbial ecology by increasing concentrations of beneficial microbial populations (e.g., bifidobacteria, lactobacilli) and decreasing

concentrations of potential pathogens (e.g., *Clostridium perfringens*). It also enhanced indices of gut health by increasing fecal butyrate and lactate concentrations and decreasing several putrefactive compounds present in feces. On the other hand, *Lactobacillus acidophilus* enhanced some beneficial microbial populations and increased total tract nutrient digestibility. *Lactobacillus acidophilus* plus FOS resulted in effects of larger entity on the concentration of bacterial metabolites. In particular, the synbiotic was effective in reducing the fecal concentrations of ammonia and putrefactive compounds including branched chain fatty acids, biogenic amines, phenols, and indoles to a greater extent as compared to administration of either probiotic or prebiotic alone (Swanson et al. 2002).

Although supplemental synbiotics may be useful in introducing beneficial bacterial populations and have potential to encourage proliferation of autochthonous-specific strains in the intestinal tract, there are limited studies using synbiotics as a supplement in the prevention or treatment of diarrhea in companion animals. Gagné et al. (2013) evaluated the effects of a synbiotic containing *Enterococcus faecium* SF68, *Bacillus coagulans*, and *Lactobacillus acidophilus* plus FOS and MOS and other ingredients on fecal quality, SCFAs concentrations, and the microbiome and its potential to reduce the episodes of diarrhea in training healthy sled dogs. Administration of synbiotic resulted in a significant rise in *Lactobacillaceae*, positive correlation between *Lactobacillaceae* and overall butyrate concentration, and improvement in fecal score which was associated with a decrease in the prevalence of diarrhea in dogs. In contrast, in a previous study, no changes in the major bacterial phyla were identified in healthy dogs and cats following administration of a commercial synbiotic containing a mixture of seven different probiotic species (*Enterococcus faecium* NCIMB 30183, *Streptococcus salivarius* subsp. *thermophilus* NCIMB 30189, *Bifidobacterium longum* NCIMB 30179, *Lactobacillus acidophilus* NCIMB 30184, *Lactobacillus casei* subsp. *rhamnosus* NCIMB 30188, *Lactobacillus plantarum* NCIMB 30187, *Lactobacillus delbrueckii* subsp. *bulgaricus* NCIMB 30186) and a blend of FOS and arabinogalactans daily for 21 days. The synbiotic had no adverse gastrointestinal effects during the study period. None of the evaluated serum (cobalamin, folate, IgA, trypsin-like immunoreactivity, and pancreatic lipase immunoreactivity) or fecal (IgA and α_1-proteinase inhibitor) markers of gastrointestinal and immune function were influenced by the synbiotic administration. However, administration of the synbiotic resulted in an increased abundance of probiotic bacteria in the feces of healthy dogs and cats (Garcia-Mazcorro et al. 2011). Lack of changes in the major bacterial phyla of the synbiotic-administered dogs and cats has been attributable to significantly less prebiotic supplied in the diet. It has been shown that to cause significant increases in the fecal microbiome of dogs such as *Lactobacillus*, at least 0.5–1% increase in soluble fiber is required (Swanson et al. 2002; Gagné et al. 2013).

Diarrhea is also the most common disease affecting kenneled dogs in animal shelters. In a recent study, Rose et al. (2017) assessed the efficacy of a synbiotic supplement on the incidence of diarrhea in a dog shelter in a randomized, double-blind, placebo-controlled trial. Supplementing healthy dogs entering an animal shelter with *Enterococcus faecium* NCIMB 10415 4b1707 plus a combination of FOS and acacia was demonstrated to significantly decrease the incidence of diarrhea. It was suggested that animal shelters can use synbiotic supplements to improve animal welfare and decrease costs involved in cleaning and housing animals as well as potentially decreasing veterinary intervention.

Studies addressing the efficacy of synbiotics in domestic cats with diarrhea are very scarce. Hart et al. (2012) examined the effect of a synbiotic formulation comprising of multispecies probiotics (*Enterococcus faecium* NCIMB 30183, *Streptococcus salivarius* subsp. *thermophilus* NCIMB 30189, *Bifidobacterium bifidum* NCIMB 30179, *Lactobacillus acidophilus* NCIMB 30184, *Lactobacillus casei* subsp. *rhamnosus* NCIMB 30188, *Lactobacillus plantarum* NCIMB 30187, *Lactobacillus delbrueckii* subsp. *bulgaricus* NCIMB 30186) plus two prebiotics in client-owned adult cats with naturally occurring chronic diarrhea. The synbiotic improved stool character, and the mean fecal score decreased from 6.0 to 4.4, representing a significantly firmer stool character. Following a 21-day course of synbiotic supplementation, 72% of owners perceived an improvement in diarrhea of their cats. This synbiotic product had no adverse effects and was found to be safe and well tolerated.

Although probiotic use is beneficial in the prevention of antibiotic-associated gastrointestinal signs (AAGS) in humans (Blaabjerg et al. 2017), little is known about efficacy of synbiotics in mitigating AAGS in cats. Stokes et al. (2017) conducted a randomized, double-blinded, placebo-controlled, two-way, two-period, crossover trial of prevention of clindamycin-induced gastrointestinal signs using a synbiotic (a commercial mixture of probiotics and prebiotics) in healthy research cats. Administration of the synbiotic 1 h after clindamycin administration decreased hyporexia and vomiting in cats. In addition, the clinical benefits of synbiotic administration persisted for at least 6 weeks after discontinuation, decreasing the severity of AAGS in cats that subsequently received clindamycin with placebo. However, synbiotic administration did not decrease antibiotic-associated diarrhea.

3 Concluding Remarks and Future Directions

In view of increasing consumer awareness and the ever-growing demand for animal products from antibiotic-free livestock production systems, synbiotics and other feed

additives are now being considered important tools to help in the improvement of animal performance and health and counteracting diseases. Although available data regarding effects of synbiotics on animal health and production are inadequate and require further studies, the majority of studies dealing with synbiotics have been conducted in poultry, but increasing research has been focused in ruminants, pigs, and pet animals. There are evidences to suggest that synbiotics are having favorable influence on the intestinal microbiota of animals and play role in the improvement of performance and maintenance of optimal health of farm and companion animals. High potential of synbiotic products is attributable to the concurrent use of probiotics and prebiotics with the ultimate aim, besides others to improve the survival and implantation of probiotic microorganisms and maintain favorable balance in the ecosystem in the gastrointestinal tract, which is crucial for the improved health and productivity of the host animal. Besides other favorable effects, improvement in the number of beneficial intestinal microorganisms and the reduction of the potential pathogen load, stimulation of immunological response, and increased production capacity have been demonstrated following administration of synbiotics in animals. They are also effective in improving the intestinal architect, blood indices, and absorption of minerals and nutrients. The use of synbiotics may reduce the demand for antibiotic-based growth promoters. Selection of most appropriate probiotic and prebiotic in synbiotic products and better understanding about the intestinal microbiota of animals will pave the way in designing more efficacious synbiotics. Further research is needed regarding preventive and therapeutic health benefits, mechanisms of action, optimal doses, duration of treatment, improved delivery systems, and augmentation of in vivo efficacy of synbiotics which may lead to the enhancement of their beneficial effects on animal health and production.

References

Abdel-Hafeez HM, Saleh ESE, Tawfeek SS, Youssef IMI, Abdel-Daim ASA (2017) Effects of probiotic, prebiotic, and synbiotic with and without feed restriction on performance, hematological indices and carcass characteristics of broiler chickens. Asian-Australas J Anim Sci 30(5):672–682

Abdelqader A, Irshaid R, Al-Fataftah AR (2013) Effects of dietary probiotic inclusion on performance, eggshell quality, cecal microflora composition, and tibia traits of laying hens in the late phase of production. Trop Anim Health Prod 45(4):1017–1024

Abdel-Raheem SM, Abd-Allah SMS, Hassanein KMA (2012) The effects of prebiotic, probiotic and synbiotic supplementation on intestinal microbial ecology and histomorphology of broiler chickens. IJAVMS 6(4):277–289

Abdel-Wareth AA (2016) Effect of dietary supplementation of thymol, synbiotic and their combination on performance, egg quality and serum metabolic profile of Hy-line Brown hens. Br Poult Sci 57 (1):114–122

Adil S, Magray SN (2012) Impact and manipulation of gut microflora in poultry: a review. J Anim Vet Adv 11(6):873–877

Alizadeh M, Munyaka P, Yitbarek A, Echeverry H, Rodriguez-Lecompte JC (2017) Maternal antibody decay and antibody-mediated immune responses in chicken pullets fed prebiotics and synbiotics. Poult Sci 96(1):58–64

Al-Sultan SI, Abdel-Raheem SM, El-Ghareeb WR, Mohamed MHA (2016) Comparative effects of using prebiotic, probiotic, synbiotic and acidifier on growth performance, intestinal microbiology and histomorphology of broiler chicks. Jpn J Vet Res 64(Suppl 2):S187–S195

Anadón A, Martínez-Larrañaga MR, Caballero V, Castellano V (2010) Assessment of prebiotics and probiotics: an overview. In: Watson RR, Preedy VR (eds) Bioactive foods in promoting health: probiotics and prebiotics. Academic, Oxford, pp 19–41

Andrejčáková Z, Sopková D, Vlčková R, Kulichová L, Gancarčíková S, Almášiová V, Holovská K, Petrilla V, Krešáková L (2016) Synbiotics suppress the release of lactate dehydrogenase, promote non-specific immunity and integrity of jejunum mucosa in piglets. Anim Sci J 87(9):1157–1166

Anwar H, Rahman ZU, Javed I, Muhammad F (2012) Effect of protein, probiotic, and synbiotic supplementation on serum biological health markers of molted layers. Poult Sci 91(10):2606–2613

Ashayerizadeh A, Dabiri N, Mirzadeh Kh, Ghorbani MR (2011) Effects of dietary inclusion of several biological feed additives on growth response of broiler chickens. J Cell Anim Biol 5(4):61–65

Awad W, Ghareeb K, Böhm J (2008) Intestinal structure and function of broiler chickens on diets supplemented with a synbiotic containing *Enterococcus faecium* and oligosaccharides. Int J Mol Sci 9 (11):2205–2216

Awad WA, Ghareeb K, Abdel-Raheem S, Böhm J (2009) Effects of dietary inclusion of probiotic and synbiotic on growth performance, organ weights, and intestinal histomorphology of broiler chickens. Poult Sci 88(1):49–56

Aziz Mousavi SMA, Seidavi A, Dadashbeiki M, Kilonzo-Nthenge A, Nahashon SN, Laudadio V, Tufarelli V (2015) Effect of a synbiotic (Biomin®IMBO) on growth performance traits of broiler chickens. Eur Poult Sci 79. https://doi.org/10.1399/eps.2015.78

Baffoni L, Gaggia F, Di Gioia D, Santini C, Mogna L, Biavati B (2012) A *Bifidobacterium*-based synbiotic product to reduce the transmission of *C. jejuni* along the poultry food chain. Int J Food Microbiol 157(2):156–161

Baffoni L, Gaggìa F, Garofolo G, Di Serafino G, Buglione E, Di Giannatale E, Di Gioia D (2017) Evidence of *Campylobacter jejuni* reduction in broilers with early synbiotic administration. Int J Food Microbiol 251:41–47

Baines D, Sumarah M, Kuldau G, Juba J, Mazza A, Masson L (2013) Aflatoxin, fumonisin and Shiga toxin-producing *Escherichia coli* infections in calves and the effectiveness of Celmanax®/Dairyman's choice™ applications to eliminate morbidity and mortality losses. Toxins (Basel) 5(10):1872–1895

Blaabjerg S, Artzi DM, Aabenhus R (2017) Probiotics for the prevention of antibiotic-associated diarrhea in outpatients-a systematic review and meta-analysis. Antibiotics (Basel) 6(4):pii: E21

Bomba A, Nemcová R, Gancarcíková S, Herich R, Guba P, Mudronová D (2002) Improvement of the probiotic effect of micro-organisms by their combination with maltodextrins, fructo-oligosaccharides and polyunsaturated fatty acids. Br J Nutr 88(Suppl 1):S95–S99

Calik A, Ceylan A, Ekim B, Adabi SG, Dilber F, Bayraktaroglu AG, Tekinay T, Özen D, Sacakli P (2017) The effect of intra-amniotic and posthatch dietary synbiotic administration on the performance, intestinal histomorphology, cecal microbial population, and short-chain fatty acid composition of broiler chickens. Poult Sci 96 (1):169–183

Cesari V, Mangiagalli MG, Giardini A, Galimberti P, Carteri S, Gallazzi D, Toschi I (2014) Egg quality and productive performance

of laying hens fed different levels of skimmed milk powder added to a diet containing *Lactobacillus acidophilus*. Poult Sci 93 (5):1197–1201

Chae JP, Pajarillo EA, Oh JK, Kim H, Kang DK (2016) Revealing the combined effects of lactulose and probiotic enterococci on the swine faecal microbiota using 454 pyrosequencing. Microb Biotechnol 9 (4):486–495

Chen Y, Wen C, Zhou Y (2018) Dietary synbiotic incorporation as an alternative to antibiotic improves growth performance, intestinal morphology, immunity and antioxidant capacity of broilers. J Sci Food Agric 98(9):3343–3350

Cheng Y, Chen Y, Li X, Yang W, Wen C, Kang Y, Wang A, Zhou Y (2017) Effects of synbiotic supplementation on growth performance, carcass characteristics, meat quality and muscular antioxidant capacity and mineral contents in broilers. J Sci Food Agric 97 (11):3699–3705

Chu GM, Lee SJ, Jeong HS, Lee SS (2011) Efficacy of probiotics from anaerobic microflora with prebiotics on growth performance and noxious gas emission in growing pigs. Anim Sci J 82(2):282–290

Dibaji SM, Seidavi A, Asadpour L, da Silva FM (2014) Effect of a synbiotic on the intestinal microflora of chickens. J Appl Poult Res 23(1):1–6

Dunislawska A, Slawinska A, Stadnicka K, Bednarczyk M, Gulewicz P, Jozefiak D, Siwek M (2017) Synbiotics for broiler chickens-*in vitro* design and evaluation of the influence on host and selected microbiota populations following *in ovo* delivery. PLoS One 12(1): e0168587. https://doi.org/10.1371/journal.pone.0168587

EFSA (2013a) The European union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2011. EFSA J 3129:1–250

EFSA (2013b) Scientific opinion on the maintenance of the list of QPS biological agents intentionally added to food and feed (2013 update). EFSA J 3449:1–108

EFSA (2017) Scientific opinion on the update of the list of QPS-recommended biological agents intentionally added to food or feed as notified to EFSA (2017 update). EFSA J 15(3):1–177. https://doi.org/10.2903/j.efsa.2017.4664

El-Ghany WAA (2010) Comparative evaluation on the effect of coccidiostate and synbiotic preparations on prevention of *Clostridium perfringens* in broiler chickens. Global Vet 5(6):324–333

El-Sissi AF, Mohamed SH (2011) Impact of symbiotic on the immune response of broiler chickens against NDV and IBV vaccines. Global J Biotechnol Biochem 6(4):186–191

Erdoğan Z, Erdoğan S, Aslantaş Ö, Çelik S (2010) Effects of dietary supplementation of synbiotics and phytobiotics on performance, caecal coliform population and some oxidant/antioxidant parameters of broilers. J Anim Physiol Anim Nutr (Berl) 94(5):e40–e48

Fan G, Chang J, Yin Q, Wang X, Dang X (2015) Effects of probiotics, oligosaccharides, and berberine combinations on growth performance of pigs. Turk J Vet Anim Sci 39(6):637–642

Fernandes BCS, Martins MRFB, Mendes AA, Milbradt EL, Sanfelice C, Martins BB, Aguiar EF, Bresne C (2014) Intestinal integrity and performance of broiler chickens fed a probiotic, a prebiotic, or an organic acid. Rev Bras Cienc Avic 16(4):417–424

Fleige S, Preißinger W, Meyer HHD, Pfaffl MW (2007) Effect of lactulose on growth performance and intestinal morphology of pre-ruminant calves using a milk replacer containing *Enterococcus faecium*. Animal 1(3):367–373

Food and Agriculture Organization (FAO) (2002) Guidelines for the evaluation of probiotics in food. Report of a Joint FAO/WHO Working Group on Drafting Guidelines for the evaluation of probiotics in food; FAO, London, Ontario, Canada, 30 April–1 May 2002

Gadde U, Kim WH, Oh ST, Lillehoj HS (2017) Alternatives to antibiotics for maximizing growth performance and feed efficiency in poultry: a review. Anim Health Res Rev 18(1):26–45

Gaggia F, Mattarelli P, Biavati B (2010) Probiotics and prebiotics in animal feeding for safe food production. Int J Food Microbiol 141 (Suppl 1):S15–S28

Gagné JW, Wakshlag JJ, Simpson KW, Dowd SE, Latchman S, Brown DA, Brown K, Swanson KS, Fahey GC Jr (2013) Effects of a synbiotic on fecal quality, short-chain fatty acid concentrations, and the microbiome of healthy sled dogs. BMC Vet Res 9:246. doi:https://doi.org/10.1186/1746-6148-9-246

Garcia-Mazcorro JF, Lanerie DJ, Dowd SE, Paddock CG, Grützner N, Steiner JM, Ivanek R, Suchodolski JS (2011) Effect of a multi-species synbiotic formulation on fecal bacterial microbiota of healthy cats and dogs as evaluated by pyrosequencing. FEMS Microbiol Ecol 78(3):542–554

Ghasemi HA, Shivazad M, Esmaeilnia K, Kohram H, Karimi MA (2010) The effects of a synbiotic containing *Enterococcus faecium* and inulin on growth performance and resistance to coccidiosis in broiler chickens. J Poult Sci 47(2):149–155

Ghasemi HA, Shivazad M, Mirzapour Rezaei SS, Karimi Torshizi MA (2016) Effect of synbiotic supplementation and dietary fat sources on broiler performance, serum lipids, muscle fatty acid profile and meat quality. Br Poult Sci 57(1):71–83

Gourbeyre P, Denery S, Bodinier M (2011) Probiotics, prebiotics, and synbiotics: impact on the gut immune system and allergic reactions. J Leukoc Biol 89(5):685–695

Guerra-Ordaz AA, González-Ortiz G, La Ragione RM, Woodward MJ, Collins JW, Pérez JF, Martín-Orúe SM (2014) Lactulose and *Lactobacillus plantarum*, a potential complementary synbiotic to control postweaning colibacillosis in piglets. Appl Environ Microbiol 80 (16):4879–4886

Hamasalim HJ (2016) Synbiotic as feed additives relating to animal health and performance. Adv Microbiol 6:288–302. https://doi.org/10.4236/aim.2016.64028

Hart ML, Suchodolski JS, Steiner JM, Webb CB (2012) Open-label trial of a multi-strain synbiotic in cats with chronic diarrhea. J Feline Med Surg 14(4):240–245

Hashem MA, Mohamed MH (2009) Haemato-biochemical and pathological studies on aflatoxicosis and treatment of broiler chicks in Egypt. Vet Ital 45(2):323–337

Hassanpour H, Zamani Moghaddam AK, Khosravi M, Mayahi M (2013) Effects of synbiotic on the intestinal morphology and humoral immune response in broiler chickens. Livest Sci 153 (1–3):116–122

Heinrichs AJ, Jones CM, Elizondo-Salazar JA, Terrill SJ (2009) Effects of a prebiotic supplement on health of neonatal dairy calves. Livest Sci 125(2–3):149–154

Hofacre CL, Beacorn T, Collett S, Mathis G (2003) Using competitive exclusion, mannan-oligosaccharide and other intestinal products to control necrotic enteritis. J Appl Poult Res 12(1):60–64

Jung SJ, Houde R, Baurhoo B, Zhao X, Lee BH (2008) Effects of galacto-oligosaccharides and a *Bifidobacteria lactis*-based probiotic strain on the growth performance and fecal microflora of broiler chickens. Poult Sci 87(9):1694–1699

Kearney SM, Gibbons SM (2018) Designing synbiotics for improved human health. Microb Biotechnol 11(1):141–144

Koc F, Samli H, Okur A, Ozduven M, Akyurek H, Senkoylu N (2010) Effects of *Saccharomyces cerevisiae* and/or mannanoligosaccharide on performance, blood parameters and intestinal microbiota of broiler chicks. Bulg J Agric Sci 16(5):643–650

Kolodziejski PA, Sassek M, Chalupka D, Leciejewska N, Nogowski L, Mackowiak P, Jozefiak D, Stadnicka K, Siwek M, Bednarczyk M, Szwaczkowski T, Pruszynska-Oszmalek E (2018) GLP1 and GIP are involved in the action of synbiotics in broiler chickens. J Anim Sci Biotechnol 9:13. https://doi.org/10.1186/s40104-017-0227-8

Krause DO, Bhandari SK, House JD, Nyachoti CM (2010) Response of nursery pigs to a synbiotic preparation of starch and an anti-*Escherichia coli* K88 probiotic. Appl Environ Microbiol 76 (24):8192–8200

Krueger LA, Spangler DA, Vandermyde DR, Sims MD, Ayangbile GA (2017) Avi-Lution® supplemented at 1.0 or 2.0 g/kg in feed improves the growth performance of broiler chickens during

challenge with bacitracin-resistant *Clostridium perfringens*. Poult Sci 96(8):2595–2600
Kwiatkowska K, Winiarska-Mieczan A, Kwiecień M (2017) Feed additives regulating calcium homeostasis in the bones of poultry—a review. Ann Anim Sci 17(2):303–316
Lee DY, Seo YS, Rayamajhi N, Kang ML, Lee SI, Yoo HS (2009) Isolation, characterization, and evaluation of wild isolates of *Lactobacillus reuteri* from pig feces. J Microbiol 47(6):663–672
Li X, Qiang L, Liu XC (2008) Effects of supplementation of fructooligosaccharide and/or *Bacillus subtilis* to diets on performance and on intestinal microflora in broilers. Arch Anim Breed 51(1):64–70
Liao SF, Nyachoti M (2017) Using probiotics to improve swine gut health and nutrient utilization. Anim Nutr 3(4):331–343
Liong M-T, Dunshea FR, Shah NP (2007) Effects of a synbiotic containing *Lactobacillus acidophilus* ATCC 4962 on plasma lipid profiles and morphology of erythrocytes in hypercholesterolaemic pigs on high- and low-fat diets. Br J Nutr 98(4):736–744
Luoma A, Markazi A, Shanmugasundaram R, Murugesan GR, Mohnl M, Selvaraj R (2017) Effect of synbiotic supplementation on layer production and cecal *Salmonella* load during a *Salmonella* challenge. Poult Sci 96(12):4208–4216
Madej JP, Bednarczyk M (2016) Effect of *in ovo*-delivered prebiotics and synbiotics on the morphology and specific immune cell composition in the gut-associated lymphoid tissue. Poult Sci 95(1):19–29
Madej JP, Stefaniak T, Bednarczyk M (2015) Effect of *in ovo*-delivered prebiotics and synbiotics on lymphoid-organs' morphology in chickens. Poult Sci 94(6):1209–1219
Maiorano G, Sobolewska A, Cianciullo D, Walasik K, Elminowska-Wenda G, Slawinska A, Tavaniello S, Zylinska J, Bardowski J, Bednarczyk M (2012) Influence of in ovo prebiotic and synbiotic administration on meat quality of broiler chickens. Poult Sci 91 (11):2963–2969
Malik JK, Ahmad AH, Kalpana S, Prakash A, Gupta RC (2016) Synbiotics: safety and toxicity considerations. In: Gupta RC (ed) Nutraceuticals: efficacy, safety and toxicity. Academic Press/Elsevier, Amsterdam, pp 811–822
Marcondes MI, Pereira TR, Chagas JC, Filgueiras EA, Castro MM, Costa GP, Sguizzato AL, Sainz RD (2016) Performance and health of Holstein calves fed different levels of milk fortified with symbiotic complex containing pre- and probiotics. Trop Anim Health Prod 48(8):1555–1560
Markowiak P, Śliżewska K (2018) The role of probiotics, prebiotics and synbiotics in animal nutrition. Gut Pathog 10:21. https://doi.org/10.1186/s13099-018-0250-0
McReynolds J, Waneck C, Byrd J, Genovese K, Duke S, Nisbet D (2009) Efficacy of multistrain direct-fed microbial and phytogenetic products in reducing necrotic enteritis in commercial broilers. Poult Sci 88(10):2075–2080
Midilli M, Alp M, Kocabach N, Muglah OH, Turan N, Yilmaz H, Cakir S (2008) Effects of dietary probiotic and prebiotic supplementation on growth performance and serum IgG concentration of broilers. South Afr J Anim Sci 38(1):21–27
Min YN, Yang HL, Xu YX, Gao YP (2016) Effects of dietary supplementation of synbiotics on growth performance, intestinal morphology, sIgA content and antioxidant capacities of broilers. J Anim Physiol Anim Nutr (Berl) 100(6):1073–1080
Miśta D, Króliczewska B, Pecka-Kiełb E, Kapuśniak V, Zawadzki W, Graczyk S, Kowalczyk A, Łukaszewicz E, Bednarczyk M (2017) Effect of *in ovo* injected prebiotics and synbiotics on the caecal fermentation and intestinal morphology of broiler chickens. Anim Prod Sci 57(9):1884–1892
Mohnl M, Acosta Aragón Y, Acosta Ojeda A, Rodríguez Sánchez B, Pasteiner S (2007) Effect of synbiotic feed additive in comparison to antibiotic growth promoter on performance and health status of broilers. Poult Sci 86(Suppl 1):217
Mookiah S, Sieo CC, Ramasamy K, Abdullah N, Ho YW (2014) Effects of dietary prebiotics, probiotic and synbiotics on performance, caecal bacterial populations and caecal fermentation concentrations of broiler chickens. J Sci Food Agric 94(2):341–348
Morrison SJ, Dawson S, Carson AF (2010) The effects of mannan oligosaccharide and *Streptococcus faecium* addition to milk replacer on calf health and performance. Livest Sci 131(2–3):292–296
Murarolli VDA, Burbarelli MFC, Polycarpo GV, Ribeiro PAP, Moro MEG, Albuquerque R (2014) Prebiotic, probiotic and symbiotic as alternative to antibiotics on the performance and immune response of broiler chickens. Rev Bras Cienc Avic 16(3):279–284
Naghi Shokri A, Ghasemi HA, Taherpour K (2017) Evaluation of *Aloe vera* and synbiotic as antibiotic growth promoter substitutions on performance, gut morphology, immune responses and blood constitutes of broiler chickens. Anim Sci J 88(2):306–313
Nemcová R, Bomba A, Gancarčíková S, Herich R, Guba P (1999) Study of the effect of *Lactobacillus paracasei* and fructooligosaccharides on the faecal microflora in weanling piglets. Berl Munch Tierarztl Wochenschr 112(6–7):225–228
Nemcová R, Bomba A, Gancarcíková S, Reiffová K, Guba P, Koscová J, Jonecová Z, Sciranková L, Bugarský A (2007) Effects of the administration of lactobacilli, maltodextrins and fructooligosaccharides upon the adhesion of *E. coli* O8:K88 to the intestinal mucosa and organic acid levels in the gut contents of piglets. Vet Res Commun 31(7):791–800
Ogué-Bon E, Khoo C, McCartney AL, Gibson GR, Rastall RA (2010) *In vitro* effects of synbiotic fermentation on the canine faecal microbiota. FEMS Microbiol Ecol 73(3):587–600
Ogué-Bon E, Khoo C, Hoyles L, McCartney AL, Gibson GR, Rastall RA (2011) *In vitro* fermentation of rice bran combined with *Lactobacillus acidophilus* 14 150B or *Bifidobacterium longum* 05 by the canine faecal microbiota. FEMS Microbiol Ecol 75(3):365–376
Olveira G, González-Molero I (2016) An update on probiotics, prebiotics and synbiotics in clinical nutrition. Endocrinol Nutr 63 (9):482–494
Pandey KR, Naik SR, Vakil BV (2015) Probiotics, prebiotics and synbiotics—a review. J Food Sci Technol 52(12):7577–7587
Parvaneh K, Jamaluddin R, Karimi G, Erfani R (2014) Effect of probiotics supplementation on bone mineral content and bone mass density. Sci World J 2014:595962. https://doi.org/10.1155/2014/595962
Pinna C, Biagi G (2014) The utilisation of prebiotics and synbiotics in dogs. Ital J Anim Sci 13(1). https://doi.org/10.4081/ijas.2014.3107
Piva A, Casadei G, Gatta PP, Luchansky JB, Biagi G (2005) Effect of lactitol, lactic acid bacteria, or their combinations (synbiotic) on intestinal proteolysis in vitro, and on feed efficiency in weaned pigs. Can J Anim Sci 85(3):345–353
Płowiec A, Sławińska A, Siwek MZ, Bednarczyk MF (2015) Effect of in ovo administration of inulin and *Lactococcus lactis* on immune-related gene expression in broiler chickens. Am J Vet Res 76 (11):975–982
Pruszynska-Oszmalek E, Kolodziejski PA, Stadnicka K, Sassek M, Chalupka D, Kuston B, Nogowski L, Mackowiak P, Maiorano G, Jankowski J, Bednarczyk M (2015) In ovo injection of prebiotics and synbiotics affects the digestive potency of the pancreas in growing chickens. Poult Sci 94(8):1909–1916
Radzikowski D (2017) Effect of probiotics, prebiotics and synbiotics on the productivity and health of dairy cows and calves. World Sci News 78:193–198
Roodposhti PM, Dabiri N (2012) Effects of probiotic and prebiotic on average daily gain, fecal shedding of *Escherichia coli*, and immune system status in newborn female calves. Asian-Australas J Anim Sci 25(9):1255–1261
Rose L, Rose J, Gosling S, Holmes M (2017) Efficacy of a probiotic-prebiotic supplement on incidence of diarrhea in a dog shelter: a randomized, double-blind, placebo-controlled trial. J Vet Intern Med 31(2):377–382
Salavati Schmitz S, Allenspach K (2017) Effects of different oligosaccharides on growth of selected probiotic bacterial strains. J Microb

Biochem Technol 9:054–058. https://doi.org/10.4172/1948-5948.1000343

Salehimanesh A, Mohammadi M, Roostaei-Ali Mehr M (2016) Effect of dietary probiotic, prebiotic and synbiotic supplementation on performance, immune responses, intestinal morphology and bacterial populations in broilers. J Anim Physiol Anim Nutr (Berl) 100 (4):694–700

Sassone-Corsi M, Nuccio SP, Liu H, Hernandez D, Vu CT, Takahashi AA, Edwards RA, Raffatellu M (2016) Microcins mediate competition among Enterobacteriaceae in the inflamed gut. Nature 540 (7632):280–283

Sattler VA, Bayer K, Schatzmayr G, Haslberger AG, Klose V (2015) Impact of a probiotic, inulin, or their combination on the piglets' microbiota at different intestinal locations. Benef Microbes 6 (4):473–483

Scavuzzi BM, Henrique FC, Miglioranza LHS, Simão ANC, Dichi I (2014) Impact of prebiotics, probiotics and synbiotics on components of the metabolic syndrome. Ann Nutr Disord Ther 1(2):1009

Scholz-Ahrens KE, Ade P, Marten B, Weber P, Timm W, Açil Y, Glüer CC, Schrezenmeir J (2007) Prebiotics, probiotics, and synbiotics affect mineral absorption, bone mineral content, and bone structure. J Nutr 137(3 Suppl 2):838S–846S

Sekhon BS, Jairath S (2010) Prebiotics, probiotics and synbiotics: an overview. J Pharm Educ Res 1(2):13–36

Shim SB, Verstegen MWA, Kim IH, Kwon OS, Verdonk JMAJ (2005) Effects of feeding antibiotic-free creep feed supplemented with oligofructose, probiotics or synbiotics to suckling piglets increases the preweaning weight gain and composition of intestinal microbiota. Arch Anim Nutr 59(6):419–427

Sławińska A, Siwek M, Zylińska J, Bardowski J, Brzezińska J, Gulewicz KA, Nowak M, Urbanowski M, Płowiec A, Bednarczyk M (2014a) Influence of synbiotics delivered *in ovo* on immune organs development and structure. Folia Biol 62(3):277–285

Sławińska A, Siwek MZ, Bednarczyk MF (2014b) Effects of synbiotics injected in ovo on regulation of immune-related gene expression in adult chickens. Am J Vet Res 75(11):997–1003

Sobolewska A, Bogucka J, Dankowiakowska A, Elminowska-Wenda G, Stadnicka K, Bednarczyk M (2017) The impact of synbiotic administration through in ovo technology on the microstructure of a broiler chicken small intestine tissue on the 1st and 42nd day of rearing. J Anim Sci Biotechnol 8:61. https://doi.org/10.1186/s40104-017-0193-1

Sohail MU, Hume ME, Byrd JA, Nisbet DJ, Ijaz A, Sohail A, Shabbir MZ, Rehman H (2012) Effect of supplementation of prebiotic mannan-oligosaccharides and probiotic mixture on growth performance of broilers subjected to chronic heat stress. Poult Sci 91 (9):2235–2240

Sohail MU, Ijaz A, Younus M, Shabbir MZ, Kamran Z, Ahmad S, Anwar H, Yousaf MS, Ashraf K, Shahzad AH, Rehman H (2013) Effect of supplementation of mannan oligosaccharide and probiotic on growth performance, relative weights of viscera, and population of selected intestinal bacteria in cyclic heat-stressed broilers. J Appl Poult Res 22(3):485–491

Sopková D, Hertelyová Z, Andrejčáková Z, Vlčková R, Gancarčíková S, Petrilla V, Ondrašovičová S, Krešáková L (2017) The application of probiotics and flaxseed promotes metabolism of n-3 polyunsaturated fatty acids in pigs. J Appl Anim Res 45 (1):93–98

Spring P, Wenk C, Dawson KA, Newman KE (2000) The effects of dietary mannanoligosaccharides on cecal parameters and the concentrations of enteric bacteria in the ceca of Salmonella-challenged broiler chicks. Poult Sci 79(2):205–211

Stokes JE, Price JM, Whittemore JC (2017) Randomized, controlled, crossover trial of prevention of clindamycin-induced gastrointestinal signs using a synbiotic in healthy research cats. J Vet Intern Med 31 (5):1406–1413

Sun Y, Kim SW (2017) Intestinal challenge with enterotoxigenic *Escherichia coli* in pigs, and nutritional intervention to prevent postweaning diarrhea. Anim Nutr 3(4):322–330

Swanson KS, Grieshop CM, Flickinger EA, Bauer LL, Chow J, Wolf BW, Garleb KA, Fahey GC Jr (2002) Fructooligosaccharides and *Lactobacillus acidophilus* modify gut microbial populations, total tract nutrient digestibilities and fecal protein catabolite concentrations in healthy adult dogs. J Nutr 132 (12):3721–3731

Talebi A, Amani A, Pourmahmod M, Saghaei P, Rezaie R (2015) Synbiotic enhances immune responses against infectious bronchitis, infectious bursal disease, Newcastle disease and avian influenza in broiler chickens. Vet Res Forum 6(3):191–197

Tang SGH, Sieo CC, Kalavathy R, Saad WZ, Yong ST, Wong HK, Ho YW (2015) Chemical compositions of egg yolks and egg quality of laying hens fed prebiotic, probiotic, and synbiotic diets. J Food Sci 80(8):C1686–C1695

Tang SGH, Sieo CC, Ramasamy K, Saad WZ, Wong HK, Ho YW (2017) Performance, biochemical and haematological responses, and relative organ weights of laying hens fed diets supplemented with prebiotic, probiotic and synbiotic. BMC Vet Res 13(1):248

Tanner SA, Lacroix C, Del'Homme C, Jans C, Zihler Berner A, Bernalier-Donadille A, Chassard C (2015) Effect of *Bifidobacterium thermophilum* RBL67 and fructo-oligosaccharides on the gut microbiota in Göttingen minipigs. Br J Nutr 114(5):746–755

Tayeri V, Seidavi A, Asadpour L, Phillips CJC (2018) A comparison of the effects of antibiotics, probiotics, synbiotics and prebiotics on the performance and carcass characteristics of broilers. Vet Res Commun 42(3):195–207

Uyeno Y, Shigemori S, Shimosato T (2015) Effect of probiotics/prebiotics on cattle health and productivity. Microbes Environ 30 (2):126–132

Vahdatpour T, Ebrahimnezhad Y, Vahdatpour S (2014) Effects of dietary functional additives on characteristics and minerals of tibia bone and blood parameters of Japanese quails (*Coturnix coturnix japonica*). Int J Plant Anim Environ Sci 4(2):690–695

Vicente J, Wolfenden A, Torres-Rodriguez A, Higgins S, Tellez G, Hargis B (2007) Effect of a *Lactobacillus* species-based probiotic and dietary lactose prebiotic on Turkey poult performance with or without *Salmonella* enteritidis challenge. J Appl Poult Res 16 (3):361–364

Villaluenga CM, Wardeńska M, Pilarski R, Bednarczyk M, Gulewicz K (2004) Utilization of the chicken embryo model for assessment of biological activity of different oligosaccharides. Folia Biol (Krakow) 52(3–4):135–142

Vitali B, Ndagijimana M, Cruciani F, Carnevali P, Candela M, Guerzoni ME, Brigidi P (2010) Impact of a synbiotic food on the gut microbial ecology and metabolic profiles. BMC Microbiol 10:4. https://doi.org/10.1186/1471-2180-10-4

Wang Y, Dong Z, Song D, Zhou H, Wang W, Miao H, Wang L, Li A (2018) Effects of microencapsulated probiotics and prebiotics on growth performance, antioxidative abilities, immune functions, and caecal microflora in broiler chickens. Food Agric Immunol. https://doi.org/10.1080/09540105.2018.1463972

Weaver CM (2015) Diet, gut microbiome, and bone health. Curr Osteoporos Rep 13(2):125–130

Yang Y, Iji PA, Choct M (2009) Dietary modulation of gut microflora in broiler chickens: a review of the role of six kinds of alternatives to in-feed antibiotics. Worlds Poult Sci J 65(1):97–114

Yasuda K, Hashikawa S, Sakamoto H, Tomita Y, Shibata S, Fukata T (2007) A new synbiotic consisting of *Lactobacillus casei* subsp. *casei* and dextran improves milk production in Holstein dairy cows. J Vet Med Sci 69(2):205–208

Yitbarek A, Echeverry H, Munyaka P, Rodriguez-Lecompte JC (2015) Innate immune response of pullets fed diets supplemented with prebiotics and synbiotics. Poult Sci 94(8):1802–1811

Enzymes in Feed and Animal Health

Arturo Anadón, Irma Ares, Maria Rosa Martínez-Larrañaga, and Maria Aŕanzazu Martínez

Abstract

Exogenous enzymes are potentially important alternatives to antibiotics for improving growth performance, particularly in poultry and swine, although research in other animals such as ruminants, fish, fur-bearers, and pets has been done in recent years. The value of added feed enzymes in promoting growth and efficiency of nutrient utilization in animal production is clearly recognized. This chapter covers several reaction conditions that need to be met for the enzyme to act, as well as feed enzymes and gastrointestinal microbiota, mode of action for enzymes, dietary substrates, and enzyme types to be used in animal feed (for example, phytase, and enzyme classes such as xylanases, β-glucanases, pectinases, amylases, and proteases) which improve the feed utilization of dietary components such as protein, amino acids, starch, lipids, and energy. Feed enzymes can affect gastrointestinal tract (GIT) microbial ecology by reducing undigested substrates and anti-nutritive factors and producing oligosaccharides in situ from dietary non-starch polysaccharides (NSP) with potential prebiotic effects.

Keywords

Veterinary nutraceuticals · Enzymes in feed · Poultry feed · Swine feed · Phytase · Glucanases · Amylases · Proteases

1 Introduction

Enzymes as feed additives for food production animals are biologically active proteins that facilitate chemical breakdown of nutrients to smaller compounds for further digestion and absorption (Thacker 2013). Enzymes added to feed are broken down in the digestive tract in the same way as other proteins. Enzymes are biologically active proteins that break specific chemical bonds to release nutrients for further digestion and absorption. The enzymes used in animal feed are commonly produced by bacteria (i.e., *Bacillus subtilis*), fungus (i.e., *Trichoderma reesei*, *Aspergillus niger*), or yeast (*Saccharomyces cerevisiae*). The potential benefits of various exogenous feed enzymes, derived from microbes (bacteria and fungi) through traditional submerged liquid fermentation or solid-state fermentation, have been used in poultry and swine feeds for the past several years, and their value for improving nutrient digestion and growth, and efficiency of utilization of dietary components (i.e., protein, amino acids, starch, lipids, energy) in feedstuffs, are well documented (Woyengo and Nyachoti 2011). The potential for use of enzymes in feed, as alternatives to antibiotics, to improve performance in poultry and swine is significant.

Enzymes extracted from microorganisms are of great importance in the manufacturing of animal feed. Currently, new molecular techniques are used to discover microbial enzymes that are used in animal feeds to improve their quality.

A number of efficacy trials indicate that the adverse consequences of discontinuance of the use of antimicrobial growth promoters (AGPs) could be compensated to some extent by administering enzymes to facilitate the digestion of polysaccharides. Specific enzymes added to the diet are able to depolymerize these polysaccharides. A variety of enzymes are obtained from plants, bacteria, and fungi but there is also a wide variety of enzymes marketed commercially for poultry feed additives, many of which are produced as recombinant proteins in yeast commercially and sold as a lysate. In poultry, feed enzymes generally increase its digestibility, which could be particularly beneficial to young animals; thus, young birds may benefit from a wide spectrum of enzymes, such as lipase, proteases, and amylases. The benefits of enzymes generally diminish with age category, and responses are usually greater during the broiler chicken

A. Anadón (✉) · I. Ares · M. R. Martínez-Larrañaga · M. A. Martínez
Universidad Complutense de Madrid, Madrid, Spain
e-mail: anadon@vet.ucm.es

R. C. Gupta et al. (eds.), *Nutraceuticals in Veterinary Medicine*, https://doi.org/10.1007/978-3-030-04624-8_21

starter phase compared with the finisher phase. The aim of adding enzymes to poultry feed is to improve performance and profitability through enhanced digestion of dietary components. In attempts to reduce the ecological footprint, enzymes as proteases are an interesting option; the enzymes allows for more effective improvements to nutritional management, thus mitigating the risks and particularly in the "post-antibiotic environment." The different classes of feed enzymes commonly employed include phytase, carbohydrases in maize-based diets, xylanase (from *Trichoderma longibrachiatum*), cellulose (from *Aspergillus niger* and *Trichoderma longibrachiatum*), α-galactosidase, β-mannanase, α-amylase (from *Aspergillus oryzae*, *Bacillus amyloliquefaciens*, *Bacillus subtilis*) and pectinase, β-glucanase (from *Aspergillus niger*), proteases, and lipases. The most common reasons for enzyme supplementation include degrading feed components resistant to endogenous enzymes (i.e., β-glucanase, xylanase, mannanase, pectinase, galactosidase), inactivating ANFs (i.e., phytase), and supplementing endogenous enzymes that may be present in insufficient amounts (i.e., proteases, lipases, amylases) (Thacker 2013). It is thought that xylanases in wheat-based diets can improve the performance of chickens by initiating the breakdown of pentosans into smaller compounds and as a result reducing their viscosity properties.

Many other enzyme classes can significantly improve the utilization of feed such as xylanases, β-glucanases, pectinases, amylases, and proteases. In this respect, endo-1,4-β-xylanase may improve the nutritive value of wheat-based diets for poultry by degrading dietary arabinoxylans (Beg et al. 2001). However, a response of broiler chickens to supplementation of wheat-based diets with exogenous endo-1,4-β-xylanase is not always observed. It is thought that endoxylanase improves the apparent metabolizable energy value and nutrient digestibility of wheat-based diets and reduces the variability of wheat.

Although there initially may be concerns over using recombinant DNA-produced enzymes as feed additives for food-producing animals, recombinant synthesized enzymes such as phytases and carbohydrases are commercially produced and sold as feed additives in monogastric food animal production (Adeola and Cowieson 2011). Proteases added to broiler chicken feed were reported to have a beneficial effect by increasing the feed conversion ratio (FCR) and lowering levels of *Clostridium perfringens* in the ileum.

Enzymes are highly effective biological catalysts capable of accelerating chemical reactions millions of times over, acting on specific substrates or reactants. Chemically, they are proteins with a highly complex three-dimensional molecular structure. The protein nature of enzymes has important implications for their stability during high-temperature feed manufacture and transit through the gastrointestinal tract (GIT). As proteins, they can be denatured by heat and pH and they can also be subject to proteolysis by digestive enzymes. Enzymes are affected by the conditions of their surroundings (e.g., water concentration, temperature, pH) with respect to enzyme activity as well as stability.

The role of feed enzymes in improving the productive value of diets for single-stomached animals is based on several modes of action (Adeola and Cowieson 2011; Slominski 2011), namely, (1) hydrolysis of specific chemical bonds in feedstuffs that are not sufficiently degraded or indeed not at all by the animal's own enzymes (e.g., mixed salts of phytic acid); (2) elimination of the nutrient-encapsulating effect of the cell wall polysaccharides and therefore increased availability of starches, amino acids, and minerals; (3) breakdown of anti-nutritional factors (ANFs) that are present in many feed ingredients [e.g., non-starch polysaccharides (NSP) and phytic acid]; (4) solubilization of insoluble NSP for more effective hindgut fermentation and thus improved overall energy utilization; and (5) complementation of the enzymes (e.g., amylase, lipase, protease) produced by young animals in which, because of the immaturity of their own digestive system, endogenous enzyme production may be inadequate.

A unique feature of enzymes is their high substrate specificity. Each enzyme breaks down onto highly specific substrates at specific reaction sites. Thus, to achieve maximal benefits from enzyme addition, it is necessary to ensure that the enzymes are chosen on the basis of substrates in the ingredients used in feed formulations. Moreover, the enzymes need to meet several reaction conditions to act (Adeola and Cowieson 2011; Ravindran 2013):

1. *Moisture content*. Enzymes require an aqueous environment to initiate their activity; thus, moisture may be essential for the mobility of the enzyme, solubility of the substrate and enzyme, or both (Ravindran 2013). When ingested feed contains exogenous enzyme, the condition for humidity is quickly met and feed moves down the digestive tract (Svihus 2011).
2. *Temperature*. In general, enzyme activity increases up to 40 °C and then sharply decays because of the loss of structure through denaturing, which renders the enzyme inactive (Ravindran 2013). For example, in the bird body most enzymes are active in a range between 40 °C and 60 °C without being a limiting factor.
3. *pH*. In general, the most exogenous enzymes have an optimum pH of 4–6 (Ravindran 2013), but variation can appear between different enzyme sources, which may result in catalytic activity at both lower and higher pH. In poultry, the exogenous enzymes are active and degrade their substrates in the forestomach (i.e., crop, proventriculus, or glandular stomach, and gizzard or muscular stomach) before they are exposed to hydrolysis by endogenous proteolytic enzymes in the proventriculus site of acid secretion (Selle and Ravindran 2007).

Table 1 Average transit time and pH in different segments of the digestive tract of broiler chickens

Segment	pH	Transit time (min)
Crop	5.5	10–50
Proventriculus and gizzard	2.5–3.5	30–90
Duodenum	5–6	5–10
Jejunum	6.5–7.0	20–30
Ileum	7.0–7.5	50–70
Caecum/colon	8.0	20–30

The pH condition becomes the first physiological limitation for the activity and stability enzymes. The second limitation is the short interval between feed ingestion and the time to reach the lower ileum. The feed pH is typically close to neutral, the crop of the chicken is mildly acidic, the proventriculus and gizzard are acidic, and the intestine is mildly acidic at the proximal end, becoming mildly alkaline to neutral towards the distal part (see Table 1). Acid secretion of chickens is high relative to mammals, possibly because of the rapid digestive transit time. Under discontinuous feeding systems, the role of the crop of poultry resembles a food storage organ, not being essential for digestion, whereas in continuous feeding systems this function does not exist. A less-developed gizzard serves as a transit organ rather than a grinding organ, with implications for reduced retention time. There is evidence that meal feeding, instead of ad libitum access to feed, may markedly increase the retention time in the crop, together with a rapid moisturization and a reduction in pH (i.e., pH between 4 and 5). In the pig, the stomach pH is usually between 2 and 3.5, so that the enzymes have been developed to resist over a broad pH range, and exhibit thermostability, that is, are resistant to endogenous pepsin and trypsin, and viable under simulated gastric conditions (Thacker 2013).

4. *Enzyme concentration*. In theory, the reaction rate is directly proportional to enzyme concentration and increases when enhancing the concentration of the enzyme, because more available active sites exist, and this will continue until no more enzyme–substrate complex can be formed. In practice, however, because of other limitations within the digestive tract of animals, this linear relationship does not occur (Ravindran 2013).
5. *Substrate concentration*. In the presence of adequate enzyme concentrations, the reaction rate increases with augmenting substrate concentration until the maximum turnover is reached. The enzymes considerably vary with the reaction conditions needed and depend on their source (fungal vs. bacterial vs. yeast). Therefore, the source has a major influence on how closely particular enzymes are adapted to the prevalent conditions in the digestive tract and their effectiveness (Ravindran 2013).

Table 2 The effectiveness of a feed enzyme in the digestive tract of a bird

Prerequisites	
Enzymes	Source, specific catalytic activity, resistance to pepsin proteolytic action
Substrate characteristics	Concentration and accessibility
Digestive tract conditions	Moisture content, pH, temperature, and the time digesta remains in the tract, especially in the early gastric phase where most of the enzyme action occurs

The effectiveness of a feed enzyme in the digestive tract of a bird depends on several prerequisites (Table 2). An aqueous environment is needed for the enzymes to initiate their activity.

2 Feed Enzymes and Gastrointestinal Microbiota

Efficacy in animal feed applications depends very much on a different set of criteria based on the mechanism of action in animal nutrition (Bedford and Schulze 1998). The interaction between feed enzymes and the GIT microbiota/microbiome can be better understood from two points of view. On one hand are the effects of substrates by themselves on the digesta biochemical characteristics and GIT physiology (i.e., increased viscosity of soluble NSP, increased digesta transit time of soluble NSP, increased endogenous secretion and cell turnover (e.g., mucus), increased undigested fat, proteins, and starches, and on the other hand is the modification of these effects by feed enzymes [decreased viscosity, decreased transit time (NSPases), decreased endogenous secretion (NSPases, phytase), decreased undigested fat, protein, and starches (NSPases, phytase, amylase, proteases)] to the extent that the substrates are degraded or modified in the GIT (Kiarie et al. 2013), which explains feed enzymes acting on specific components of feed ingredients,which in most cases explicates the role of feed enzymes in modulating the gut microbiota.

Viscosity reduction can be effectively achieved with a single enzyme provided that it is active against the backbone of the polymer and not against a side chain. Because viscosity is, in part, a function of chain length, it is not necessary to degrade the polymer fully; relatively few breaks in the chain will substantially reduce or destroy a gel-forming capacity. Thus, problems, such as sticky droppings, which relate solely to gelation are relatively simple to alleviate with any enzyme preparation containing the necessary activity. For example, the problems of wet droppings associated with oats and rye-based diets to broiler chickens can be overcome by

inclusion of enzyme preparations rich in β-glucanase and endo-β-xylanase (pentosanase) activity, respectively.

The latest endo-β-xylanase improves litter quality as well. In conclusion, a close relationship exists between viscosity data obtained in vitro by extraction of soluble polysaccharide and those ANFs properties that can be reduced or eradicated by enzyme treatment (Annison 1991). The viscosity aspect is important because the rationale for the development and application of feed enzymes is to target certain dietary substrates (i.e., phytate and NSP) that are not degraded sufficiently or indeed not at all by the endogenous digestive enzyme array in the GIT. For instance, an accepted paradigm in broiler chickens is that increased intestinal viscosity from soluble NSP as indicated previously is the most important cause of for poor growth and feed utilization. Consequently, it has been suggested that feed enzymes might influence the intestinal microbiota through two main mechanisms: reducing the undigested substrates; and creating (in situ) short-chain oligosaccharides from cell wall NSP with potential prebiotic effects (Kiarie et al. 2013).

For feed digestion by enzymes, there is, however, a physiological limit to the extent that enzymes can improve digestion, and these barriers relate to pH and retention time within the GIT. The use of exogenous feed enzymes in poultry diets is becoming a norm to overcome the adverse effects of anti-nutritional factors (ANFs) that are present in plant-based feedstuffs such as phytic acid, NSP, and cell wall complex carbohydrates, and to improve digestion of dietary components and bird performance. The cell walls of cereal grains, legumes, and oilseed meals are made up of complex carbohydrates commonly referred to as NSP (Choct 2009). NSP are a complex group of components differing widely in chemical composition, physical properties, and physiological activity. NSP consist of a wide range of polymers including (hemi)cellulose, pectins, β-glucans (consisting of either a more soluble or a nonsoluble fraction), α-galactosides (raffinose, stachnyose, verbascose), and xylans (Thacker 2013). Many NSP have negative effects on growth and performance and reduce the nutritional value of feed ingredients in a number of ways: (1) they are indigestible by mammalian enzymes and therefore dilute the energy and nutrient content of the feed, and (2) NSP exhibit a so-called cage effect whereby normally highly digestible nutrients such as starch, fat, and protein are entrapped in a coating of NSP that prevent access of the endogenous enzymes to these substrates (Metzler et al. 2005). Further, certain NSP may increase intestinal viscosity. The anti-nutritive effects exerted by NSP are complex, but their viscous nature is considered a primary cause for their anti-nutritive effect in poultry. The increased bulk and viscosity of the intestinal contents decrease the rate of diffusion of substrates and digestive enzymes and hinder their effective interaction at the mucosal surface (Choct et al. 1996). In addition to the direct effect of viscous NSPs on gut physiology and morphology, there appear to be some indirect effects that have important implications for the efficient use of nutrients by the chicken (Dänicke et al. 1999). One such indirect effect may be related to stimulation of fermentation of NSPs by the gut microbiota, leading to volatile fatty acid (VFA) production in the small intestine. On a NSP-rich diet, the VFA concentration increases, mainly in the distal ileal lumen, because of excess fermentation combined with a proliferation of the fermentative microflora with a rather limited effect on the activity of the hindgut microbiota (Choct et al. 1999). Fermentation in the small intestine indicates competition with the host for digestible nutrients. Dietary NSP enzymes reduce the viscosity of the digesta in the small intestine, so that digesta passage and nutrient digestion rate increase, allowing less substrate and less time for the fermentation organisms to proliferate. This change may restore the normal and efficient endogenous enzymatic digestion of nutrients in the small intestine. The enzymes partially counterbalance the adverse effects of soluble NSP on performance (Bedford and Classen 1992). Additionally, NSP-degrading enzymes will also reduce the proliferation of pathogenic bacteria such as *Clostridium perfringens* (Jackson et al. 2003). Presently, all broiler chicken feed contains enzymes such as xylanases and β-glucanases that break down NSPs. Different cereal types contain variable NSP levels with concomitant differences in chemical composition. For example, maize contains almost exclusively insoluble NSPs, whereas wheat and barley contain NSPs of which the ratio of soluble to insoluble is about 1:6. This ratio is about 3:4 in rye, making this cereal one with particularly high levels of soluble NSPs (Choct 2002). Diet formulation has to be adjusted and conditions have to be created to ensure maximum response to added enzymes.

3 Mode of Action for Enzymes

It must be recognized that different feed enzymes (Table 1) have nonidentical modes of action. Despite their increasing acceptance as feed additives, the exact mode(s) of action of feed enzymes remains to be elucidated. The possible mechanisms of action of in-feed enzymes include the following: (1) increase in the digestibility of nutrients that are otherwise not degraded by host enzymes (e.g., phytic acid); (2) elimination of the nutrient-encapsulating effect of cell wall polysaccharides and increase in the availability of starches, amino acids, and minerals; (3) inactivation of anti-nutritional factors (e.g., phytic acid or soluble NSP) and reduced intestinal viscosity; (4) increase in the solubility of nonsoluble NSP and promotion of caecal fermentation; and (5) supplementation of endogenous enzymes that may occur in insufficient amounts, especially in young animals in which the digestive system is not fully developed (Choct 2009;

Kiarie et al. 2013). In addition to the enzymes, effects have been observed on nutrient digestibility; they are also thought to influence the composition of the gut microbiota. The enzyme-induced microbiota changes are mostly indirect and are thought to be mediated by two main mechanisms: (1) reducing the undigested substrates and (2) generating short-chain oligosaccharides from cell wall NSP with potential prebiotic effects (Bedford 2000; Bedford and Cowieson 2012; Kiarie et al. 2013). These mechanisms influence the nutrient supply and intestinal environment, thus altering selection pressures on bacterial species (Bedford and Cowieson 2012; Cheng et al. 2014).

The general consensus is that one or more of the following mechanisms are responsible for the observed benefits (Bedford and Partridge 2011): (1) degradation of specific bonds in ingredients that are not usually hydrolyzed by endogenous digestive enzymes; (2) degradation of anti-nutritional factors that limit nutrient digestion directly, increase intestinal digesta viscosity indirectly, or both; (3) disruption of endosperm integrity and release of nutrients that are bound to or entrapped by the cell wall; (4) shift of digestion to more efficient digestion sites; (5) reductions in endogenous secretions and protein losses from the gut, resulting in reduced maintenance requirements (Cowieson et al. 2009); (6) reduction in the weight of the intestinal tract and changes in intestinal morphology (Jaroni et al. 1999); and (7) changes in the microflora profile in the small intestine. The proven value of the addition of enzyme preparations, capable of hydrolyzing the mixed-linked β-glucan content of barley endosperm walls (part of a storage structure) to poultry diets containing barley (classed as a low-energy cereal) has stimulated interest in both the mechanism underlying the productive response and the application of enzymes to other cereals as an important component of farm animal diets. Cell walls having a structural function are intrinsically more resistant to degradation.

β-Glucanase alone is sufficient to disrupt barley endosperm walls: multi-enzyme preparations containing high levels of enzymes active against cellulose and arabinoxylan are required to maximize the release of protein from the aleurone layer (Mulder et al. 1991). The endosperm walls of maize, triticale, and wheat also contain arabinoxylans and glucans. The disruption of intact walls and release of entrapped nutrients is the major factor in any improvement in nutritive value ascribed to exogenous enzymes. β-Glucan and arabinoxylans, which, in varying proportions, form the endosperm wall of all cereals, act to restrict access to nutrients found in the endosperm. For that reason, enzyme additions in both young pigs and poultry support the digestibility values for fat, starch, and nitrogen.

As enzymes influence the amounts and form of substrate present within the gut, their use has a direct effect on the bacteria that make up the microfloral populations (Apajalahti et al. 2004; Vahjen et al. 1998); alteration of gut flora towards favourable bacterial species, which improves gut health, provides a protective effect on the overall health of the animal that arises, in part, from the influence of flora on immune function. Augmentation of endogenous digestive enzymes, which are either insufficient or absent in the bird, results in improved digestion, especially in newly hatched chicks with immature digestive systems.

4 Dietary Substrates and Enzyme Types

In birds, substrates in feed ingredients can be mainly classified into three groups: (1) substrates for which suitable enzymes are produced in their own digestive tract (e.g., starch, proteins, lipids) [some of these incomplete endogenous enzymes (i.e., 10–20%) of these substrates are not digested and are excreted]; (2) substrates for which enzymes are not produced by the bird and not digested (e.g., cellulose); and (3) substrates for which enzymes are not produced by the bird and, in addition not being digested, have anti-nutritive effects (e.g., β-glucans, pentosans, phytate). Pentosans (arabinoxylans) in wheat have an anti-nutritive activity in broiler chickens. In feed ingredients, the substrates (nutrients and antinutrients) exist as complexes, limiting the accessibility to enzymes (Ravindran 2013).

Despite advances, the chemistry and structure of most target substrates in feed ingredients are still poorly defined. It is known that the chemistry of NSP in different ingredients varies widely. Although basic quantitative information on the type of sugars making up the NSP is available for cereals, corresponding information for other ingredients is lacking. The high NSP and indigestible protein contents in cereal co-products can limit their inclusion in pig feed; however, supplemental NSP-degrading enzymes and proteases might allow high inclusion of such feedstuffs. The efficacy of enzymes can be greatly improved if the chemistry of the target substrates is more precisely understood. Matching an enzyme, however, does not guarantee the efficacy of the enzyme in degrading the substrate, and affinity must also be considered. Using three fungal xylanases (xylanase A derived from *Thermomyces lanuginosus*, xylanase B from *Humicola insolens*, and xylanase C from *Aspergillus aculeatus*), of different substrate affinities, showed that substrate specificity is dependent on the source of the enzyme. It may be concluded that wheats with low or normal metabolizable energy values vary in their responses to xylanase supplementation (Choct et al. 2004).

For an enzyme to be effective, an adequate enzyme-to-substrate ratio must be present in the diet. A complicating factor is that a particular substrate in one ingredient is not exactly the same as the one found in another ingredient. The substrates differ and the same substrate in different

Table 3 Type of commercial feed enzymes and target substrates

Enzyme	Target substrate	Target feedstuff
Phytases	Phytic acid	All plant-derived ingredients
β-Glucanases	β-Glucan	Barley, oats, and rye
Xylanases (carbohydrase)	Arabinoxylans	Wheat, rye, triticale, barley, fibrous plant materials
α-Galactosidases	Oligosaccharides	Soybean meal, grain legumes
Proteases	Proteins	All plant protein sources
α-Amylase	Starch	Cereal grains, grain legumes
Lipases	Lipids	Lipids in feed ingredients
β-Mannanases, cellulases (carbohydrase), hemicellulases, pectinases	Cell wall matrix (fiber components)	Plant-derived ingredients, fibrous plant materials

ingredients may respond differently to the enzyme. Such differences arise from the location of the substrate in the ingredient matrix, the presence of other limiting factors, and differences in accessibility or solubility. In the case of phytate it was shown that phytates from different ingredients are not similarly susceptible to dephosphorylation and that the reactive, and not total, phytate content is critical in determining the responses to supplemental phytase (Leske and Coon 1999). It was found, for example, that canola meal contained a relatively high level of total phytate, but a less reactive phytate, and does not respond well to added phytase (Ravindran 2013).

Feed enzymes are actually the most researched feed additive. The current usage of feed enzymes is very broad, given the number of feed enzymes (Table 3) and, within each enzyme, a large number of commercial products is available, varying in their source, enzyme activities, and characteristics (Ravindran 2013). Not surprising is the success of enzyme addition to broiler chicken diet containing barley, which has stimulated interest in the application of feed enzymes to other diets and diet ingredients, notably wheat and soybean.

Currently, four distinct categories of enzyme products are commercially available for use by the feed industry: (1) microbial phytases; (2) glycanases or glycan hydrolases (xylanases, β-glucanases) targeting viscous cereals (e.g., wheat, barley) (these enzymes degrade polysaccharides and show little or no activity against oligosaccharides); (3) enzymes targeting nonviscous cereals (e.g., corn, sorghum); and (4) enzymes targeting non-cereals (e.g., soybean meal, grain legumes). With the exception of microbial phytases, most other enzyme products contain a mixture of enzymes that may be produced by one or more organisms (Cowieson et al. 2006; Selle and Ravindran 2007). The most widely used feed enzymes are a mixture of a variety of glycanases, and the single-use degrading enzyme is phytase (Ravindran 2013). There is also evidence to suggest that preparations with multiple enzyme activities may provide a competitive strategy to improve nutrient utilization in poultry diets (Selle and Ravindran 2007). The combined application of enzymes may result in additive, sub-additive, or synergistic effects on nutrient utilization and animal performance (Ravindran et al. 1999; Juanpere et al. 2005). Such enzyme combinations, rather than pure single enzymes, represent the next generation of feed enzymes, because feed ingredients are exceedingly structurally complex. In the native stage, nutrients in raw materials are not isolated entities, but exist as complexes with various linkages to protein, fat, fiber, and other complex carbohydrates. For example, in wheat-based diets, merely targeting the arabinoxylans with xylanases may not provide full benefits (Ravindran 2013).

The benefits of simultaneous inclusions of a carbohydrase enzyme with predominantly xylanase activity and a microbial phytase in wheat-based broiler chicken diets, in terms of both protein and energy utilization and growth performance, have been reported (Ravindran et al. 1999; Zyla et al. 1999; Selle et al. 2009). It appears that the activity of one type of feed enzyme may be facilitated by the other, possibly in a reciprocal fashion, by providing greater substrate access, and also by reducing the anti-nutritive effects of the substrates and phytate) on nutrient utilization. It is thought that these anti-nutritional effects are manifested by depression in performance and wet droppings, connected with the high viscosity of NSPs. The simultaneous inclusion of phytase with α-galactosidases, protease, β-glucanase, and xylanase in corn-, barley-, or wheat-based diets has been investigated: it was found that the phytase in combination with carbohydrase and protease had additive effects in nutritionally marginal broiler chicken diets (Cowieson and Adeola 2005; Juanpere et al. 2005).

In this context, various enzymes, derived from microbes (bacteria and fungi) through fermentation, have been used in swine and poultry feeds for the past several years, and their value in enhancing growth and feed efficiency is widely known. The diverse classes of enzymes commonly employed include phytase, carbohydrases, and proteases. The enzymes do not directly attack bacteria, but only reduce substrates for the growth of bacteria.

5 Main Enzymes

5.1 Phytase

Phosphorus is the third most expensive nutrient in diets for non-ruminants; however, the majority (>65%) of the phosphorus in feedstuffs of plant origin is bound in mixed salts of phytic acids and is unavailable to the animal without

enzymatic dephosphorylation (Kiarie and Nyachoti 2009). The plant phosphate, to be unavailable to the animals, acts as an ANF by forming complexes with minerals such as calcium, zinc, and iron. Phytase, an activity derived from *Aspergillus ficuum*, is able to release phosphate from phytic acid (hexa-phosphorylated myoinositol). The requisite enzyme to hydrolyze phytic acids is insufficient in avian and mammalian pancreatic and intestinal secretions, present in some feedstuffs, and ubiquitous in microbial systems (Selle and Ravindran 2007, 2008). Phytic acid is the major storage form of phosphorus in plant seeds and is particularly abundant in cereals, where it occurs as crystals associated with protein bodies found principally in the aleurone layer (Graf 1986). Approximately two thirds of the phosphorus in feeds for pigs and poultry is present as phytic acid, which is unavailable to non-ruminants. So, monogastric animals (i.e., swine and poultry) poorly utilize the phosphorus present in phytate as they have little to no endogenous phytase activity. The application of phytase, either as a pre-treatment or as a feed supplement, allows diets to be formulated with substantially less supplemental inorganic phosphorus and reduces the discharge of faecal phosphorus to the environment. Consequently, to provide adequate phosphorus to non-ruminant farm animals it is necessary to include feedstuffs with high phosphorus bioavailability such as inorganic supplements (e.g., dicalcium phosphate) or animal-based feedstuffs (e.g., meat and bone meal) in the diet. It is known phytase has significant effects on the digestibility of calcium, phosphorus, and minerals as well as the intestinal mucin production and endogenous losses, all of which influence the nutrient supply and the intestinal environment, which will alter the selection pressure on bacterial species (Bedford and Cowieson 2012).

Nowadays, phytase dominates the market of feed enzymes and rapid growth has been associated with the acceptance of phytase in replacing inorganic phosphates (Kiarie et al. 2013). The use of phytases in improving phosphorus availability from plant feed ingredients is very well known in animal nutrition. A simple example is dietary phosphorus level and microbial phytases; if the diets contain excess amounts of phosphorus, then the chance of any animal response to phytase addition will be low. The use of microbial phytase is potentially useful and appropriate only for diets with suboptimal phosphorus levels and containing significant levels of plant-derived ingredients. The phytase feed additive is used to increase phosphorus absorption, to digest more than 30% more phosphate in its ratios, by pigs. Less of the mineral is therefore required in feed and less is wasted, thus reducing the emission of mineral phosphate in slurry and easing the burden in groundwater. It also offers substantial saving for natural resources of mineral phosphate. Phytase in pig rations can cleave phosphate output in manure and increase the digestion efficiency for phosphorus and other minerals in the diet including calcium, magnesium, and zinc.

The pollution potential from too high levels of phosphate in pig and poultry manure begins with the way in which phosphate is presented in most vegetable feed materials as phytate-phosphorus. This phytate-phosphorus can only be broken down for digestion through the activity of phytase enzymes.

These enzymes can be found in the stomachs of ruminants such as cows where they are naturally produced by microorganisms. But, in monogastric animals such as pigs and poultry, the enzymes are not present, or present only in very small amounts. A feeding pig on conventional rations excretes more than 60% of the phosphorus present in its feed and a breeding sow some 85%. When fed to growing slaughter pigs at the rate of about 100 g per tonne of feed mix, this phytase only offers around 33% reduction in phosphate excretion. Because it promotes more efficient digestion of dietary phosphorus, any supplementation of mineral phosphorus in feed can be reduced.

The addition of phytase enzymes to animal diets has the potential to improve the phytic acid-phosphorus utilization by the animal. Phytase enzymes catalyze the hydrolysis of feed grain and fiber phytic acid to myoinositol and phosphorus and thereby allow reduction in mineral phosphate supplementation and excretion. A wide variety of techniques follow before the phytase product is ready: screening, characterization, protein engineering, genetic modification, genomics, expression technology, fermentation, and testing. The next generation of the products is even better because they are more stable and less excess phosphate is released into the environment.

5.2 Carbohydrases

Carbohydrases include all enzymes that catalyze a reduction in the molecular weight of polymeric carbohydrate, but more than 80% of the global carbohydrase market is accounted for by xylanase and β-glucanase (Adeola and Cowieson 2011). Other commercially available carbohydrases include α-amylase, cellulose, β-mannanase, α-galactosidase, and pectinase. These carbohydrases have widespread application in the poultry industry but are used less commonly in feeds for swine.

The effect of carbohydrase supplementation on the performance of pigs is contradictory (Thacker 2013). Although positive effects on performance are observed, they are commonly associated with increases in nutrient digestibility, probably a result of increased accessibility of endogenous enzymes to nutrients as a result of inhibition of the "cage effect" as well as hydrolysis or partial hydrolysis of the NSP.

Hydrolysis of NSP results in increased sugar release in the large and small intestine and thereby stimulates the growth of lactobacilli, which produce lactic acid. Increased proportions of lactic acid promote gut health by suppressing the growth of coliforms such as pathogenic *E. coli*.

It is known that the response of pigs to carbohydrase supplementation is less consistent than has been observed with poultry, differences resulting from the physiology of the pig and the chicken. One clear difference is the pH in the gut. In the pig, the duration that feed is exposed to a low pH is significantly longer than in the chicken (Baas and Thacker 1996). It is possible that exposure to low pH in the stomach of the pig is either partially or totally denaturing the enzyme, accounting for the lower magnitude of responses obtained when carbohydrases are fed to pigs compared with poultry (Thacker 2013).

5.3 Proteases

Proteases have the capacity of breaking down proteins. The use of proteases in feed has not been analyzed as extensively as other enzyme classes, and in many cases the proteases investigated have been present as part of an enzyme mixture (e.g., *Bacillus* wild-type fermentations). Monogastric animals, such as swine and poultry, produce digestive proteases, for example, pepsin, trypsin, chymotrypsin, and carboxypeptidases, which digest feed proteins to a greater extent. Nevertheless, a fraction of the ingested feed protein is excreted in the faeces, representing an opportunity for an exogenous protease to improve the utilization of protein in chickens for fattening (Glitsoe et al. 2015). Protease reduces the proportion of indigestible amino acids in feedstuffs, resulting in a corresponding increase in digestible amino acids. Generally, the more indigestible the level of amino acid present, the greater the increase in digestibility when proteases are added. The greater the level of undigested protein, as measured by the level of digestibility of amino acids, the more variability a particular feedstuff will exhibit.

Protease is a very modern feed additive that supports better nutrition, health, and environmental conditions in pig production, which are closely interrelated through the mode of action of protease. The use of protease can contribute significantly to reduce nitrogen emissions during livestock production. Improved gut health, for example, also reduces the requirement for amino acids and energy. Nutritional benefits are based firmly on digestible amino acids, in terms of both animal requirements and availability from feedstuffs. Health benefits are linked to the potential to reduce dietary protein levels and therefore reduce the level of undigested proteins in the hindgut. Excess protein is a fermentation substrate that may produce breakdown products such as phenols, amines, ammonia, and indoles that in turn increase the pH, which are all undesirable outcomes. Likewise, anti-nutritional factors (ANFs), such as trypsin inhibitors, might set up conditions to predispose the growth of *Clostridium perfringens.* Higher ammonia levels in animal buildings could predispose animals to risks of respiratory disease.

Protease added to broiler chicken feed was reported to have a beneficial effect by increasing the FCR and lowering levels of *Clostridium perfringens* in the ileum. There are a wide variety of enzymes for poultry feed additives, many of which are produced as recombinant proteins in yeast used as a lysate. Production of enzymes by *Pichia pastoris* can serve as a potential source for biochemical or animal feed studies (Johnson et al. 2010), and dietary use of encapsulated lysozyme (Zhong and Jin 2009) as a feed additive in the diet of chickens significantly reduced the concentration of *Clostridium perfringens* and gastrointestinal lesions because of the organism in the ileum (Liu et al. 2012).

Abrupt piglet weaning around the fourth week of life causes a dramatic fall in the output of amylase and a significant check in the production of protease, whether measured as activity per unit pancreas weight or on a total pancreas basis (Lindemann et al. 1986). The weaned pig is ill prepared to digest the starch and protein found in a cereal-based weaner diet and could benefit from enzyme augmentation immediately after weaning (Owsley et al. 1986). However, recovery of endogenous enzyme production is rapid and the benefit of enzyme supplementation is likely to be short lived.

At weaning, the transition from highly digestible to less digestible sources of protein is worsened by a decrease in secretion of endogenous protease and changes in gut lining morphology. These changes include the reduction of villus height and crypt hyperplasia, which results in impaired absorption of nutrients and gut health issues. Therefore, additive protease supplementation can help piglets to digest proteins, which reduces the amount of undigested protein reaching the hindgut. Feeding exogenous protease additive to piglets can improve gut morphology when compared to nonsupplemented piglets. Thus, the integrity of tight junctions can be preserved, leading to a reduction in post-weaning mortality. By minimizing the levels of nondigested protein, protease enzyme also establishes the conditions for a healthier gut, reduced nitrogenous excretion, and lower protein diets, all of which have the potential to improve performance and reduce feed and production costs (Aehle 2007). This functionality is unique to protease, which is why it has been called the “next amino acid.” The indirect nutritional effects of protease enzyme provide some further health benefits. The potential reduction in putrefaction protein in the hindgut lessens the risk of generation of a range of harmful metabolites, such as 3-methylindole and hydrogen sulfide. The protease supplementation resulted in a significant increase in the thickness of the adherent mucus layer in the

GIT of broiler chickens and promoted the ability to survive with a coccidiosis infection.

It was observed that supplemental protease increased gut tensile strength, villus height, and crypt depth, and reduced epithelial thickness and goblet cell numbers (Cowieson et al. 2009). These morphological changes are indicative of enhanced gut integrity and resilience and are suggestive of beneficial effects of protease that extend beyond increased amino acid recovery. The presence of protease has been shown to enhance the numbers of lactobacilli and reduce the levels of *Escherichia coli* in the colon in both high- and low-protein diets.

A life cycle analysis was carried out to determine the environmental impact of protease in the whole broiler chickens production chain using the life cycle assessment methodology described in ISO 1404017 and 1404418. The study showed significant benefits for all the environmental impacts that were considered. The most important was the potential to reduce water and air pollution with nitrous compounds, which can lead to eutrophication and acidification. The greatest effects were observed when the protease was used in diets to compensate for reduced protein content (Oxenboll et al. 2011).

Low-protein diets reduce disease risk. The use of protease allows confidence in achieving compatible performance from lower protein diets and, hence, supporting a better environment and reducing risk from respiratory disease produced by *Pasteurella multocida*; a lower-protein diet produces manure with a much lower ammonia, hydrogen sulfide, and odor content.

In pig farming, it has been proposed to use "precision feeding techniques" as an essential approach to improve the utilization of dietary nitrogen, phosphorus, and other nutrients, and also to reduce nutrient excretion. For example, addition of enzymes (e.g., phytases) improves the environmental traits in comparison with the traditional feed formulation. The enzyme feed addition can promote the sustainability of the improving the environmental sustainability of pork production, animal well-being, and meat product quality by reducing nutrient excretion in swine operations with small increases in feeding costs (Pomar et al. 2009). Using these "precision feeding techniques," pigs are individually fed with a daily adjusted diet that can improve dietary nutrient efficiency. The estimated feeding costs can be reduced in an amount higher than 4.6%, and nitrogen and phosphorus excretion can both be reduced more than 38% (Pomar et al. 2009).

The role of protease, therefore, becomes increasingly important in improving the availability of multiple essential amino acids from feedstuffs. An additional benefit of protease is in reducing the negative effects of ANFs, such as trypsin inhibitors, on the efficiency of production. A further outcome is that gut mucin production may be reduced so that absorbed amino acids are used for useful protein building through lean gain or milk production and not for unnecessary production of intestinal mucin protein. Mucin production also uses energy that would otherwise be supporting protein building. Consequently, energy is used more efficiently, which makes this a doubly valuable result.

Protease additives reduce the proportion of indigestible amino acids in feedstuffs, resulting in a corresponding increase in digestible amino acids. Generally, the more indigestible level of amino acids present, the greater the increase in digestibility when proteases are added. A meta-analysis revealed that the improvement of amino acid digestibility was about 10% when the overall digestibility in a control diet was less than 70%. As the overall digestibility of the control diet improved, opportunity for the benefit from protease was reduced. At 90% dietary digestibility, the average response from protease was about 2% (Cowieson and Ross 2014). The apparent metabolizable energy was significantly increased by 49 kcal/kg and fat digestibility was improved by 1%. It is worth noting that the inherent digestibility in the control diet explained approximately 047% of the variance in the response.

Previously, following in vitro addition of protease, higher hydrolysis of allergenic proteins, glycinin, and β-conglycinin from soybean meal has been shown (Wang et al. 2004). It is important to note that nutrient levels in a diet affect the response to protease and that the best responses appear to occur when there are higher levels of indigestible protein and soy in the diet. Therefore, response to protease may be more consistent in the presence of phytase and xylanase. Furthermore, enzyme interactions with a corn source indicated the influence of extractable salt-soluble protein content of corn on accessibility to protein and starch. The magnitude of phytase responses was greater during the first week posthatch compared with broiler chickens at 4 weeks of age. Responses with exogenous enzymes cannot be assumed to be constant and are influenced by the source of corn used as well as the age of broiler chickens. One caveat with this particular study relates to the fact that between day 9 and 21 the birds were all fed the same enzyme treatments but a constant and different source of corn (Gehring et al. 2013).

5.4 Xylanase

Interestingly, xylanase added to a wheat-based diet alleviated the pathological effects of *Clostridium perfringens* in broiler chickens (Liu et al. 2012).

5.5 β-Galactomannans

The dietary inclusion of β-galactomannans has become a promising strategy to control and prevent intestinal infections. The effect of various β-galactomannan-rich products on intestinal morphology in chickens challenged with *Salmonella enteritidis* has been investigated, indicating the beneficial effects of these enzymes on intestinal morphology give more evidence of the positive effects of these supplements in poultry nutrition (Brufau et al. 2015).

6 Concluding Remarks and Future Directions

The potential for using enzymes in poultry diets to improve the nutritional value of feeds has been recognized for many years. The limitations on enzyme activity are imposed by the nature of the substrate and by the host animal. The scientific basis for enzyme addition is established for some feedstuffs although for others it is not. The β-glucanases and xylanases have been used as feed additives for more than 20 years and their ability to improve the FCR and weight gain of monogastric animals (poultry and pigs) has been demonstrated in numerous reports. The use of these feed enzymes has been restricted primarily to poultry and pigs where the majority of feed in intensive farming contains feed enzymes, although research focusing on supplemental enzymes for ruminants and fish, as well as fur and pet animals, has been also carried out during recent years. During the past years investigations into the mode of action of feed enzymes have continued rapidly, particularly in phytase research, where it is clear that the valuable benefit of this enzyme is not simply through the provision of phosphate, but also via the destruction of phytic acid, a potent anti-nutrient. Similarly, a better understanding between feed enzyme function and digestive physiology has positively influenced application recommendations for feed enzymes. The confirmation of feed enzymes reduces the environmental impacts of agriculture as a complex area needing consideration and specific studies. Feed enzymes are applied to improve feed utilization and their benefits include reduced feed cost, improved animal performance, and reduced environmental impact of animal production. Additionally, effects of feed enzymes on the microbiota or microbiome of the GIT need further research. Feed enzymes may cause an impact on GIT microbial ecology by reducing undigested substrates and anti-nutritive factors and producing oligosaccharides in situ from dietary NSP. Furthermore, investigations using specific enteric pathogen challenge models have demonstrated the efficacy of feed enzymes in modulating gut health. Because feed enzymes probably change the substrate characteristics along the GIT, subsequent microbiota responses will vary according to the populations present at the time of administration and their reaction to such changes. Nowadays, the role of major feed enzymes (for example, carbohydrases and phytase) on the gut health of poultry and swine species with a specific focus on the impact on GIT microbiota is of great interest. The application of genetic engineering in the process of enzyme production allows the development of enzymes targeted for specific purposes. Establishment of specific substrates (and therefore enzyme activity), substrate effects, factor affecting substrate importance, and the stability of enzyme cocktails are required to fully exploit this new engineering technology. However, before a feed enzyme can be put on the market, it must be submitted to a number of toxicological and safety examinations and fulfill the requirements of the registration procedures.

Acknowledgments This work was supported by Project S2013/ABI-2728 (ALIBIRD-CM Program) from Comunidad de Madrid, and by Project Ref. RTA2015-00010-C03-03 from Ministerio de Economía, Industria y Competitividad, Spain.

References

Adeola O, Cowieson AJ (2011) Opportunities and challenges in using exogenous enzymes to improve non-ruminant animal production. J Anim Sci 89:3189–3218

Aehle W (2007) Enzymes in industry: production and applications, 3rd edn. Wiley VCH, Weinheim. ISBN:978-3-527-31689-2

Annison G (1991) Relationship between levels of soluble nonstarch polysaccharides and the apparent metabolizable energy of wheats assayed in broiler chickens. J Agric Food Chem 39:1252–1256

Apajalahti J, Kettunen A, Graham H (2004) Characteristics of the gastrointestinal microbial communities, with special reference to chickens. World Poult Sci J 60:223–232

Baas TC, Thacker PA (1996) Impact of gastric pH on dietary enzyme activity and survivability in swine fed β-glucanase supplemented diets. Can J Anim Sci 76:245–252

Beg Q, Kappor M, Mahajan L et al (2001) Microbial xylanases and their industrial applications: a review. Appl Microbiol Biotechnol 56(3–4):326–338

Bedford M (2000) Removal of antibiotic growth promoters from poultry diets: implications and strategies to minimise subsequent problems. World Poult Sci J 56:347–365

Bedford MR, Classen HL (1992) Reduction of intestinal viscosity through manipulation of dietary rye and pentosanase concentration is effected through changes in the carbohydrate composition of the intestinal aqueous phase and results in improved growth rate and food conversion efficiency of broiler chicks. J Nutr 122:560–569

Bedford MR, Cowieson AJ (2012) Exogenous enzymes and their effects on intestinal microbiology. Anim Feed Sci Technol 173:76–85

Bedford MR, Partridge GG (2011) Enzymes in farm animal nutrition. CAB International, Wallingford

Bedford MR, Schulze H (1998) Exogenous enzymes for pigs and poultry. Nutr Res Rev 11:91–114

Brufau MT, Martín-Venegas R, Guerrero-Zamora AM et al (2015) Dietary β-galactomannans have beneficial effects on the intestinal morphology of chickens challenged with *Salmonella enterica* serovar *enteritidis*. J Anim Sci 93:238–246

Cheng G, Hao H, Xie S et al (2014) Antibiotic alternatives: the substitution of antibiotics in animal husbandry? Front Microbiol 5:217

Choct M (2002) Non-starch polysaccharides: effect on nutritive value. In: McNab JM, Boorman KN (eds) Poultry feedstuffs. CABI Publishing, New York

Choct M (2009) Managing gut health through nutrition. Br Poult Sci 50:9–15

Choct M, Hughes RJ, Wang J et al (1996) Increased small intestinal fermentation responsible for the anti-nutritive activity of non-starch polysaccharides in chickens. Br Poult Sci 37:609–621

Choct M, Hughes RJ, Bedford MR (1999) Effects of α-xylanase on individual bird variation, starch digestion throughout the intestine, and ileal and caecal volatile fatty acid production in chickens fed wheat. Br Poult Sci 40:419–422

Choct M, Kocher M, Waters DLE et al (2004) A comparison of three xylanases on the nutritive value of two wheats for broiler chickens. Br J Nutr 92:53–61

Cowieson AJ, Adeola O (2005) Carbohydrase protease and phytase have an additive beneficial effect in nutritionally marginal diets for broiler chicks. Br Poult Sci 84:1860–1867

Cowieson AJ, Ross FF (2014) Bioefficacy of a mono-component protease in the diets of pigs and poultry: a meta-analysis of effect on ileal amino acid digestibility. J Appl Anim Nutr 2:1–8

Cowieson AJ, Singh DN, Adeola O (2006) Prediction of ingredient quality and the effect of a combination of xylanase, amylase, protease and phytase in the diets of broiler chicks. 1. Growth performance and digestible nutrient intake. Br Poult Sci 47(4):477–489

Cowieson AJ, Bedford MR, Selle PH et al (2009) Phytate and microbial phytase: implications for endogenous nitrogen losses and nutrient availability. World Poult Sci J 65:401–418

Dänicke S, Vahjen W, Simon O et al (1999) Effects of dietary fat and xylanase supplementation to rye-based broiler diets on selected bacteria groups adhering to the intestinal epithelium, on transit time of feed, and on nutrient digestibility. Poult Sci 78:1292–1299

Gehring CK, Bedford MR, Dozier WA (2013) Interactive effects of phytase and xylanase supplementation with extractable salt-soluble protein content of corn in diets with adequate calcium and nonphytate phosphorus fed to broilers. Poult Sci 92(7):1858–1869

Glitsoe V, Ruckwbusch J-P, Knap I (2015) White paper. Innnovation in enzyme development. DSM, Health Nutrition Materials

Graf E (1986) Chemistry and applications of phytic acid. In: Graf E (ed) Phytic acid: chemistry and applications. Pilatus Press, Minneapolis, MN, pp 1–21

Jackson ME, Anderson DM, Hsiao HY et al (2003) Beneficial effect of β-mannanase feed enzyme on performance of chicks challenged with *Eimeria* sp. and *Clostridium perfringens*. Avian Dis 47:759–763

Jaroni D, Scheideler SE, Beck MM et al (1999) The effect of dietary wheat middlings and enzyme supplementation. II: Apparent nutrient digestibility, digestive tract size, guts viscosity, and gut morphology in two strains of Leghorn hens. Poult Sci 78:1664–1674

Johnson SC, Yang M, Murthy PP (2010) Heterologous expression and functional characterization of a plant alkaline phytase in *Pichia pastoris*. Protein Expr Purif 74:196–203

Juanpere J, Pere-Vendrell AM, Angulo E et al (2005) Assessment of potential interaction between phytase and glycosidase enzyme supplementation on nutrient digestibility in broilers. Poult Sci 84:571–580

Kiarie E, Nyachoti CM (2009) Bioavailability of calcium and phosphorous in feedstuffs for farm animals. In: Vitti DMSS, Kebreab E (eds) Phosphorus and calcium utilization and requirements in farm animals. CAB International Wallingford, pp 76–83

Kiarie E, Romero LF, Nyachoti CN (2013) The role of added feed enzymes in promoting gut health in swine and poultry. Nutr Res Rev 26:71–88

Leske KL, Coon CN (1999) A bioassay to determine the effect of phytase on phytate phosphorus hydrolysis and total phosphorus retention of feed ingredients as determined with broilers and laying hens. Poult Sci 78:1151–1157

Lindemann MD, Cornelius SG, El Kandelgy SM et al (1986) Effect of age, weaning and diet on digestive enzyme levels in the piglet. J Anim Sci 62:1298–1307

Liu D, Guo Y, Wang Z, Yuan J (2012) Exogenous lysozyme influences *Clostridium perfringens* colonization and intestinal barrier function in broiler chickens. Avian Pathol 39:17–24

Metzler B, Bauer B, Mosenthin R (2005) Microflora management in the gastrointestinal tract of piglets. Asian-Aust J Anim Sci 18:1353–1362

Mulder MM, Lomax JA, Hotten PM et al (1991) Digestion of wheat aleurone by commercial polysaccharidases. Anim Feed Sci Technol 32:185–192

Owsley WF, Orr DE, Tribble LF (1986) Effect of age and diet on the development of the pancreas and the synthesis and secretion of pancreatic enzymes in the young pig. J Anim Sci 63:497–504

Oxenboll KM, Pontoppidan K, Fru-Nij F (2011) Use of a protease in poultry feed offers promising environmental benefits. Int J Poult Sci 10(11):842–848

Pomar C, Hauschild L, Zhang G-H et al (2009) Applying precision feeding techniques in growing-finishing pig operations. Rev Bras Zootec 38:226–237

Ravindran V (2013) Feed enzymes: the science, practice, and metabolic realities. J Appl Poult Res 22:628–636

Ravindran V, Selle PH, Bryden WL (1999) Effects of phytase supplementation, individually and in combination, with glycanase on the nutritive value of wheat and barley. Poult Sci 78:1588–1595

Selle PH, Ravindran V (2007) Microbial phytase in poultry nutrition. Anim Feed Sci Technol 135:1–41

Selle PH, Ravindran V (2008) Phytate-degrading enzymes in pig nutrition. Livest Sci 113:99–122

Selle P, Ravindran V, Partridge GG (2009) Beneficial effects of xylanase and/or phytase inclusions on ileal amino acid digestibility, energy utilization, mineral retention and growth performance in wheat-based broiler diets. Anim Feed Sci Technol 53:303–313

Slominski BA (2011) Recent advances in research on enzymes for poultry diets. Poult Sci 90:2013–2023

Svihus B (2011) The gizzard: function, influence of diet structure and effects on nutrient availability. World Poult Sci J 67 (2):207–224

Thacker PA (2013) Alternatives to antibiotics as growth promoters for use in swine production: a review. J Anim Sci Technol 4(1):35

Vahjen W, Gläser K, Schäfer K et al (1998) Influence of xylanase-supplemented feed on the development of selected bacterial groups in the intestinal tract of broiler chicks. J Agric Sci 130:489–500

Wang Z, Li L, Yuan D et al (2004) Reduction of the allergenic protein in soybean meal by enzymatic hydrolysis. Food Agric Immunol 25 (3):301–310

Woyengo TA, Nyachoti CM (2011) Supplementation of phytase and carbohydrases to diets for poultry. Can J Anim Sci 91(2):177–192

Zhong Q, Jin M (2009) Nanoscalar structures of spray-dried zein microcapsules and in vitro release kinetics of the encapsulated lysozyme as affected by formulations. J Agric Food Chem 57:3886–3894

Zyla K, Gogol D, Koreleski J et al (1999) Simultaneous application of phytase and xylanase to broiler feeds based on wheat: in vitro measurements of phosphorus and pentose release from wheat and wheat-based feeds. J Sci Food Agric 79:1832–1840

Nutraceuticals Used as Antibacterial Alternatives in Animal Health and Disease

Arturo Anadón, Irma Ares, Maria Rosa Martínez-Larrañaga, and Maria Aŕanzazu Martínez

Abstract

In the past decades, an accumulative amount of research has been concentrated on the development of alternatives to antimicrobials to maintain animal welfare and health and performance. A high level of internal and external biosecurity for farms, herds, or flocks is critical because they constantly receive and deliver biological material. Many efforts have been dedicated to demonstrate how viruses or bacteria are shed from poultry or pigs and transmitted among farms, herds, or flocks. A good external biosecurity is crucial to avoid introduction of new biological agents. To overcome the increased rate of mortality and morbidity resulting from the ban of in-feed antibiotics, a number of antibacterial alternatives have been designed. The classes of alternative substances described in this chapter include organic acids and short- and medium-chain fatty acids, phytobiotics and essential oils, antimicrobial peptides, bacteriophages and their endolisins, and immunomodulatory compounds (vaccines). Prebiotics and probiotics, and enzymes, are also notable alternatives to antibacterials but are described in two other chapters. These nutraceuticals are valuable tools in antibiotic-free production systems to support the intestinal health of the animal. Based on a literature search, it is evident that a long and growing list of compounds have been reported for their ability to replace antibacterials as feed additives in animal diets. Research is still needed in the area of nutraceuticals used as antibacterial alternatives in animal health because the perfect alternative to antibacterials does not yet exist. The mechanism of action for these compounds needs to be better defined. Regulations concerning feed additives in the European Union for these nutraceuticals used in animal production are also described.

A. Anadón (✉) · I. Ares · M. R. Martínez-Larrañaga · M. A. Martínez
Universidad Complutense de Madrid, Madrid, Spain
e-mail: anadon@vet.ucm.es

Keywords

Veterinary nutraceuticals · Antibacterial alternatives

1 Introduction

Antimicrobials have been used worldwide in animal husbandry including livestock, poultry, and fish farming for many decades because of their favorable economic effects in livestock production to maintain health and productivity. Antibiotics added in low doses (i.e., sub-therapeutic doses) to the feed of farm animals improved growth and performance (feed conversion ratio) and hence were known as "antimicrobial growth promoters" (AGP). Growth promotion involves the modification of the gut microflora population and changes in the host metabolism. Concerns exist that these practices in antibiotics use as growth promoters in animal feed contributed to the development of antimicrobial resistance, spreading drug-resistant pathogens in both farm animals and humans, posing a significant potential threat to public health.

The problems associated with the use of antimicrobials in food animals are growing worldwide without clear evidence of the need for or benefit from it, leading to increasing recognition that urgent action is needed. It is accepted that the prevalence of antibiotic resistance in the livestock sector is generally higher in animal species reared under intensive production systems. The prudent use of antimicrobials (also referred to as "judicious," "rational," or "antimicrobial stewardship") in animals is very important to help reduce the development of antimicrobial resistance (AMR) because, as in humans, inappropriate use can result in the development of resistant microorganisms, which can be subsequently transmitted from animals to humans (or vice versa) and to the environment. "Prudent or judicious" and "rational" terms are frequently used to suggest a responsible attitude to antimicrobial use, aimed at minimizing the development and spread of AMR while maximizing therapeutic efficacy.

R. C. Gupta et al. (eds.), *Nutraceuticals in Veterinary Medicine*, https://doi.org/10.1007/978-3-030-04624-8_22

Antimicrobial stewardship is a concept developed as an interdisciplinary approach to ensure the efficacy of antibacterials and their use in future generations. The core principles of the concept are the "5 R's": Responsibility (shared responsibility involves a drug-prescribing veterinarian and livestock producers), Reduction (avoid prophylactic and metaphylactic use and use antibacterials only for treatment outbreak), Refinement (combination of reliable diagnostics followed by correct use of antibacterials), Replacement (use of alternatives at the farmers' disposal, pre- and probiotics, organic acids and butyrate, secondary plant compounds), and Review (regularly checking the applied measures with the aim to eventually reduce antibiotic use to zero). The prudent use of antimicrobials is part of good veterinary and good animal husbandry practice and takes into consideration disease prevention practices such as the use of vaccination and improvements in husbandry conditions. The different applications of antibiotics in food animals have been described as prophylactic use, therapeutic use, and metaphylactic and sub-therapeutic use. The use of antimicrobials for mass medication, in sub-therapeutic doses or in other inappropriate manners, amplifies this risk. Thus, the European Commission in 2015 published guidelines for the prudent use of antimicrobials in animals that set out many potential factors to be considered in establishing policies and actions which reflect the multi-faceted, complex issues involved in tackling AMR. They define the prudent use of antimicrobials as leading to more rational and targeted use of antimicrobials, thereby maximizing their therapeutic effect and minimizing the development of AMR (EC 2015, 2018). Mixed opinions still exist on the transfer of antibiotic resistance genes from animal to human pathogens (Gadde et al. 2017). Several studies showed that there might be a link between the practice of using sub-therapeutic doses of antibacterials and the development of AMR among the microflora (Cosby et al. 2015). Because of the emergence of microbes resistant to antibiotics (AMR) that are used to treat human and animal infections, the European Commission (EC) decided to phase out, and ultimately ban since the 1 January 2006 decision taken on "precautionary principles," the marketing and use of antibiotics as growth promoters in animal feed (Anadón 2006). Since then, the use of antibacterials is only allowed on veterinary prescription for direct applications or as medicated feed. These restrictions are deemed necessary as antibacterials may lead to the selection of resistant bacterial strains in animals that could be transferred to humans, by direct contact or via foodstuffs, and subsequently lead to an impairment of the efficacy of antibiotics used in therapy of human infectious diseases (Anadón 2006).

The impact of phasing out animal growth promoters could be minimized provided that adequate attention is given to alternative disease prevention strategies and management factors, such as alternative husbandry practices in food animal production. Livestock performance and feed efficiency are closely interrelated with the qualitative and quantitative microbial load of the animal gut, the morphological structure of the intestinal wall, and the activity of the immune system (Huyghebaert et al. 2011). In the US, antibiotic use in livestock and poultry feeds is under great scrutiny as a result of increasing consumer awareness and the demand for livestock products from antibiotic-free production systems. In 2013, the US Food and Drug Administration (FDA) called for major manufacturers of medically important animal drugs to voluntarily stop labeling them for growth promotion in animals and revise the labels such that veterinary supervision is required for therapeutic uses (FDA 2013). The FDA continued to strengthen its agenda on promoting judicious use of antimicrobials in food-producing animals and published its final rule of the Veterinary Feed Directive (VFD) in early 2015, bringing the use of critically important antimicrobials (CIAs) (for example, third- and fourth-generation cephalosporins and/or fluoroquinolones) according to their degree of risk to man in feed under veterinary supervision, so that they are used only when necessary to ensure the health of the animals. Medicated feed containing antibacterial veterinary medicinal products shall not be used to supplement food-producing animals nor to enhance their performance. Particular issues should be considered before using CIAs. (1) Many of the antimicrobials used in animals are also used in humans. Some of these antimicrobials are critical for preventing or treating life-threatening infections in humans. (2) Special consideration is necessary to ensure the continued efficacy of such antimicrobials and to minimize the development of resistance. (3) Before using these antimicrobials in animals, consideration should be given to the following: these antimicrobials should only be used in situations for which a veterinarian has assessed, on the basis of antimicrobial susceptibility testing (AST) and relevant epidemiological data, that there is no non-critically important effective antimicrobial available. (4) In exceptional cases where the use of these antimicrobials under "off-label" (extra-label) use (cascade system) is unavoidable and legally permissible, prescription and final use should be sufficiently justified and recorded. Such use should be based on clinical grounds: that is, the prescribing veterinarian considers the use of a particular CIA necessary to avoid the suffering of diseased animals, and should also take into consideration ethical and public health concerns. The use of CIAs should be limited to cases where no other alternative is available (EC 2015).

Antimicrobial resistance is a well-documented knock-on effect of the overuse of antibiotics as a preventive method to maintain animal health and performance. The majority of

antibiotics used in animal husbandry are not used to treat sick animals, but are used to treat unhealthy production systems. For example, in dairy production (e.g., mastitis treatments are responsible for most antibiotics used on a dairy farm to control inflammation of the mammary gland and udder tissues, usually caused by bacterial infections), dairy cows receive on average two antibiotic treatments annually, one to prevent and one to treat mastitis. This practice often involves "blanket" use of antibiotics across all cows to prevent the occurrence of mastitis during the "dry" period; when dairy cows are rested between the end of one lactation and the start of the next (typically around 60 days), with penicillins, cephalosporins (e.g., cefquinome, a third-generation cephalosporin), or other β-lactam drugs. The most commonly used antibiotics for dry-cow therapy are older cephalosporins, with modern cephalosporins used by about 16% of farmers (Rose and Nunan 2016). Another area where antibacterials use is too high is in pre-weaned dairy calves reared at the dairy; antibiotics are added to the milk/milk replacer and to routinely treat diarrhoea. Similarly, beef calves that enter feedlots receive antibiotics for the treatment of clinical respiratory disease or to prevent liver abscesses that negatively impact growth with macrolide tylosin (Walker et al. 2012).

Growing swine receive antibiotics, commonly tetracyclines or tylosin, in their feed for disease prevention and growth promotion purposes. Finally, the most common use of antibiotics in poultry production is to support marginal baby chicks that have become contaminated in the yolk sac or at the navel during hatch. The systematic injection of antimicrobials (e.g., cephalosporins) into eggs or day-old chicks in hatcheries is often practiced but should be avoided entirely, unless justified for exceptional reasons. For example, the use of third- and fourth-generation cephalosporins in poultry (including eggs) should be prohibited, in accordance with the Commission's decision following the referral procedure of 13 January 2012 and in line with the European Food Safety Authority's (EFSA) scientific opinion on the public health risks of bacterial strains producing extended-spectrum beta-lactamases (ESBL) and/or AmpC β-lactamases in food and food-producing animals and due to the risk of AMR spreading to humans (EMA 2012). The prophylactic use of antimicrobials at this stage can be avoided by ensuring good hatchery hygiene and through good management of day-old chick production (e.g., temperature control, hygiene, and stimulation of drinking and eating) (EC 2015). For the newly hatched chick, access to feed is also very important. Neonatal nutrition is a critical component in the establishment of normal gut function, from digestion and absorption to barrier function and development of the immune system. The intestinal epithelium is constantly exposed to commensal or resident pathogenic microbes and antigens that are important for the development of immunity.

2 Impact of Phasing Out Animal Growth Promoters

Antimicrobials have many times been used indiscriminately, to the detriment of healthy animals. If antimicrobials are given to an animal with a healthy gut microflora, it is highly likely to result in digestive and microbial disturbances. In-feed prophylactic antibiotics are not used to treat individual sick animals but are used to treat sick and suboptimal production systems.

It is well known that after antibiotic human treatment results in severe disturbance of the intestinal microflora leading to diarrhoea and abdominal discomfort in a variable fraction of patients, depending on the age group and the antibiotic used; approximately 15–30% of patients experience a symptomatic recurrence after discontinuation of antibiotics. A treatment period with antibiotics only temporarily change the composition of the microbiota, causing environmental changes. In most cases, the cause of the diarrhoea is unknown but a varying proportion of the cases are caused by *Clostridium difficile*. The *C. difficile* toxins may cause anything from mild diarrhoea, which can be cured simply by terminating the antibiotic treatment, to the life-threatening disease pseudo-membranous colitis (Vanderhoof et al. 1999). Although these effects usually resolve with *Lactobacillus* probiotics as single species or combination probiotic products, in livestock these intestinal disturbances certainly come with a cost in terms of lost feed efficiency. So, the impact of phasing out AGP could be minimized with adequate use of alternative disease prevention strategies and management factors, such as alternative husbandry practices in food animal production (Huyghebaert et al. 2011).

The incidence of certain poultry diseases, such as (subclinical) "necrotic enteritis," also called dysbacteriosis, affects chickens and turkeys for fattening at the age of 2–16 weeks, proliferating at the age of 3–6 weeks. The subclinical necrotic enteritis leads to a slow but steady growth rate reduction. This poultry disease syndrome is clearly emerging in the EU simultaneously with the EU ban of AGP; both the incidence of bacterial enteritis and use of drug medication to treat this disease have increased. Necrotic enteritis is caused by specific gram-positive, facultative anaerobic bacteria, *Clostridium perfringens* (toxin-producing pathogen), mostly type A. The overgrowth of the toxin-producing strain of *C. perfringens* damages the intestinal wall, impairing wall functionality and chemical architecture, which reduces their capacity to absorb nutrients. Acute clinical necrotic enteritis in which the level of infection is severe may result in high mortality even in the absence of other symptoms. The most commonly recognized predisposing factors for necrotic enteritis include coccidiosis infection, high dietary levels of non-starch polysaccharides (NSP), and indigestible protein.

C. perfringens is a component of the normal gut flora and is found in litter, faeces, soil, dust, and in healthy animal guts. These spore-forming bacteria are extremely resistant against environmental influences and can survive in soil, feed, and litter for several years and even reproduce. The factors that create an intestinal environment favorable for *C. perfringens* promoting an infection with necrotic enteritis are as follows. (1) Feed: undigested non-starrch polysaccharides (NSPs) serve as substrate, and some cause higher production of mucus, also serving as substrate and providing ideal anaerobic conditions. Undigested proteins from high content in the diet also serve as substrates. (2) Stress: stresses such as feed change or high stocking density. (3) Immunosuppressive diseases (such as infective chicken anaemia, and Gumboro or Marek's) decrease resistance against intestinal infections and facilitate their colonization. Dysbacteriosis is commonly observed in broiler chickens with acute or chronic gastrointestinal (GI) disease; primary dysbiosis is defined as alterations in microbiota without an identifiable cause. Anatomical and physiological differences along the GI tract, differences between luminal and mucosa-adherent microbiota, and bacterial interactions between host and microbiota (via innate and adaptive immune system) can also take place on a functional level without obvious changes in the actual composition of microbiota (Suchodolski et al. 2005). Some pathogens exert pressure on the gut and prepare the way for *Clostridia*: *Cryptosporidia*, and *Salmonella* may result in an imbalance between commensals or residents and potentially pathogenic bacteria (*Clostridium perfringens,* among others) because of an oversupply of nutrients in the lumen, leading to interference of microbiota with mucosa and inflammation (Dibner and Richards 2005). Consequently, the gastrointestinal tract (GIT) becomes less functional, contributing to poor digestion of feed and flock performance. This condition of the gut may be synonymous with conditions such as "wet litter," "small intestinal bacterial overgrowth," "malabsorption," and "feed passage syndrome." In this syndrome, there is increased water content in the faeces and reduced digestibility with indigested residues visible in the faeces (Huyghebaert et al. 2011). Dry bedding might still have to be added to address the moisture already in the litter. Alternatives such as probiotics or slow-release butyrates are given via the feed before the wet litter problem arises, bringing some benefits in terms of feed conversion ratio (FCR) and uniformity. Avoiding "wet litter" can contribute to the air quality in the poultry house, improving conditions for workers and animals alike. Good intestinal health can prevent respiratory problems by way of drier litter.

Weaning is a critical event in the pig's life cycle, frequently associated with severe enteric infections and overuse of antibiotics, which raises serious economic and public health concerns. During the weaning period, piglets lose the benefit of passive immunity from the sow's milk and experience GI changes that accompany the change in diet, which causes them a degree of stress and leaves them vulnerable to secondary infections. Among the physiological and GI factors impacted by the weaning transition, gut microbiota disruption is likely to be recognized as a key leading to postweaning diarrhea. The pig gut microbiota is a very complex ecosystem showing dynamic composition and diversity that shifts over time and along the entire GI tract (Isaacson and Kim 2012). Colonization is initiated at birth and is shaped by consumption of the sow's milk, which provides nutritional advantages to the population of lactic acid bacteria, building a milk-oriented microbiome (Frese et al. 2015). *Escherichia coli* and *Streptococcus* spp. create an anaerobic environment favouring the establishment of other colonizers such as *Bacteroides*, *Lactobacillus*, *Bifidobacterium*, and *Clostridium* (Petri et al. 2010). During the suckling period, the brood and rearing mother further differentiate the fecal microbiota of piglets. This weaning period obliges a change to new strategies because now no protection comes from the mother. As a result of all these changes, some piglets will not eat for some period of time. Strategies have been developed to counteract the problems of this period because the feed is less digestible as it includes raw vegetable materials and it is often presented in a dry form. One strategy is to stimulate the development of the GI tract of the neonate by supplementation of the sow diet with certain biologically active substances and plants. The weaned piglet body must try to adapt to this new physiological condition by reducing the use of energy and protein and lowering the replacement rate of the intestinal epithelial cells, resulting in a shortening of the intestinal microvilli (Skrzypek et al. 2007): their stomach however is not prepared to receive large quantities of food. The time the food remains in the stomach will be shorter and the pH obtained will not be correct to activate protein enzymes to digest vegetable protein, and therefore the risk of diarrhoea will increase.

Weaning has a strong impact on the GI immune system as the moment of weaning results in three major changes for the piglet: (1) all the diet composition is changed drastically from easily digested milk to a less digestible solid-based diet; (2) the piglets are moved to a new environment; and (3) litters are mixed, which brings social stress to the animal. These factors have major impact on the piglet's health and performance and sometimes results in mortality. The gut microbiota dysbiosis, induced by abrupt changes in the diet composition and environment of piglets, emerges as a leading cause of "post-weaning diarrhea," and associated infections in piglets even if the exact underlying mechanisms remain unclear (Gresse et al. 2017). The change in diet composition might have an enormous impact on their feed and water intake, which results in a fasting period of 24 h or even 48 h. When a piglet is in a status of dysbiosis, there is an increased risk for post-weaning diarrhoea. Moreover, weaning is associated

with increased permeability of the intestinal epithelium, which increases the risk of pathogenic infections as it become easier for pathogens to cross the epithelium and enter the body (Gresse et al. 2017). For many years, the solution has been based on using antibiotics or zinc oxide as premix for medicated feedstuff, at a dosage of 100 mg/kg bw/day for 14 consecutive days, that is, 2500 mg/kg feed ppm, in pre-starter and starter diets, for the treatment or prevention and control of post-weaning diarrhea in piglets (EMA 2016a, b). According to recently published data, zinc as used in animal farming might increase the prevalence of antibiotic-resistant bacteria because of co-selection for antimicrobial resistance genes. Several published studies demonstrated, during in vivo experiments or by investigating environmental isolates, a correlation between high doses of zinc supplementation in food and the prevalence of antimicrobial-resistant bacteria (LA-MRSA) or of multi-resistant bacterial clones (*Escherichia coli*). The EMA-CVMP considers that (1) the environmental risks identified by the yearly accumulation of zinc in soil from the spreading of manure in agricultural land cannot be controlled with risk mitigation measures, and (2) the overall benefit–risk balance for veterinary medicines containing zinc oxide to be administered orally to food-producing species is negative, as the benefits of zinc oxide for the prevention of diarrhoea in pigs do not outweigh the risks for the environment. Overall, there is a risk of co-selection for resistance associated with the use of zinc oxide, but at the present time, that risk is not quantifiable (EMA 2016a, b). Among the non-antimicrobial alternatives there are essential oils, and prebiotics or probiotics, which are currently evaluated to restore intestinal balance and allow a better management of the crucial weaning transition (Gresse et al. 2017).

3 Biosecurity

Biosecurity is the term used in veterinary medicine to describe measures to prevent pathogens from entering farm premises or a group of animals (external biosecurity) or the spreading of pathogens within farm premises or groups of animals (internal biosecurity) (Amass and Clark 1999). Biosecurity generally refers to keeping infectious agents out of an animal operation or farm whereas biocontainment refers to keeping any infectious agent(s) present on a farm or within a region confined to that region (Turner 2018). Biosecurity is a kind of health animal production system or programme, which is critical to prevent the introduction of disease-causing organisms in a farm/herd/flock and prevent the spread within a farm/herd/flock, being part of sustainable livestock production practices. Biosecurity means taking steps to ensure good hygiene practices are in place so that the risk of a disease occurring or spreading is minimized. These measures should be practiced at all times and not just during a disease outbreak. The poultry industry has for a long time embraced the importance of biosecurity, and pig production has come far, whereas the importance of biosecurity has not been fully embraced in dairy and beef production. The level of biosecurity of a certain herd can be assessed by interviewing the farmer regarding biosecurity practices and collecting data by visual inspection. A high level of biosecurity is a demand, not only for farms located in pig-dense areas with high infection pressure, but for all farms because they constantly receive, and deliver, biological material (i.e., animals and feed). The risk-based weighted biosecurity scoring system translates questions regarding biosecurity into a score for a herd for its internal, external, and overall biosecurity status. This score aims at providing an objective, comprehensive, and quantitative description of the level of biosecurity and can be used to inform the farmer about possible areas for improvements, and to compare the biosecurity level with that of other farms/herds/flocks. Farmers are likely to be more motivated to implement biosecurity measures if such measures can be expected to be beneficial for their farm performance (Laanen et al. 2014), yet there are limited quantitative data available to link biosecurity and production parameters (Amass and Clark 1999; Laanen et al. 2013). Especially, studying these relationships on the basis of multiple country data has not been done previously. Biosecurity also has a role in maintaining the health of animals in farms and the challenges within farms to manage implementation of biosecurity protocols.

Antibacterials use, for example, in pig farming, is influenced by a range of risk factors, including herd characteristics, biosecurity level, farm performance, occurrence of clinical signs and vaccination scheme, as well as farmers' attitudes and habits towards antimicrobial use. Therefore, initiatives should be required to preserve antibiotic effectiveness while simultaneously ensuring food security in low- and lower- to middle-income countries.

The development of antimicrobial resistance is a normal evolutionary process for microorganisms, but the selection pressure created by the routine use of antibiotics accelerates this development. This problem threatens the achievements of modern medicine, as the pipeline for the development of new antibacterial drugs is nearly empty. Limiting the use of antimicrobial drugs in human and veterinary practice can minimize the development of resistance. In 2016, colistin-resistant *E. coli* was isolated from a commercial poultry farm in China. Following the discovery of a new colistin horizontally transferable resistance mechanism (*mcr*-1), and considering the rapidly increasing importance of colistin for the treatment of critically ill human patients, the European Medicines Agency (EMA) recommended that all countries should strive to reduce the use of polymyxins as much as possible because colistin is an antibiotic of last resort (EMA

2016a). In parallel, the EMA also recommended refusal of new marketing authorizations and the withdrawal of the existing marketing authorizations for veterinary medical products containing zinc oxide. The recommendation is primarily based on the risk for the environment in addition to the possible risk of co-selection for antimicrobial resistance associated with the use of zinc oxide (EMA 2016a, b).

Global efforts include the 2016 United Nations Political Declaration on AMR and the 2015 WHO Global Action Plan on AMR, which was subsequently adopted by the World Animal Health Organisation (OIE) and the Food and Agriculture Organization (FAO). AMR has also been addressed in the G7 and G20 forums. In addition, action plans on antimicrobial resistance, such as those launched by FAO, WHO, and the EU (EU 2017), are also enabling and motivating countries to provide supportive actions to reduce infections and thus the need for antimicrobials. The industry is clearly working together to ensure our farming systems are sustainable and healthy. According to this "European One Health Action Plan on AMR", the European Agencies' supportive actions will include infection prevention, biosecurity measures, and control practices in human healthcare and in animal husbandry, including aquaculture, to reduce infections and thus the need for antimicrobials (EU 2017). In biosecurity measures, one must monitor the following critical features.

3.1 Animal Welfare

The five freedoms proposed by FAWC (1993) encompassed (1) Freedom from thirst, hunger, and malnutrition; (2) Freedom from discomfort; (3) Freedom from pain, injury, and disease; (4) Freedom to express normal behaviour, and (5) Freedom from fear and distress, combined elements in welfare which are a very useful framework to identify the main welfare problems in a given production system and also as a starting point to select the main welfare indicators. The welfare of an individual is its state as regards its attempts to cope with its environment. Many of the behavioral changes and medical conditions associated with aging may impair the welfare of the animal as defined by the five freedoms (FAWC 1993). Animal welfare has a large impact on production efficiency and health. Although most of us know about all these stressors for animals, it is very common to come to farms, herds, or flocks where the management and stress factors leading to disease are not corrected, but the animals are fed antibiotics to reduce disease and poor performance. For example, several diseases may result in pain or interfere with the expression of normal behavior. In piglets, reducing stress particularly at the time of weaning by increasing the weaning age and optimal nutrition are also beneficial, particularly to reduce the need for antimicrobials to control post-weaning diarrhoea. In poultry, the subclinical necrotic enteritis that produces conditions such as "wet litter" is a constant challenge in broiler chicken production, strongly linked to "food pad" lesions and welfare concerns for air quality.

3.2 Disease Prevention

Animal diseases and infections should be prevented by ensuring biosecurity, following good production and good management practices, and implementing integrated disease control programmes to both minimize the occurrence of diseases and eradicate endemic disease (EC 2015). Nutritional solutions to prepare the gut for an influx of bacteria could be crucial. Biosecurity measures that ensure hygiene standards, and housing situations that adhere to the welfare and well-being of the animal, should be the highest possible. Disease prevention through biosecurity measures is believed to be an important factor for improvement of overall health status in animal husbandry. Better protected animals, in turn, diminish the need for preventative or sub-therapeutic medicine. Biosecurity measures can influence animal health for the better and reduce potential need for antimicrobials. So, implementation of biosecurity measures, good farming practices, and herd health planning that prevents infections reduce the need for antimicrobials (EC 2015).

3.3 Cleaning and Disinfection

Good biosecurity requires a disinfection protocol. A correctly implemented protocol can be a cost-effective method of reducing pathogenic organisms and is an important step in any biological risk management program. Every biosecurity protocol needs to include a correctly selected hygiene and disinfection agent, which must prevent the proliferation of pathogenic microorganisms. An effective disinfectant material reduces ammonia formation, absorbs excessive moisture, decreases the pH value of the litter, contributes to significantly better air quality through minimizing harmful emissions, and improves overall animal performance. Applied regularly, the disinfectant can improve biosecurity status and minimize the spread of disease. Hygiene and biosecurity measures, including measures designed to prevent the introduction of infections, include keeping separate clothes and boots for each unit; controlled or limiting access to minimize intra- and interspecies exposure to animals and transmission of pathogens (e.g., by arthropods and rodents); handwashing (e.g., before preparing or eating human food), with hand disinfection facilities (liquid soap, hot and cold water) available close to the workplace; ensuring quick removal of and prevention of access to dead animals; applying the 'all-in all-out' system in each unit; following a strict

schedule for cleaning and disinfection; and performing regular disinfection controls (EC 2015). One key action that stands above all in terms of preventing antibiotic resistance is establishing a better and healthier environment through improved hygiene and good biosecurity practices.

3.4 Good Hygiene Practices and Environment

In production systems with high density of animals or poor biosecurity, development and spread of infectious diseases is favoured, leading more frequently to antimicrobial treatment and prevention of those diseases and providing favourable conditions for selection, spread, and persistence of antimicrobial-resistant bacteria (Rose and Nunan 2016). The objective of reducing antimicrobials use is also in line with animal welfare, aiming to reduce the farm animal population density. Stocking density (an adequate welfare parameter), and hygienic conditions of housing, bedding material, temperature, humidity, and air quality, are just a few environmental conditions with a very large influence on production, health, and welfare of animals. Overstocking is very common, impacting environmental temperature and hygiene, and often reduces cleaning and disinfection between batches of animals. Overcrowding also imposes social stress on the animals as there may be insufficient eating and resting space. Overcrowding and very large numbers of animals facilitate disease transmission and pathogen mutation to greater virulence. Keeping stock densities low and avoiding excessive herd or flock sizes, and avoiding mixing within the herd or flock, or quarantining stock for an appropriate period before mixing, are important biosecurity measures (EC 2015). A period of quarantine and stabilization can help ensure that new stock are healthy and free from disease (Turner 2018).

A flawless environmental temperature is perfectly adapted to the age and weight of the animals. Good air quality and ventilation are essential to keep animals healthy and vigorous. Physical separation of sick and healthy animals, and handling and attending to the needs of healthy animals first, will help reduce disease transmission within a group of animals (Turner 2018). In birds, for example, ventilation must be managed adequately to minimize the negative impact from ammonia concentrations, dust, litter moisture, excessive humidity, or combustion gases. A warm, moist environment is also a perfect environment for pathogenic bacteria to thrive and propagate.

In laying hens, optimal hatchery equipment operation and optimal hatchery environment reduce the need for supportive antibiotics. The establishment and maintenance of good gut function is vitally important in reducing neonatal chick morbidity and mortality. Antimicrobials should not be used routinely on the arrival of day-old chicks at the farm. The prophylactic use of antimicrobials at this stage can be avoided by ensuring good hatchery hygiene and through good management of day-old chick production (e.g., temperature control, hygiene, stimulation of drinking and eating) (EC 2015).

In bovines and small ruminants, mass or group medication of cattle is rare, although veal calves can be subjected to group treatment using antimicrobials. Treatment given to cows at drying-off is of particular importance. These measures include (1) avoiding prophylactic use of antimicrobials in newborn calves (e.g., antimicrobials added to milk replacers) by implementing good farming practices (e.g., ensure high standards of hygiene); (2) developing preventive strategies (e.g., vaccinations, feeding colostrums to calves), especially for the allotment of veal calves and beef cattle; (3) avoiding the systematic treatment of cows at drying-off, considering and implementing alternative measures on a case-by-case basis; (4) establishing thorough hygiene measures and good farm practice and management strategies to minimize the development and spread of mastitis in dairy cows; (5) promoting rapid diagnostic tests (e.g., standardized tests with chromogenic media) for identifying mastitis-causing pathogens to minimize both intramammary and injectable antimicrobials in milking cows; and (6) avoiding feeding calves with waste milk from cows treated with antimicrobials (EC 2015). In dairy cows, hard flooring, inadequate bedding, and crowded conditions can increase the likelihood of dairy cows developing mastitis or lameness. Housing should therefore be designed around cow comfort, with good ventilation, suitable humidity, high-quality bedding, and good hygiene practices (Rose and Nunan 2016).

3.5 Immunity

Vaccines have proven to be very important and cost-effective in preventing the onset and spread of infectious diseases and thereby have great potential to reduce the incidence of AMR; thus, good systemic immunity is essential. The European Commission states in the "One Health Action Plan" (EU 2017) that vaccines should be boosted even further to decrease the use of antimicrobials in those sectors. However, immune-enhancing measures are not limited to vaccinations but include genetic selection, pathogen-free animals, nutrition, mycotoxin prevention, stress reduction, stocking density, and environmental factors. Biosecurity and increased use of vaccination were perceived to be the most promising alternatives to antimicrobials in industrial pig and poultry production, based on combined effectiveness, feasibility, and return on investment. In baby chicks, for example, training hatchery personnel to monitor factors critical to chick quality as well as training in vaccination technique enable poultry producers to start with healthy birds. When possible, alternative strategies for controlling disease that have been

proven to be equally efficient and safe (e.g., vaccines) should be preferred over antimicrobial treatment. Vaccination management should also include measures to avoid stress reaction and improvements for the availability of autogenous vaccines (EC 2015).

3.6 Management

Management is probably the best alternative, but it is not so easy to apply as it includes many different characteristics. Management has to ensure good hygiene and environment (e.g., when pigs are weaned and located in nurseries). The pigs have to find the feed and water as easily as possible, and feeders have to allow them to eat in groups, as they do during lactation. Probably the most important management improvement is teaching the pig how to eat feed. It is recognized that if pigs know how to eat and drink before weaning, the fasting period after is reduced and with it, all the derived problems. Training the young pig to eat should start very early on, during lactation (Oostindjer et al. 2011). Ensuring that animal feed is preserved and that water is clean and not contaminated will minimize the spread of disease within a group of animals (Turner 2018). Stimulating them to eat creep feed should include nutritional skills to formulate an attractive and digestible diet, but also allow them to take feed and water at the same time, as when they nurse from their mother. Liquid presentation in a concentration similar to the mother's milk can make training easier and increase feed intake while they are still lactating, allowing them to achieve their maximum genetic potential. This presentation before weaning is not simple as it must guarantee that the feed remains clean and attractive to the piglets. Weaning management in cases of recurrent weaning diarrhoea should be reassessed, considering in particular hygiene, age of pigs, use of 'all-in all-out' systems, reducing stress suffered by the animals, and alternatives to the prophylactic use of antimicrobials (EC 2015).

3.7 Nutrition

Feed business operators must comply with the legal requirements for feed hygiene, implement best practices in the production of safe and nutritionally balanced feed, and ensure adequate feed formulation. They must also ensure that all ingredients meet the required standards and that the manufacturing process does not allow the feed to be contaminated with deleterious agents, which could compromise the safety of the feed. Feed business operators producing medicated feed must be approved for its manufacture. They must follow all legal requirements for medicated feeds and may only produce medicated feed from authorized veterinary medicinal products and in accordance with a veterinarian's prescription. They must follow good manufacturing practices and ensure appropriate mixing to guarantee the homogeneity of antimicrobials in the feed as well as avoid cross-contamination and minimize the transfer of antimicrobials to subsequent batches of feed (Dorne et al. 2013; EC 2015).

Highly digestible diets have always been regarded as a good instrument to prevent digestive problems. The bioavailability of synthetic amino acids has made it possible to formulate low crude protein diets that are considered safer, but pig performance remains unaltered. Even low-protein diets will not totally prevent protein fermentation in the gut, as the digestibility of the protein will not be 100%. The inclusion of higher levels of fibre could help in maintaining intestinal stability. The type or what level of fibre would be most interesting in post-weaning diets but needs further investigation (Gloaguen et al. 2014). It must be highlighted that the colostrum fed to the piglet or calf during the first 24 h after birth is the most important meal of the animal's life as it is vital for the development of the gut. A hygienic environment and feed are crucial for young animals with underdeveloped immune systems.

Besides good nutrition and a proper anti-coccidial program, fattening chickens requires eubiotics to prevent wet litter problems and limit antibiotic usage. The synergistic effect of certain organic acids with essential oils is well known, and these are now widely used as "gut health enhancers." The combination of specific essential oil compounds and benzoic acid is particularly effective in improving nutrient utilization, therefore improving performance and reducing flock treatments.

The eubiotic approach must be included within a complete feed strategy accurately involving all the other critical nutritional components including micronutrients and enzymes. Nutrition is only part of the solution and needs to be integrated in a sustainable holistic approach including proper vaccination, farm management, and biosecurity.

3.8 Stress

Transport and trade in live animals impose high physiological stress on the animals and create a high risk of spread of diseases, and this creates a need for antimicrobial medication to protect the animals from disease. Current production systems have enabled live animal transport and trade to the detriment of the animals. Transport of young animals often includes prophylactic antibiotic medications. Antimicrobials are most often used in pigs to relieve weaning diarrhoea, intestinal infections associated with *Lawsonia intracellularis*, and respiratory diseases often associated with transport, plus the stress caused when pigs originating from different farms are brought together or when animals are housed with

inadequate ventilation, unsuitable feeding, or insufficient biosecurity measures (EC 2015). These production systems must minimize all unnecessary transport of animals during rearing. However, there are advantages in segregated production systems, and therefore more focus should be placed on minimizing stress and spread of disease through animal trade and transport and minimizing the length of transports. Stressful situations can weaken animal immune systems and make them more susceptible to infections; thus, to avoid stress, animal transport should be limited by minimizing transport time and avoiding overcrowding) (EC 2015).

4 Reducing Antibiotic Use

Prophylactic antibiotics have been used for various digestive tract challenges in animal production, such as necrotic enteritis in poultry, pre-weaning calf diarrhoea, and pre- and post-weaning diarrhoea in piglets. Thus, a focus on gut health is critical to achieving a sustainable, efficient production without the routine use of antibiotics. For example, butyric acid decreases the incidence of subclinical necrotic enteritis caused by *C. perfringens* (Timbermont et al. 2009). To reduce antibiotic use, the production system needs to be healthy, sustainable, and respectful of animal needs.

Reduced risk of contracting an infection can allow reducing the need for antimicrobial treatment of animals. A clear link between biosecurity and both production- and antimicrobial treatment-related criteria in pig herds has been demonstrated, indicating substantial differences in the current application of biosecurity in pig herds. Aspects of both external and internal biosecurity were positively associated with daily weight gain and negatively associated with FCR, respectively. Internal scores were negatively associated with disease treatment incidence, suggesting that improved biosecurity might reduce the prophylactic use of antimicrobials (Laanen et al. 2013). Reduced and prudent usage of antimicrobials in livestock production is an arguable point, mainly in relationship to public health. It is known that previously effective antibiotics are losing their power, and healthcare is approaching a situation similar to the "pre-antibiotic era". Antibacterials have been, and are, the best treatment options for serious bacterial disease. International, national, regional, and local antibacterial stewardship campaigns have been developed to encourage prudent and responsible use of antibiotics, limiting unnecessary exposure, with the ultimate desire of preserving their effectiveness for serious and life-threatening infections.

In June 2017, the EU adopted "the European One Health action plan against antimicrobial resistance (AMR)" (EU 2017). This policy was reinforced with the 2011 Commission action plan, notable for its "One Health approach," addressing AMR in both humans and animals. "One health" is a term used to describe a principle which recognizes that human and animal health are interconnected, that diseases are transmitted from humans to animals and vice versa and must therefore be tackled in both. The "One Health approach" also encompasses the environment, another link between humans and animals and likewise a potential source of new resistant microorganisms. The EU new one health action plan is based on three pillars: (1) making the EU a best practice region on AMR: better evidence and awareness; better coordination and implementation of EU rules; better prevention and control; better addressing the role of the environment; a stronger partnership against AMR and better availability of antimicrobials; (2) boosting research, development, and innovation on AMR: new economic models and incentives, better detection and control measures; new antimicrobials, rapid diagnostic tests, vaccines, and alternative therapies; AMR in the environment; and (3) shaping the global agenda on AMR: stronger EU global presence, partnering; stronger bilateral partnership for stronger cooperation; cooperating with developing countries; and developing a global research agenda (EU 2017). This new action plan will enable and motivate countries to provide supportive actions including infection prevention, biosecurity measures, and control practices in human healthcare and in animal husbandry to reduce infections and thus the need for antimicrobials.

The greatest flaw of animal production systems is that they have been designed and utilized in a way that requires antibacterials to maintain production and the health and welfare of animals. The majority of antibacterials used in animal production are not used to treat sick animals but rather treat unhealthy production systems. The goal to reduce antibiotic use should be rephrased to a goal to produce healthy production systems. The knowledge and tools to create these systems and many producers can demonstrate high productivity and cost-effectiveness in production systems that have eliminated prophylactic and metaphylactic use of antibiotics. In striving for a higher level of health and productivity, and providing the quality products that modern-day consumers are requesting, it is time for alternatives to antibiotics before taking action. A key to success in an antibiotic reduction programme is to assemble a team including veterinarians, nutritionists, consultants (governmental, academic, or industry), building engineer experts, owners, managers, and workers. This "One Health" term is globally recognized, having been widely used in the EU and in the 2016 United Nations Political Declaration of the high-level meeting of the General Assembly on antimicrobial resistance. The draft political declaration recognizes the "One Health approach," emphasizing that this requires coherent, comprehensive, and integrated multi-sectoral action, because human, animal, and environmental health are interconnected. Two key messages were raised by the Inter-Agency Coordination Group (IACG) on AMR: (1) antimicrobial resistance (AMR) is a global,

multi-sectoral issue that affects all countries and requires coherent, comprehensive action on human, animal, plant, and environmental health in the framework of a "One Health approach", and (2) a successful response to AMR will address not only antimicrobials but also diagnostics, vaccines, and alternatives to antibiotics for human and animal health (IACG 2018).

The reduction of antibiotic use in food animal production has become a significant aim of all livestock producers. Veterinarians and domestic animal producers, when using a veterinary drug, must apply the dosage form, route of administration, or dosage regimen as stated; the withdrawal period stated in the product labeling applies only to that particular formulation when administered via the recommended route and in accordance with the dose regime to ensure that animal products are not contaminated with drug residues. Alteration to any of these factors affects the pharmacokinetics behavior of the drug in the animal and invalidates the withdrawal period (Anadón et al. 2018). Concerns about the development and spread of resistant genes, regardless of the prudent and responsible use of antibacterials, have created a climate where overall antibiotic reduction has become a goal of food animal producers, retailers, and consumers alike.

The first step toward the objective of antibiotic reduction is better preventive disease strategies. Preventive use of antibiotics or use to enhance the performance of food-producing animals should in particular not be allowed. The period after weaning is known for regular antibiotic application and is a perfect target for antibiotic reduction. The World Organisation for Animal Health (OIE) and the European Commission (EC), as well as the WHO, have proposed strategies for the issue of antimicrobial resistance (EU 2017): (1) good governance and usage principles for antibiotics; (2) monitoring of antimicrobial usage and resistance; (3) nonspecific prevention, interventions at the production level to reduce risks of disease development on the farm (good housing and management to reduce environmental challenges and stress, correct nutrition, diets to biosecurity); and (4) specific prevention, disease- or pathogen-specific interventions to eliminate or reduce disease incidence and therefore avoid the need for antibiotics. This step could be focused on control of primary bacterial infections or control of viral diseases that can trigger more severe clinical outcomes. Veterinarians and farmers have a direct impact on implementation of prevention programmes to reduce antibiotic reliance using four main steps. (1) Diagnosis: Specialist veterinarians with a thorough understanding of the disease situation on a farm can collect samples and carry out testing to determine the cause of an infection or disease. Often such diseases are multifactorial and the whole farm should be considered in the analysis (infection chain). If a bacterial cause is found, the laboratory can test for the most appropriate antimicrobial treatment (i.e., conventional cultures and antimicrobial susceptibility testing, or C&AST): each bacterium can be reported as susceptible, intermediate, or resistant. More accurate is determination of the minimum inhibitory concentration (MIC), the lowest concentration of an antimicrobial that inhibits the growth of the bacteria. (2) Advice on control: Once the situation is fully understood, interventions can be implemented. Specific interventions, for example, may be curative treatment with an antibiotic using prudent use principles to stop the disease and suffering and mortality. Usage of appropriate vaccinations can result in less antibiotic use on the farm. General interventions are often as important, for example, minimizing exposure to pathogens (i.e., internal biosecurity: ventilation and hygiene to reduce the presence, load, and spread of pathogens) and good external biosecurity will avoid new pathogens entering the production system. A recent work study that measured levels of implementation of biosecurity measures in pig production in four EU countries (Postma et al. 2016b) also examined associations between biosecurity compliance and farm and production characteristics. The results suggested improvements in biosecurity. Improved biosecurity and management practices focused on prevention could reduce antimicrobial use and allow better overall health status and higher animal production and welfare. Prevention is better than a cure (Collineau et al. 2017). (3) Monitor treatment outcomes and antimicrobial susceptibility testing (AST) results by routine and periodic follow-up, with revision of disease control plans to optimize prevention, so a veterinarian can advise the best antibiotic treatment. (4) Education: Pork producers need to be informed of the best practices and to understand legislation and public health developments that impact their business.

In an effort to reduce antimicrobial use, there is an excellent opportunity to optimize production animal health, welfare, and productivity. Biosecurity, herd-level immunity, feeds and feeding systems, stress levels, individual pig health, and environmental conditions all influence animal health and productivity and all interact with each other. Many resources and information sources can assist an individual producer to optimize and maintain high levels of health and welfare in production systems that do not necessitate antimicrobial medication. European pig health experts recently ranked alternative solutions to antimicrobials into a consortium. The top five measures in terms of perceived effectiveness were (1) improved internal biosecurity; (2) improved external biosecurity; (3) improved climate/environmental conditions; (4) high health/specific pathogen-free/disease eradication; and (5) increased vaccination (Postma et al. 2015). These experts have indicated that biosecurity is the highest priority as an alternative solution to antimicrobials (Postma et al. 2016b). Higher levels of biosecurity in pig farms have been shown to be associated with higher levels of production and decreased antimicrobial use and resistance (Postma et al. 2016a). Good

developed immunity to various diseases is essential in industrial pig production. Vaccines have proven very cost-effective in preventing the onset and spread of infectious diseases and are therefore crucial in the modern health production system. On the other hand, stress can have a negative impact on the development of the intestinal barrier.

In animal production systems with a high density of animals or poor biosecurity, development and spread of infectious diseases is favoured, leading more frequently to antimicrobial treatment and prevention of those diseases (Davies et al. 2009), because these conditions favour the selection, spread, and persistence of antimicrobial-resistant bacteria. Some of these bacteria are capable of causing infections in animals and, if zoonotic, also in humans. Bacteria of animal origin can also be a source for transmission of resistance genes to human and animal pathogens. In many cases, such disease can be prevented by good husbandry, a good environment, and hygiene, rather than by the routine prophylactic use of antibiotics (Aarestrup 2004). Positive measures that can reduce disease in farmed animals include the following. (1) Switching to extensive production systems: High-welfare, free-range, and organic systems can achieve higher levels of animal health together with lower levels of antibiotic use than intensive production systems. Recent studies in the UK, Norway, and Sweden found that organic dairy farms, where preventive antibiotic treatment of dry cows is less likely to be used, achieve the same level of mastitis control as conventional farms that typically use routine prophylactic antibiotics (Van Borel and Sorensen 2004; Cogliani et al. 2011; Hovi et al. 2003). (2) Reducing stress: Stress can cause immunocompromise of the animals whereas reducing stress can improve immunocompetence and the ability of animals to fight disease. (3) Good weaning practices: If too early or poorly managed, weaning can cause stress and lead to disease (Davies et al. 2009). Later weaning helps to ensure that animals are more independent of their mother nutritionally, immunologically, and physiologically, which reduces stress and the risk of scouring. The "Alliance to Save our Antibiotics" use in the UK dairy sector has identified three potential measures to optimize animal welfare and immunity, to mitigate the risk of zoonotic diseases, and to reduce the need for antibiotics in this sector: (1) animals should be kept on pasture; (2) move to selective dry-cow therapy; and (3) maximize herd health, welfare, and hygiene (Rose and Nunan 2016).

Veterinarians are responsible for internal biosecurity, avoiding the spread of pathogens into farms or animal groups, as well as the implementation of disease vaccinations to reduce antibiotic use. Alternative measures, in particular preventive strategies, also reduce the use of antibacterial therapy. Herd-specific interventions to substantially reduce antimicrobial usage in pig production were followed without negative impact on overall farm performance. A median reduction of 47% of the antibiotic treatment from birth to slaughter time was achieved (more than 30% median antimicrobial expending reduction). During the study there were no significant changes in mortality, daily weight gain, and feed conversion ratio; on the other hand, there was a slight improvement in the number of weaned piglets per sow per year. Pork producers, veterinarians, and consultants could see reduction of antibacterial use policy as a chance to optimize production and disease prevention approaches improving farming sustainability and animal health and welfare (Collineau et al. 2017).

It has become prevalent to develop alternatives to in-feed antibiotics, as demanded by consumers and by legislation. When it comes to antibiotic-free animal production, veterinarians, nutritionists, and animal producers use several substances such as organic acids, enzymes, prebiotics and probiotics, and phytogenic feed additives to support the intestinal health of the animals.

5 Gastrointestinal Microflora

The gut is the largest internal organ and has the most extensive exposed surface within the body. It is a selective barrier with physical, chemical, immunological, and microbiological aspects where digested feed and water are absorbed but disease-causing agents should be kept under control. The gut is also the largest immune organ of the body and the location of 70% of the immune cells, being the body's first line of active defence against pathogens. A healthy gut is therefore not only a disease-free gut; but is also an effective digestive organ that can mount good protection against disease and cope with changes and stresses. The gastrointestinal (GI) microbiota are crucial in the host immune system, its physiological development, health, nutrition, and productivity. A healthy gut with a balanced microbial composition is critical for optimal digestion and nutrient uptake. The most important tool for good healthy gut is providing the best feed possible that meets the nutritional needs for the specific age and stage of production of the animal. Multi-phase feeding systems and precision feeding are valuable tools, not only for productivity but also for health. Appropriate feed for the various stages of development is very important, and weaning times and weaning systems in piglets and dairy calves should allow the animals to transition from milk to grain-based diets.

The presence of disease-causing microorganisms (pathogens) in the gut is not sufficient to cause disease. Trillions of microorganisms inhabit the intestinal tract (collectively called "the gut microflora"), forming a complex ecosystem that can influence the immune system both inside and outside the gut and the animal. When this gut microflora

is disturbed (dysbiosis), there is a microbial imbalance between the beneficial microflora and potential pathogens, and disease can occur. Dysbiosis therefore makes it easier for pathogens to damage gut structures and functions. Any dietary change leads to changes in the microflora, so dietary changes should always be gradual to allow the microflora to adapt. Probiotics and mannan-oligosaccharides have been shown to be beneficial for microflora and the gut structures, being valuable tools in antibiotic-free production systems.

The animal diet must incorporate eubiotic substances such as organic acids, probiotics, prebiotics, and essential oils to optimize GI functionality, either by acting on the intestinal barrier or tight junctions, or by modulating the microflora composition. For example, Short-chain fatty acids (SCFAs) reduce paracellular permeability in the caco-2 cell line, possibly from promotion of a more differentiated phenotype; if there is such an effect in vivo, it may have consequences for the biology and pathobiology of the colonic mucosa (Mariadason et al. 1997). Overall, the GI microbiota has great influence on the host immune system, its physiological development, health, and nutrition, and on animal productivity. The control of the microbial community of the GIT with the inclusion of feed additives embracing prebiotics, probiotics, phytobiotics, and phages can enhance animal growth and control of both human and animal pathogens. In fact, the gut of the young animal is exposed to bacteria from the environment through the mother, pen, and feed, nutrition being mother dependable. Good gut condition can help to overcame environmental, management, and nutritional stresses, helping animals to stay healthy. Weaning creates a stress point on the immature gut and can impact the microflora, so initial intakes are important. A diverse microflora, for instance, in chickens, can provide benefits such as pathogen competitive exclusion and natural disease resistance (Patterson and Burkholder 2003). In pigs, the gut microbiome in young animals may be modified by the composition of dietary glycans, resulting in a different functional capacity of the microbiome before and after weaning. Glycan degradation pathways differ significantly among diets (Frese et al. 2015).

Evidence suggests that eubiotics can be great contributors to optimal GI functionality, reducing the need for antibiotics. During the past few decades, the concept of gut health has been of increasing interest to animal nutritionists. However, the lack of a clear definition has redirected scientific efforts towards GI functionality, defined as a steady state where the microbiome and the host intestinal tract exist in symbiotic equilibrium, meaning that the welfare and performance of the animal is not constrained by intestinal dysfunction. Occasionally, pathogenic bacteria have a change to grow, immune barriers are disrupted, and the host animal becomes sick. Antibiotics use has an influence on microbial populations as it can kill both pathogenic and harmless bacteria. GI functionality embraces factors such as effective digestion, absence of gut disorders, a balanced intestinal microbiota, and optimal gastrointestinal chemical and physical barriers (Kamada et al. 2013).

An overall state of well-being, an effective immune system, and optimal inflammatory and oxidative status are also directly related to a healthy GI tract. A fully integrated approach needs to be adopted in which management and health practices include nutrition for success. Alterations in the composition of commensal bacterial communities are associated with enhanced susceptibility to multiple inflammatory, allergic, metabolic, and infectious diseases. Commensal bacteria-derived signals can influence the host immune response to invasive pathogens by acting as an adjuvant to boost the immune response to infection or by providing tonic stimulation to induce basal expression of factors required for host defense. Conversely, some pathogens have evolved mechanisms that can utilize commensal bacteria to establish a replicative advantage within the host. Thus, examining the dynamic relationship between the mammalian host, commensal bacteria, and invasive pathogens can provide insights into the etiology of pathogenesis from an infection (Abt and Artis 2013).

Eubiotics are becoming increasingly popular and are being used globally. The term “eubiotic” was introduced many years ago to encompass all the different product categories. Organic acids and essential oils act directly on gut flora modulation, leading to improved digestibility, whereas probiotics are notably able to enhance the resilience of young animals to enteric disease or stress by establishing, maintaining, or even restoring a balanced gut flora. Prebiotics act specifically in the hindgut, indirectly reducing pathogen pressure, enhancing gut morphology and modulating immune response. It is essential to understand the challenges that poultry and swine producers might face to propose the most relevant and efficient eubiotics combination. Their integrated use in animal feeding has great potential for animal nutrition.

Antibiotics are one of the most important medical discoveries of the twentieth century and will remain an essential tool for treating animal and human diseases in the twenty-first century. However, antimicrobial resistance (AMR) among bacterial pathogens and concerns over their extensive use in food animals has garnered global interest in limiting antibiotic use in animal agriculture.

There is worldwide concern over the present state of AMR among zoonotic bacteria that circulate among food-producing animals including poultry, beef and dairy cattle, goats, sheep, and aquacultural populations (Gyles 2008; Prescott 2008). Although antibiotic growth promoters (AGPs) have been successfully utilized during food animal production since their efficacy was first described during the 1940s, the exact modes of action are not fully understood and are probably multi-factorial. In recent years, many national

veterinary associations have produced "prudent and responsible use guidelines" to improve antimicrobial drug use and decrease resistance, but the impact of these guidelines is unknown. Within the evolving global movement for "antimicrobial stewardship" many aspects of antimicrobial use in animals can be improved, including infection control and reduction of use, with a view to reducing resistance and its spread and to preserving antimicrobial drugs for the future (Prescott 2008). Novel intervention methods including narrow-spectrum antimicrobials and probiotics that selectively target pathogenic organisms while avoiding killing of beneficial organisms are needed.

The urgent need for effective alternatives to AGPs to maintain current animal production levels without threatening public health should stimulate new research (Millet and Maertens 2011). Consequently, the discovery of additional antimicrobials from nature could lead to even more wide-ranging novel veterinary and medical alternatives to common antibiotics.

6 Antibacterial Alternatives in Animal Health

Alternatives to antibacterials are wide, but probably there is not one as powerful, consistent in results, and easy to use as are antibiotics. The following different alternatives to be explored here include management, feed formulation, vaccines, organic acids, enzymes, prebiotics, probiotics, and phytogenic feed additives.

6.1 Organic Acids

Organic acids and their salts are distributed in nature and have been applied widely worldwide for more than 50 years as feed additives (acidifiers) and drinking water supplements among a variety of candidates for the replacement and reduction of antibiotic consumption in animal production. These acids occur as normal constituents of animal or plant tissues and some, particularly short-chain fatty acids (SCFA), are produced naturally by the body as by-products in the hindgut of food animals and human through large intestinal bacterial fermentation of dietary fibres, starches, and sugar by the microbiota-bacteria, viruses and fungi that colonise the GI tract. SCFA and medium-chain fatty acids (MCFA) are primarily absorbed through the portal vein during lipid digestion (Huyghebaert et al. 2011). It has been common practice to add organic acids to animal feed; they may have significant benefits in poultry and swine production over the years because of their preservative effect and the positive influence on growth rate and FCR (Panda et al. 2009; Adil et al. 2011; Chowdhury et al. 2009). Organic acids have been used in rearing diets to improve growth rate and the FCR (e.g., 5% in piglets and 1–5% in pigs) during the early nursery period of piglets (Table 1). Dietary acids inclusion may be particularly beneficial when there is a problem with the enterobacteria *Escherichia coli* in the nursery period. The mechanism of action is unclear, but it may be related to a reduction in the pH in the upper intestinal tract, and therefore decrease potential pathogen proliferation of undesirable microorganisms in the stomach and small intestine. Acidification of the feed (sanitation effect of feed) and of the GIT through the proper use of acidifiers improves digestion and absorption and influences intestinal flora.

It can be assumed that drinking water is the most prominent risk factor for the spread of *Campylobacter* infection in broiler chicken flocks, and acidification in drinking water using organic acids could affect *Campylobacter* infection in young chicks. Organic acids in drinking water were able to keep the water free from *Campylobacter*. Animal welfare concerns about the consumption of acidified drinking water were taken into account by evaluating the lesions of the epithelium of the gastrointestinal tract. Consumption of acidified water showed no differences in number of damaged epithelial cells in the digestive tract. In crop and caecal contents of chickens, no difference of volatile fatty acid (VFAs) levels was observed between treatment and control groups (Chaveerach et al. 2004).

Research has shown that the beneficial effects of organic acids can be enhanced by using them as blends rather than a single acid, for example, in improving the FCR in chickens for fattening (Samanta et al. 2008). Blends of organic acids represent an array of pKa values and are used because of the broader spectrum of activity. The use of benzoic acid as an animal feed additive is not permitted at present. Nevertheless, results suggest that benzoic acid supplementation has a positive effect on both ammonia emission and feed conversion. Chemically, organic acid used in food-producing animals are listed in Table 2.

The mechanism of action of organic acids is not clearly understood, but it can be attributed to their antibacterial activity. Several possible mechanisms have been summarized: (1) reducing the pH level of the upper GI tract (crop, pro-ventriculus, gizzard) and associated physiological changes in the gut mucosa; (2) altering the gut microflora either by directly killing through cell-wall penetration or by indirectly modifying pH and reducing the numbers of pathogenic bacteria, increasing acid-tolerant beneficial species

Table 1 Organic acids used in food animals

Piglets	Acetic acid, citric acid, formic acid, fumaric acid, propionic acid, tartaric acid
Poultry	Citric acid, formic acid, lactic acid, malic acid, sorbic acid, tartaric acid

Table 2 Organic acids groups used in food animals

Monocarboxylic acids	Acetic acid, butyric acid, formic acid, propionic acid
Carboxylic acids	Citric acid, lactic acid, malic acid, tartaric acid

Table 3 Variables that influence antibacterial activity of organic acids

• Chemical formula
• pKa value of the organic acid
• Chemical form (esterified or not; acid salt, coated or not)
• Molecular weight
• The microorganism-related MIC value of the acid
• The nature of the microorganism
• Animal species
• Buffering capacity of the feed

such as *Lactobacillus* spp., and reducing competition for nutrients by the altered microbes; (3) increasing nutrient digestibility by elevating protein and dry matter retention, improving mineral absorption and phosphorus utilization; and (4) improving gut health through direct effects on epithelial cells (e.g., SCFA are a direct energy source for the growth of epithelial cells) (Gadde et al. 2017). Overall, variables that influence antibacterial activity (Huyghebaert et al. 2011) are listed in Table 3.

SCFA, with a chain length less than 6 carbon atoms, and mid-chain fatty acids (MCFA), with a chain length between 8 and 12 carbon atoms, are broadly used in animal nutrition. The fields of application and the effect of the respective acids vary widely; each organic acid can be used for different aims, and they have diverse properties according to their chemical characteristics. Feed supplements of mixtures of organic acid are used to make their spectrum broader and combine the good qualities of the different acids. However, synergistic effects can be created by targeted selection and combination of acids (Huyghebaert et al. 2011). The beneficial effects can be attributed to several factors such as the inclusion rates, the source of the organic acids, and the buffering capacity of other dietary ingredients. SCFA positively affect the microbiota and modulate immune responses in the gut. The antimicrobial action of organic acids in feed or drinking water occurs through lowering of pH, reduction of the feed buffering capacity, and a direct effect on the microbial population. Yet, more important is the activity of organic acids inside the animal GI tract.

The antimicrobial activity of organic acids depends on the pKa value of each acid. At a pH equal to the pKa value, half the acid present is dissociated. Strong organic acids (low pKa) at normal intestinal pH are mostly in a dissociated form. Thus, they are pH-reducing acids and have a bacteriostatic effect. Weak organic acids (high pKa) at normal intestinal pH are mostly in an undissociated form. This undissociated organic acid form is a requisite to approach the bacteria, can easily penetrate the lipid of bacteria cell membrane, where they dissociate and thus have a bactericidal effect. Inside the bacterial cell (pH is maintained near 7), with a pH above the pKa of the acid, it will lower the cytoplasmic pH, consequently inhibiting normal enzyme activity and causing cell leakage (Ricke 2003). There is a synergistic effect between strong and weak acids. The pH-reducing acids will ensure better action of the bactericidal acids because they promote weak acids to stay in the undissociated form. In this way, organic acids can cope with enteropathogenic bacteria, acting as modulators of the intestinal microflora. Cell enzymes are suppressed and energy depleted as bacteria try to maintain a neutral cytoplasm by pumping out protons. Antimicrobial activity of organic acids generally improves with higher pKa values. Organic acids with higher pKa values are more effective antibacterial compounds and their efficacy is generally improved with increasing chain length and degree of unsaturation (Huyghebaert et al. 2011): SCFA are weak acids with a pKa value below 4.8. In the hindgut, where the pH is close to neutral, they are present as anions and have lesser antimicrobial properties compared to MCFA. The microorganism-related MIC value of the acid is another factor determining its antimicrobial activity. *Clostridium perfringens* is susceptible to most MCFA with the lowest MIC value for lauric acid, whereas resistance occurs against fumaric, lactic, acetic, and propionic acids. The bacterial cell wall is less penetrated by these SCFA at intestinal pH, around 6, because of their lower pKa. It is very important to differentiate the antimicrobial action of organic acids as a preservative in feed or drinking water, from the effect on the complex microbial community along the intestine and on the integrity of the intestinal mucosa. In conclusion, organic acids may help the intestinal mucosa maintain its integrity; they reduce the buffering capacity of the diets helping to keep stomach pH low, resulting in increased pepsin activity and slowing of stomach emptying.

The antibacterial activity of organic acids is mainly against *Campylobacter* spp., *Escherichia coli*, *Salmonella*, *Clostridium perfringens*, and *Listeria monocytogenes* (Over et al. 2009). SCFA can also offer a way to resist or limit the effects of a *Salmonella* infection. Dietary supplementation of organic acids increased the counts of CD4 cells and T-cell receptor II lymphocytes, which corresponds to a faster immune response (Khan and Iqbal 2016). Organic acids are also being studied for their part in improvement of phytate phosphorus utilization in chickens (Rafacz-Livingston et al. 2005). SCFAs have been reported to upregulate genes involved in epithelial cell growth, division, differentiation, proliferation, and apoptosis (Hashemi and Davoodi 2011).

Butyric acid mainly has an impact on intestinal physiology development and recovery of intestinal integrity, but also provides indirect effects on animal metabolism beyond compromised digestive and absorptive capacity, intestinal

barrier function, and tight junction quality, which also deteriorate at weaning. Butyric acid induces the production of host defence peptides in mucus and repair of the intestinal tract architecture through increased cell proliferation, tight junction assembly, and immune cell regulation. Butyric acid stimulates the expression of tight junction proteins (Mariadason et al. 1997). At weaning of piglets, activation of the intestinal immune system and upregulation of genes of pro-inflammatory cytokines engender a significant inflammatory reaction, resulting in intestinal mucosal injury and dysfunction. The energy needs for an activated immune system increase more than 20%. The antiinflammatory properties of butyric acid and alkaloid-rich plant extracts can largely temper the inflammatory reaction, which contributes to an energy-saving and growth-promoting effect. Weaning stress is also related to increased reactive oxygen species (ROS). Glutathione, positively influenced by butyric acid, is critical in many biological processes as a major redox buffer in mammalian cells. The intensive renewal process of the intestinal cells (2–7 days) requires an adequate energy supply. SCFA and MCFA stimulate mitosis, maturation, and differentiation of intestinal mucosal cells and inhibit their apoptosis (Isaacson and Kim 2012).

Similar effects of growth performance improvement were seen when butyric acid was included in the broiler chicken diet (Panda et al. 2009). To guarantee the presence of butyric acid in the intestine, esterified forms of butyric acid are used. The esterified forms automatically bypass the stomach, and the butyric acid molecules are enzymatically released (mainly from di-and tributyrins) by lipase into the small intestine. Polar monobutyrins pass the hydrophilic membrane of pathogenic bacteria (*E. coli, Salmonella, Clostridium perfringens*), disturbing their metabolism and inactivating them.

Butyrate is one of the most important SCFAs. Butyrate is a very small molecule, easily absorbed in the stomach and the upper part of the small intestine. However, to improve gut health and integrity it must be delivered to the lower part of the intestinal tract. The solution to this problem lies in the use of butyric acid as a coated salt (i.e., sodium, potassium, or calcium salts) or partially esterified (e.g., butyric acid glyceride) to reach the lower part of the intestinal tract. Glycerides have the advantage over butyric acid salts that they are very easily absorbed, and very palatable because of the sweet taste of glycerol. This chemical form does not depress feed intake and does not have an odor. Another advantage of esterified fatty acids is the gradual release of the corresponding acid throughout the gut by the enzymatic activity of pancreas lipase. For supplementary butyrate to increase the levels achieved by endogenous production it must be protected from the digestive processes (i.e., coated forms of butyrate could provide the protection needed to go through the acidic stomach). Butyrate released in the first part of the intestinal tract also has an impact on colon inflammation, attributable to the action of the gut as a sensory organ which communicates through effector systems that include the enteroendocrine hormonal signalling system, gut innervation, the gut immune system, and local tissue defences. Butyrate is important in improving gut integrity and in the development and improvement of intestinal functions in mammals and birds. Butyric acid is released in the intestine, nourishing the gut mucosa, decreasing pathogen proliferation, and inactivating negative bacteria, greatly improving the quality of the intestinal microbiome and mucosal integrity (Castillo et al. 2006). Among the SCFA, butyric acid has received particular attention for its extraordinary beneficial effects on general health status. Butyrate is naturally present at high concentrations in the large intestine, as a product of bacterial fermentation, and in milk. It serves as an important source of energy for enterocytes, especially for colon epithelial cells. It also substantially increases epithelial cell proliferation and differentiation while reducing apoptosis of normal enterocytes. Butyrate seems to have a positive influence. In chickens, butyrate seems be involved in satiety management and the maintenance of colonic homeostasis. Various studies confirm a stimulating effect of butyric acid and MCFA on beneficial microflora in early-weaning pigs while decreasing the number of coliforms (*E. coli*).

The immense surface area of the intestinal mucosa is constantly in direct contact with the environment. The intestine is a selective barrier between animal metabolism and the luminal environment, providing adequate protection against invasion of pathogenic bacteria, but also ensures an efficient absorption of nutrients. To accomplish both functions requires simultaneously maintaining an exquisite balance between gut microbial components and adequate numbers of bacteria and their location. Any factor compromising the intestinal mucosal integrity will affect animal health and productivity (Mariadason et al. 1997). It is important to appreciate the particulars of how butyrate can support health against bacterial challenges and restore the equilibrium of the microbiota when it is perturbed, for example, by antibacterials. However, the effects of butyrate on the immune system are potentially involved in animal production. The gut is the largest immunologically active organ. SCFAs are a crucial link in maintaining a healthy microbiota and immune system against pathogenic invasion. In that way, the SCFAs act as a vital link between microbiota and host immunity.

The intestinal mucosal surface is an immunological component that protects the host from pathogenic invasion challenge, is tightly regulated with regard to its permeability, and can influence the systemic energy balance. The activation or suppression of the toll-like receptors (TLRs) by microbial signals can dictate the tone of the immune response, and the TLRs are implicated in regulation of energy homeostasis. The SCFAs are a group of molecules that can both modulate

the intestinal barrier and escape the gut to influence systemic health. As modulators of immune response, microbiota-derived signals influence functions of distant organs and can alter susceptibility to metabolic diseases (Spiljar et al. 2017). The immune system is constituted with innate and adaptive responses which rely on activation by the innate immune system, including specific responses by antibody formation. Innate immunity includes physical epithelial barriers (i.e., intestinal cells, and sentinel cells as macrophages and chemical messengers). The intestinal epithelial barrier, constituted of epithelial cells, tight junction proteins, and intestinal secretions, can prevent entrance of luminal substances and antigens throughout the paracellular space (Kamada et al. 2013). It is known that dysfunction of the intestinal barrier integrity induced by toxins and pathogens is associated with a variety of GI disorders including diseases. Butyrate modifies intestinal barrier function in IPEC-J2 cells through a selective upregulation of tight junction proteins and activation of Akt signaling pathways. Moreover, butyrate increases both mRNA expression and protein–junction (i.e., claudin-3 and claudin-4), influencing intracellular ATP concentration in a dose-dependent relationship (Yan and Ajuwon 2017). At the GI tract, butyrate has a regulatory capacity on trans-epithelial fluid transport, improves mucosal inflammation and oxidative stress status, fortifies the epithelial defense barrier, and adjusts visceral sensitivity and intestinal motility (Canani et al. 2011). It has been described as synergy supplied by the application of exogenous ketone and beta-hydroxybutyrate in combination with SCFA butyrate in the context of cellular and physiological outcomes (Cavaleri and Bashar 2018). Butyrate also exerts a beneficial effect on the immune system through antiinflammatory and anti-oxidant activity (Hodin 2000). Another important feature of SCFA in broiler chickens is its influence on the intestinal microflora. Supplementation of acetate, butyrate, and propionate increase from undetectable levels in 1-day-old broiler chicks to high concentration in 15-day-old-broiler chickens, after which they stabilize. The results indicated that significant negative correlations could be calculated between the number of *Enterobacteriaceae* and concentrations of undissociated SCFAs (van Der Wielen et al. 2000). Butyrate has been the most studied SCFA, being the preferred metabolite for colonocytes. On this basis, butyrate and glucose metabolism by colonocytes has been evidenced in experimental mouse colitis, and it was concluded that colonocyte metabolism of butyrate, but not of glucose, is deteriorated in colitis, which may be important in the interpretation of colitis pathophysiology (Ahmad et al. 2000). Preclinical evidence corroborates the SCFAs as modulator substances in colonic function and in multiple inflammatory and metabolic processes. SCFAs are also associated with autoimmune, allergic, and metabolic diseases (Gill et al. 2017).

6.2 Phytobiotics and Essential Oils

The need to reduce the use of antibiotics in livestock arising from increased concerns over the spread of AMR, has raised interest in phytobiotics. Phytogenic also referred as "phytobiotics", "botanicals", or "plant extracts", which are natural biologically active constituents that are derived from plants and incorporated into animal feed to enhance productivity (Windisch et al. 2008). Phytochemical medicines are recognized as useful and viable alternatives to chemotherapy because they are economical, effective, non-resistance-forming, renewable, and environmentally friendly. Dietary phytochemicals are small molecules with high structural diversity that cause selective stress to or stimulate the resident microbiota. SCFAs are correlated with specific microorganisms of the phylum of intestinal Firmicutes. Potentially effective plant extracts could be used in combination with antibiotics to lower the required antibiotic dose and increase their effectiveness (Shin and Park 2018).

Phytobiotics constitute a large number of compounds or variety of herbs, species, and products derived thereof, such as essential oils. A common feature of phytobiotics is that they are a highly complex blend of bioactive components. Phytobiotics are described as primary or secondary components of plants that contain bioactive constituents which exert a positive effect on the productivity and health of animals.

Phytobiotics have been exploited in animal nutrition in particular for their antimicrobial, antiinflammatory, anti-oxidative, and anti-parasitic activities. Primary components include the base nutrients, such as protein, fats, and carbohydrates; secondary compounds include essential (lipophilic or volatile) oils, bitters, colorants, and phenolic compounds (Grashorn 2010) that may be classified into four groups: (1) herbs (products from flowering, non-woody, and non-perennial plants); (2) botanicals (whole plants or processed parts); (3) essential oils (hydro-distilled extracts of volatile plant compounds); and (4) oleoresins (extracts based on nonaqueous solvents) (Clavijo and Vives Flórez 2017). Biologically active constituents of plants are mostly secondary metabolites, such as terpenoids (mono- and sesquiterpenes, steroids, etc.), phenolics (tannins), glycosides, and alkaloids (present as alcohols, aldehydes, ketones, esters, ethers, lactones, etc.) (Huyghebaert et al. 2011). Useful antimicrobial phytochemicals can be divided into several categories, such as phenolics/polyphenols, terpenoids/essential oils, and lectins/polyphenols (Windisch et al. 2008). Essential oils have two major classes of compounds, terpenes (e.g., carvacrol and thymol) and phenylpropenes (e.g., cinnamaldehyde and eugenol) (Omonijo et al. 2018). Flavonoids such as resveratrol, epigallocatechin gallate, and phenols such as galangin, puerarin, and ursolic

acid are proven to be effective as antimicrobial agents (Shin and Park 2018).

Many plants have beneficial multifunctional properties derived from their specific bioactive components. Large variations in composition result from (1) different factors (i.e., plant species, growing location, harvest conditions), (2) manufacturing (i.e., extraction/distillation, stabilization), and (3) storage conditions (i.e., light, temperature, oxygen tension, time). The challenge is to identify and quantify the multitude of actions and claims improving feed utilization, animal physiology, and health status.

A wide range of plants and their products are in this beneficial category and, based on their origin (part of the plant), can be broadly classified as herbs (flowering, non-woody, non-persistent plants from which leaves and flowers are used) or spices (non-leaf parts of plants, including seeds, fruits, bark, or root with intensive taste or smell) (Windisch et al. 2008). They can be used in solid, dried, and ground form or as extracts (crude or concentrate) (Gadde et al. 2017). A common feature of phytobiotics is that they are a very complex blend of bioactive components. The biologically active constituents are compounds derived from plants or parts thereof (leaves, roots, flowers, etc.) (Huyghebaert et al. 2011). The wide range of modes of action of different phytogenic activities can optimize nutrient digestibility and support intestinal health. Phytogenics can promote the growth of beneficial intestinal bacteria for the host and promote increased concentrations of SCFA in the ileum and colon while reducing potentially harmful bacteria and the production of protein-derivative catabolites.

Phytogenics, or plant-based compounds, are specifically essential oils of herbs and spices known to have a narrow range of biologically active properties that can be applied in modern animal production. Phytogenics are potent to improve nutrient utilization, stimulate digestive enzymatic activity (i.e., intestinal and pancreatic enzyme production), increase bile flow, and even show anti-oxidant, antimicrobial, antiinflammatory, and immunomodulatory effects as well as maintaining the gut growth (intestinal barrier functions) and health and feed palatability enhancement (Windisch et al. 2008).

Experimental data show the in vitro antimicrobial effects with respective minimal inhibitory concentration (MIC) values and spectrum of activity (Fu et al. 2007; Barbosa et al. 2009). The MIC of essential oils needed for killing enteric pathogens may not ensure the optimal feed intake in swine production. The lipophilic and volatile nature of essential oils creates a challenge in their effective delivery within the pig gut, and this challenge can be partially resolved by microencapsulation and nanotechnology (Omonijo et al. 2018). According to Adams (1999), antimicrobial activity is rather weak for ginger (*Zingiber officinale* Roscoe) and pepper (*Piper* spp.), medium for cumin (*Cuminum cyminum*), coriander (*Coriandrum sativum*) (also known as cilantro), oregano (*Origanum vulgare*), rosemary (*Rosmarinus officinalis*), sage (*Salvia officinalis*), and thyme (*Thymus vulgaris*), and strong for clove (*Syzygium aromaticum*), mustard (Lyrica), cinnamon (*Cinnamomum cassia*, *Cinnamomum zeylanicum, Cinnamomum verum*), and garlic (*Allium sativum*) (Table 4). Other biologically active plant extracts include soybean derivatives, *Allium* derivatives, carvacrol, *Curcuma longa* derivatives, *Astragalus* derivatives, *Capsicum* derivatives, *Achyranthes* derivatives, *Echinacea*, and *Origanum* derivatives.

Phytogenics are characterized by their plant-derived origin, being natural and proven to be safe. The phytogenic feed additives consist mainly of essential oils, bitter and pungent substances, saponins, flavonoids, mucilages, and tannins; it is evident that they are not only for sensorial stimulation but also stimulate various physiological processes. It is very well known that components used in phytogenics are generally approved in the EU for use in animal feed as "sensory

Table 4 Natural phytobiotic substances and chemical composition

Natural substances	Chemical composition
Cinnamon species Family Lauraceae	Monoterpenes (pineme, camphene, liminene), sesquiterpenes The main constituent of cinnamon bark oil is cinnamaldehyde
Coriander Family Apiaceae	Linalool (unsaturated terpene)
Clove Family Myrtaceae	Eugenol and β-caryiphyllene
Cumin (black) Family Apiaceae	*p*-Cymene (alkylbenzene related to a monoterpene)
Garlic Family Liliaceae	Allicin: the main constituents of garlic oil are allylpolysulfifes (diallyl sulfide, diallyl disulfide, diallyl trisulfide)
Ginger Family Ziniberaceae	6-Gingerol, 8-gingerol, 10-gingerol, methylgingerol, gingerdiol, dehydrogingerdione, gingerdiones, diarylheptanoids (curcuminoids), diterpene lactones, galanolactone)
Mustard Family Brassicaceae	Allylisothiocyanate
Oregano Family Lamiaceae	Carvacrol, *o*-cymophenol (monoterpenoid phenol)
Pepper Family Solanaceae	Capsaicin, dihydrocapsaicin, nordihydrocapsaicin, homocapsaicin, homodihydrocapsaicin
Rosemary Family Lamiaceae	Carnosic acid, carnosol, 12-*O*-methylcarnosic acid, rosmarinic acid, genkwanin, isoscutellarein-7-*O*-glucoside
Sage Family Lamiaceae	Cineol or eucalyptol (essential oil)
Thyme Family Lamiaceae	Thymol, monoterpene phenol derivative of cymene

additives" or "flavors" because of their flavor characteristics. However, it is important to understand which flavours or compounds might have a negative impact on feed intake by the animals and to know the acceptable dose, mask their taste, or replace them altogether in the formula. Composition of phytogenetic products and optimal inclusion feed levels must be based on the dose–response trials by animal species. Moreover, the bioactive components of herbs and species can have functional properties besides their flavouring properties.

Essential oils have been shown to have good potential as antibiotic alternatives in feeds for swine production. The combination of different essential oils and other compounds (synergistic effect) such as organic acids seems to be a promising approach to improve the efficacy and safety of essential oils in applications.

Although phytogenics, and especially essential oils, have demonstrated direct antibacterial effects, high concentrations are needed to guarantee such effects and are less attractive for economic or sensorial reasons. Nevertheless, small amounts of various plant-derived essential oils effectively reduce the production of virulence factors by bacteria, so-called quorum sensing (QS), resulting in reduced toxin production, adhesion factors, and biofilm formation. QS is a process of cell–cell communication that allows bacteria to share information about cell density and adjust gene expression accordingly. This process enables bacteria to express energetically expensive processes as a collective only when the impact of those processes on the environment or on a host will be maximized (Rutherford and Bassler 2012). Overall, the effects of essential oils on inflammation, oxidative stress, microbiome, gut chemosensing, and bacterial QS have led to better production performance of animals fed essential oils in a number of studies (Omonijo et al. 2018). Essential oils are aromatic oily liquids obtained from plant material and usually have the characteristic odor or flavor of the plant from which they are derived (Stein and Kil 2006). Typically mixtures of secondary plant metabolites, they may contain phenolic compounds (i.e., thymol, carvacrol, eugenol), terpenes (i.e., citric and pineapple extracts), alkaloids (capsaicine), lectins, aldehydes (i.e., cinnamaldehyde), polypeptides, or polyacetylenes (Thacker 2013). Many plants produce chemicals with therapeutic and protective properties, the isolation and concentration of which may produce potent compounds that could potentially improve animal health and performance. These phytogenics benefits are derived from improved intestinal health, including improved digestion, modification of digestive secretions, and support of the histology of the intestine (Diaz-Sanchez et al. 2015). In addition to herbs and spices, various essential oils (thymol; carvacrol; cinnamaldehyde; essential oils from clove, coriander, star anise, ginger, garlic, rosemary, turmeric, basil, caraway, lemon, and sage) have been used either individually or as blends to improve animal health and productivity (Gadde et al. 2017).

The principal use of phytobiotics in poultry and swine has been the administration of essential oils, which have been used for a long time in the preparation of feed as artificial flavors and preservatives. Most essential oils have been classified as Generally Recognized as Safe (GRAS), by the US FDA. These oils are characterized as being involved in antimicrobial activities and having growth-promoting properties. Several oils, including carvacrol and thymol obtained from oregano and eugenol from the clove plant, have been shown to inhibit a wide range of pathogenic bacteria (Dorman and Deans 2000). Several studies have reported controlled experiments in which oils have been used as feed additives to reduce the presence of different pathogens in the intestine, including, for example, *Salmonella* using capsaicin (Tellez et al. 1993; Vicente et al. 2007); *E. coli* (Jamroz et al. 2005); *Campylobacter* (Ali 2014), and *Clostridium perfringens* (Mitsch et al. 2004). These results suggest that the effectiveness of essential oils varies, principally because their active components can differ depending on the method of extraction, geographic origin, plant genotype, and storage time.

Plant extracts have been reported to alter the composition of the microbiota in poultry (Guo et al. 2004) and pigs (Castillo et al. 2006). Cinnamaldehyde, carvacrol, eugenol, and thymol have received the most interest for use in swine production. The exact mode of action of essential oils has not been established but the activity may be related to changes in lipid solubility at the surface of the bacteria. The hydrophobic constituents of essential oils allow them to disintegrate the outer membrane of *E. coli* and *Salmonella* and thus inactivate these pathogens (Stein and Kil 2006). Essential oils are aromatic, volatile, and oily liquids extracted from plant materials such as seeds, flowers, leaves, buds, twigs, herbs, bark, wood, fruits, and roots; essential oils containing phenolic compounds tend to have greater antimicrobial activity than oils containing other compounds (Brenes and Roura 2010). Based on the fact that essential oils appear to control pathogenic bacteria, several research groups have attempted to determine whether the inclusion of essential oils in swine diets can improve pig performance (Ragland et al. 2008). The results have been inconclusive; some trials have demonstrated positive results whereas others have reported no beneficial effects. Conclusive evidence for including essential oils in diets fed to swine was demonstrated by a trial that compared the performance of pigs fed an unsupplemented control diet with that of pigs fed a diet supplemented with antibiotics or a combination of thymol (*Thymus vulgaris*) and cinnamaldehyde (*Cinnamomum zeylanicum, Cinnamomum verum*) (Li et al. 2012). Weight gain, FCR, and fecal consistency of pigs fed essential oils was essentially equal to that of pigs fed antibiotics. In addition, total antioxidant capacity and levels of the cytokines

interleukin 6 (IL-6) and tumor necrosis factor-α (TNF-α) were altered by inclusion of essential oils. The variability could result from differences in the type of essential oils and dose used (Li et al. 2012). It appears that oils containing phenolic compounds have greater antimicrobial activity than those based on other compounds. In addition, if the dose used is too high, the strong smell can reduce feed intake and thereby limit pig performance (Stein and Kil 2006). Another important consideration is the stability of essential oils during pelleting.

Eucalyptus oil, a MCFA is obtained from the leaves of the eucalyptus, a tree in the plant family *Myrtaceae* that is cultivated worldwide. Eucalyptus oil has also been shown to stimulate the immune system by affecting the phagocytic ability of monocyte-derived macrophages (Serafino et al. 2008). In poultry, dietary inclusion of eucalyptus has been shown to improve production performance and stimulate the immunity of commercial laying hens (Abd El-Motaal et al. 2008).

MCFAs have been suggested as an alternative feed additive to antibiotics for piglets (Hong et al. 2012). MCFAs have shown antimicrobial activity against *Salmonella* (Rossi et al. 2010) and *E. coli* (Dierick et al. 2002). Feeding a blend of caprylic and caproic acids has been reported to improve performance and nutrient digestibility in 3- and 4-week-old weaned pigs during the first 2 weeks after weaning (Hong et al. 2012). A recent review in vitro study demonstrated the influence of various MCFAs on the growth of swine-specific pathogenic germs (Omonijo et al. 2018). The effects of essential oils on inflammation, oxidative stress, microbiome, gut chemosensing, and bacterial QS have led to better production performance of animals fed essential oils in a number of studies. It has been demonstrated that essential oils have good potential as antibiotic alternatives in feeds for swine production.

Bacteria cause many problems on pig farms. *Streptococcus suis* is a species of pathogenic gram-positive bacterial strains that represents a primary health problem in the swine industry worldwide. *S. suis* is also an emerging zoonotic pathogen that causes severe human infections clinically featuring varied diseases or syndromes such as meningitis, septicemia, and arthritis. Infections with streptococci are considered to be a prime reason for the high use of antibiotics in piglet production (Feng et al. 2014). Piglets are generally infected during or shortly after birth, with germs capable of entering the animal through even the tiniest wounds. The special feature of the dangerous streptococci is that they possess the ability to enter the bloodstream, where they settle in certain places in the body via the blood and reproduce. Once the pathogen has settled in the animal, it can cause a wide variety of disease patterns. With pneumonia, inflammation of the joints, and meningitis, streptococci can also cause sudden death. The aim must therefore be to minimize the germ pressure as early and as much as possible.

MCFAs are particularly important in animal nutrition because of their energy-supplying ingredients and antibacterial effect. These properties are highly interesting for use in pig farming. The pressure of infection on the animals, triggered by swine-specific pathogenic germs, is one of the main reasons for using antibiotic medications. Many in vitro studies displayed pronounced antibacterial effects following the use of MCFAs against both gram-negative and gram-positive bacteria. Lauric acid and glycerol monolaurate are considered to be particularly active antibacterial ingredients (Omonijo et al. 2018).

Salmonella invasion in intestinal epithelial cells is decreased and colonization of *Campylobacter jejuni* is reduced in broiler chickens when administered a mixture of MCFAs containing lauric acid. In particular, lauric acid has a strong antibacterial effect against *Clostridium perfringens* strains, significantly decreasing necrotic lesions in the gut. The MIC of the different additives were determined in vitro, showing that lauric acid, thymol, and cinnamaldehyde are very effective in inhibiting the growth of *Clostridium perfringens*. The efficacy of target-released butyric acid, MCFAs (C6 to C12, but mainly lauric acid), and essential oils (thymol, cinnamaldehyde, essential oil of eucalyptus) micro-encapsulated in a poly-sugar matrix to control necrotic enteritis was investigated. Combining butyrate and mid-chain fatty acids, such as lauric acid, gave the best protection against these lesions and can be used for controlling necrotic enteritis in broilers. These results suggest that butyric acid, medium-chain fatty acids, and essential oils may contribute to the prevention of necrotic enteritis in broiler chickens (Timbermont et al. 2010). MCFAs have a pronounced antimicrobial effect on a wide range of bacterial species, towards both gram-positive and gram-negative bacteria: this effect is higher than for SCFAs.

Micro-encapsulation of MCFAs is a process in which medium-chain fatty acids are nano-micronized to extremely small particles and then encapsulated. A product of eucalyptus extract mixed with caprylic and carpric acids and encapsulated with palm oil was tested in comparison with antibiotics or zinc oxide. The performance of pigs fed the eucalyptus–medium-chain fatty acid blend was essentially equal to that of those receiving antibiotics or zinc oxide. The performance-enhancing effects of the blend appeared to be mediated through improvements in nutrient digestibility (Han et al. 2011).

An alternative approach to support the intestinal health of farm animals may be found by combining the beneficial effects of phytogenic feed additives with the antibacterial effects of esterified SCFAs and MCFAs. Both SCFAs and MCFAs possess antimicrobial properties and influence the composition of the microbiota (Chaveerach et al. 2004; Solis

de Los Santos et al. 2008). SCFAs are produced by fermentation of indigestible oligosaccharides and may alter the pH of the intestinal environment, changing the relative proportions of susceptible bacteria, including *Salmonella* and *C. perfringens* (Chaveerach et al. 2004; Van Immerseel et al. 2006). MCFAs have potent antimicrobial properties in vitro and in vivo. For example, lauric acid has been shown to target *C. perfringens* (Skrivanova et al. 2005), whereas caprylic acid is effective against *E. coli* (Marounek et al. 2003) and *Campylobacter jejuni* (Solis de Los Santos et al. 2008).

A possible influence of phytogenics on the efficacy of antibiotics through combined administration would require a change in application recommendations of antibiotics and phytogenic feed additives. A trial evaluated to what extent this is the case.

Many antibacterials used in animals are applied via the waterline, where they are dosed in combination with other feed additives. Amongst those there are mixtures of secondary plant compounds with a proven antimicrobial efficacy against veterinary pathogenic bacteria. However, there is only sparse information available if and to which extent antibiotics and phytogenics influence each other. There are several scenarios possible: (1) no interaction (if the bacterial strain is sensitive to both, antibiotics and phytogenics, and they do not influence each other in their antimicrobial efficacy, then a combined application with their recommended dosages is possible without restrictions and limitations); (2) negative interaction (if the combined use of antibiotics with phytogenic feed additives attenuates the efficacy of antibiotics, a combined application would not be recommended); (3) positive interaction (if phytogenics increase the effectiveness of antibiotics, a combined application of both might generate a synergistic antimicrobial effect on certain veterinary pathogenic bacteria). There are reports on the adverse interactions of phytogenics with enzyme preparations (Sarica et al. 2005). An alternative approach to support the intestinal health of farm animals may be found by combining the beneficial effects of phytogenic feed additives with the antibacterial effects of esterified SCFAs and MCFAs. In a trial, this combination was shown to improve the efficacy of poultry performance, even under challenging conditions involving necrotic enteritis (Dahiya et al. 2006). In terms of performance, feeding the combination of the phytogenic feed additive and esterified SCFAs and MCFAs for 28 days was effective. The wide spectrum of modes of actions of phytogenic feed additives based on plant extracts show the synergistic effects of many different bioactives, which have not been reduced to the effects of a single lead substance.

Nonetheless, although the mechanisms of action of the most phytotherapeutic chemicals are not fully understood, their synergistic antimicrobial activity is generally assumed to be the result of bacterial membrane disruption by lipophilic compounds or their blockade of cell division by DNA synthesis inhibition (Chandar et al. 2017).

6.3 Antimicrobial Peptides

Antimicrobial peptides (AMPs) are widely distributed and are classified into two categories, non-ribosomally synthesized AMPs and ribosomally synthesized AMPs, according to the peptide synthesis mechanism. The non-ribosomal AMPs, mainly produced by bacteria, are synthesized by peptide synthetases and structural modifications (i.e., gramicidin, polymyxin, bacitracin, sugar peptide) (Cheng et al. 2014).

Reports have indicated the protective effect of antimicrobial peptides on animals. Gene-encoded natural antibiotics that have gained recent attention include host-derived antimicrobial peptides such as defensins and cathelicidins, which provide a protective response against bacterial infection and are a principal component of innate immunity in vertebrates (Sang and Blecha 2008). For instance, porcine host defense peptides are a large group of innate immune antimicrobial peptides that possess antibacterial activity (Sang and Blecha 2008). Cathelicidins are host defense peptides that were first described in mammals, and are also found in birds, that exhibit both antimicrobial and immunomodulatory activities (van Dijk et al. 2009, 2011) which could be used to control pathogens such as *Campylobacter jejuni* (van Dijk et al. 2012). Other peptides such as lactoferricin B, a 25-residue peptide derived from the N-terminal domain of bovine lactoferrin, and synthetic derivatives of this peptide cause depolarization of the cytoplasmic membrane in susceptible bacteria and have antimicrobial activity (Liu et al. 2011). Antimicrobial peptides such as cecropins (Boman 1991) and magainins (Zasloff 1987), a chimeric peptide, are derived from insects and amphibians, respectively. Cecropìn A added to the feed diet increased weight gain, feed intake, feed:gain ratio, and intestinal villus height while decreasing aerobic bacterial counts in both jejunal and caecal digests. Other antibacterial peptides are the bacteriocins, which are defined as ribosomally synthesized peptides secreted by various bacteria that have antibacterial activity against other similar or closely related bacteria. Bacteriocins produced by lactic acid bacteria (LAB) generally function to suppress competitor species that are primarily active against other gram-positive bacteria (Cotter et al. 2005). In the past, bacteriocins were used as food preservatives. Several bacteriocins have been indentified, such as nisin, lactacin, lactocin, helveticin, fermenticin, sakacin, lacticin, plantacin, and subticin, among others in genera including gram-positive and gram-negative bacteria as well as Archaea. In vitro tests show that bacteriocins have strong killing and suppressive effects on a variety of pathogens, including resistant pathogens. The

antibacterial spectrum of most bacteriocins is narrow, only effective to the related bacterial species. One of the most reported as a dietary supplement in poultry was divercin AS7 (produced by *Carnobacterium divergens* AS7), a lactic acid-producing bacterium isolated from fish, which has been extensively studied in broiler chickens for growth-promoting effect, digestibility, and modulatory effect on intestinal microbiota (Józefiak et al. 2012).

In addition, dietary nisin (produced by *Lactococcus lactis*) exerted a modulatory effect on the microbial ecology of the GIT with decreased counts of *Bacteroides* and *Enterobacteriacae*, but unchanged counts of *Clostridium perfringens*, *Lactobacillus* spp., *Enterococcus* spp., and total bacteria (Józefiak et al. 2013). Albusin B (produced by *Ruminococcus albus* 7), another bacteriocin added to poultry feed, also showed improved growth performance, increased intestinal absorption and *Lactobacillus* counts, modulated lipid metabolism, and activated systemic antioxidant defense (Wang et al. 2013). Overall, the use of antimicrobial peptides including bacteoricins have potential to enhance animal health and productivity.

6.4 Bacteriophages and Their Endolisins

Bacteriophages, which are highly species-specific viruses that kill bacteria, have been considered agents to treat bacterial infections through the production of endolysins and subsequent lysis of the bacterial cells. Bacteriophages can be considered safe antibiotic alternatives as they exhibit no activity against animal and plant cells. Bacteriophages (phages) have been utilized as treatment for bacterial diseases in Europe (Sulakvelidze 2005), particularly in poultry (i.e., to reduce mortality in poultry caused by *Staphylococcus gallinarum* and *Pullorum* (Atterbury et al. 2007) and in cattle (Smith et al. 1987). Preparations of bacteriophages are commercially available in many parts of the world. The FDA accepted the use of a bacteriophage preparation as a food additive (Bren 2007). An important extension to bacteriophage therapy is the use of phage lytic enzymes that digest the bacterial peptidoglycan, especially of gram-positive bacteria, as a novel class of alternative antimicrobials (Fischetti 2008; Schmelcher et al. 2012a). The phage lytic enzymes can be applied externally and have a variety of biochemical activities that can be fused into recombinant chimeric molecules which synergistically retain their parental activities to digest bacterial cell walls, thereby avoiding resistance development (Schmelcher et al. 2012b). Bacteriophages can replicate in host cells and are able to produce new lytic phages to keep pace with the mutation of pathogens. Many of these phage lytic enzymes are highly species specific (Simmons et al. 2010), and their cell wall-binding (CWB) domains can also be used for bacterial detection systems (Schmelcher et al. 2010). Phages can cause the release of toxins [e.g., endotoxin (lipopolysaccharides, LPS)], in large quantities from bacteria, especially gram-negative bacteria, which may account for several side effects on the host such as the development of an inflammatory cascade leading to multiple organ failure (Chen et al. 2014).

An important extension to bacteriophage therapy is the use of phage lytic enzymes that digest the bacterial peptidoglycan, particularly of gram-positive bacteria (Schmelcher et al. 2012a). Bacteriophages are defined as specific intracellular parasites of bacteria that multiply using the metabolic machinery of their hosts. There are two large kinds of phages: (1) virulent phages, with a lytic life cycle; and (2) temperate phages, with a lysogenic life cycle. In a lytic life cycle, the phage recognizes specific bacteria, injects its genetic material, and then uses the metabolic machinery of the host to replicate and assemble copies of itself. A process of cellular lysis mediated by the phage then frees the virions assembled within the interior of the cell. Once freed, these new virions can infect another cell, reinitiating the cycle. By contrast, in the lysogenic life cycle the phage recognizes the host cell, the injected DNA is incorporated into the bacterium genome, and replicates with it. Under certain conditions, this DNA can detach from the genome and initiate a lytic life cycle. As it leaves the bacterial genome, the phage DNA can take information with it which can be transferred to its next host. This process might impart undesirable characteristics to the new host, such as virulence factors or antibiotic resistance genes.

Phage therapy is defined as the use of phages to treat bacterial infections; the term is restricted to the employment of virulent phages. Its application to humans was described almost as soon as these viruses were discovered, in 1915 (Abedon et al. 2011). However, the bacteriophages use was displaced by the discovery of penicillin and continued only in some countries of the former Eastern Block (Summers 2012). Today, the problematic emergence of multi-drug-resistant (MDR) bacteria has provided a new focus on bacteriophages as a natural, nontoxic alternative treatment of bacterial infections. The advantages of phage therapy include that treatment with phages can target a specific group of bacteria, with the result that the normal microbiota is not affected, reducing, thereby, the risk of secondary infections associated with antibiotic therapies (Loc-Carrillo and Abedon 2011). Phage therapy is one of the strategies available to manipulate the gut microbiome; it has promising results and also has some advantages over the other technologies. Phages are considered to be more effective than antibiotics as they only multiply when their specific host is present, which implies that phages have the ability to increase their density in situ. Equally, following infection, once the concentration of the host has been reduced, the population of phages diminishes as well. Another important advantage is that phages can be

effective against sensitive bacteria as well as strains that are resistant to antibiotics (Nilsson 2014). Phages are able to target only certain groups of pathogenic bacteria without having any negative effect on the normal microbiota of a given niche (Sulakvelidze 2011), which, as has been shown throughout, fulfills important functions in the host.

The application of phages has been described for humans (Abedon et al. 2011), different models in animals, plants, and food (Cooper 2016). A bacteriophage cocktail that targets *Listeria monocytogenes* contaminants on ready-to-eat foods containing meat and poultry products was approved in 2006 by the US FDA (Sulakvelidze 2013). In spite of this, several studies have used bacteriophages in animals to control bacteria transmitted by foodstuffs. These models include the use of phages to control *Salmonella* and *Campylobacter* in broiler chickens (Wernicki et al. 2017). It is widely recognized that pathogens such as *Campylobacter* and *Salmonella* can be transmitted along the food chain and can be the source of human illness. In all cases, phages have been administered orally, either as a feed supplement, in water, or using a gavage after the birds have been challenged with a given concentration of the pathogen (Grant et al. 2016). Published studies show that the application of phages in higher concentrations than the targeted microorganism is more successful in reducing the presence of the latter (Bardina et al. 2012). It should also be considered that cocktails of phages are more effective than individual applications. Additionally, it has been demonstrated that treatment with phages is more effective when it precedes the exposure to the pathogen (Wong et al. 2014). On administration routes, even though most of the studies have been carried out using oral *gavages*, comparing the effectiveness of treatments conducted using a *gavage* with those in which phages were administered as a feed supplement, both approaches obtained reductions of 1.7 log10 and 2 log10 CFU/ml, respectively (Carvalho et al. 2010). In a different approach, an extremely interesting study showed that the application of phages alongside with probiotics is more effective in reducing *Salmonella* than applying each treatment separately (Toro et al. 2005).

Bacteriophages are viruses that are parasitic on bacteria, and have long been considered as one of the types of agents to treat bacterial infections. A bacteriophage cocktail that targets *Listeria monocytogenes* contaminants on ready-to-eat (RTE) foods containing meat and poultry products was granted approval during 2006, which was the first time that the US FDA accepted the use of a bacteriophage preparation as a food additive (Bren 2007). Preparations of bacteriophages are commercially available in many parts of the world to reduce mortality in poultry caused by *Salmonella gallinarum* and *S. pullorum*. Lytic phage has also been used to treat a fatal neonatal meningitis *E. coli* infection of rats (Pouillot et al. 2012). An important extension to bacteriophage therapy is the use of phage lytic enzymes (PLEs) that digest the bacterial peptidoglycan, especially of gram-positive bacteria, as a novel class of alternative antimicrobials (Fischetti 2008; Schmelcher et al. 2012a). PLEs can be applied externally and have a variety of biochemical activities that can be fused into recombinant chimeric molecules which synergistically retain their parental activities to digest bacterial cell walls, thereby avoiding resistance development (Schmelcher et al. 2012b). Many of these enzymes are highly species specific (Simmons et al. 2010), and their cell wall-binding domains can also be used for bacterial detection systems (Schmelcher et al. 2010). Currently, the main challenge for the promotion of phage preparations is the lack of data obtained from large-scale clinical trials, thus hindering their universal application. Regulatory loopholes remain another major hurdle. In addition to the inherent safety concern, neither the US FDA nor the EMA has an approval processing place that can easily accommodate the ever-changing combinations of phages that accompanies the need to continuously develop the product to stay one step ahead of evolving MDR bacteria (Miedzybrodzki et al. 2012).

Another alternative to the use of bacteriophages is the application of bacteriophage endolysins (or lysins) (glucosidase, amidase, endopeptidase, transglycosylase), which are lytic enzymes encoded by bacteriophages that decompose the bacterial cell wall peptidoglycan during the late stage of the phage reproduction lytic cycle, degrading bacterial peptidoglycan to facilitate the release of new phages from the infected bacteria. Endolysins can treat sepsis and a few gram-positive bacteria infections but is poor against gram-negative bacteria (Cheng et al. 2014). Endolysins can quickly kill susceptible strains with wider antibacterial spectrum than phages and the activities are easier to detect.

These enzymes present some advantages and disadvantages over living phages. The advantages are (1) lysins are not self-replicating, meaning they are more targeted and defined control; (2) resistance to these enzymes has not yet been reported; (3) they can be identified and used from temperate and virulent phages; and (4) lysins have the potential to be used in many environments (humans, animals, food, biofilms, etc.) (O'Flaherty et al. 2009). Among the important disadvantages of lysins are (1) there is a lack of effectiveness against gram-negative bacteria; and (2) bacteriocins are protein, therefore are susceptible to inactivation. Several reports on the antimicrobial application of endolysins along the food processing line have been carried out, mainly directed to *Staphylococcus aureus* and *Listeria monocytogenes* in dairy products (Oliveira et al. 2012).

It is also important to note that all other *Clostridium* species were resistant to lytic activity, demonstrating species specificity for *C. perfringens*. No other reports of lysins with potential use in broiler chickens were found, perhaps because

most of the food-borne contamination is caused by gram-negative bacteria (Zimmer et al. 2002).

It should be stressed that phage therapy still presents limitations, such as variability in the results obtained, which might be explained by different reasons: (1) the development of resistance to phages by target bacteria, (2) the low multiplicity of infection, (3) the inaccessibility of the target microorganism, and (4) the deactivation of phages by the host. The most important limitations to employ bacteriophages in producer farms is the lack of approval and regulation for their use with animals, and acceptance of the therapy by the producer community, given that it is a relatively new technology. However, if such approval is to be achieved, research into the effectiveness of phages in the commercial conditions of factory farming is still required (Grant et al. 2016).

Only one study using bacteriophages in broiler chicken flocks using *Campylobacter* phages is available (Kittler et al. 2013). The authors of this chicken study carried out three field trials, two in the same farm but in different sheds, and the third was carried out in another farm. The cocktail of four phages was supplied via drinking water to a final concentration of 10^5–10^7 CFU/ml. In the first trial, a reduction up to 3.2 CFU/g of *Campylobacter* load in the caecal content was achieved, compared to the control. However, no significant reduction was observed in the experimental groups of the other trials, indicating that additional research is required for large-scale application of the phages (Kittler et al. 2013).

Overall, bacteriophages represent a promising alternative for the control of *Salmonella* and *Campylobacter* in food-producing animals. However, replicable studies that demonstrate the effectiveness of the technology in intensive production systems are still required.

6.5 Immunomodulatory Compounds: Vaccines

Immunomodulatory compounds such as lactoferrin may also affect the microbiota. Lactoferrin was shown to affect the bacterial community in the caecum of poultry (Geier et al. 2011). In pigs, lactoferrin increased numbers of *Lactobacillus* and *Bifidobacteria* and decreased numbers of *E. coli* and *Salmonella* (Wang et al. 2007). Other compounds, including bacterial products such as bacteriocins (Shin et al. 2008) and immunomodulatory compounds, may also be important in modifying the intestinal microbiota.

One technique that appears to have considerable potential as an alternative to antibacterials in the presence of disease-causing organism is the use of egg yolk antibodies, usually referred to as IgY. The injection of the antigens induces an immune response in the hen, resulting in the production of antibodies. Approaches utilizing pathogen-specific antibodies in animal feeds are based on the fact that transfer of avian maternal antibodies from serum to egg yolk (the antibodies are deposited in the egg yolk) can confer passive immunity to embryos and neonates (Tini et al. 2002). Consequently, passive immunization by oral administration of specific antibodies is a possible alternative to antibiotic treatment to reduce gastrointestinal pathogens in animals. Specifically, based on treatment with specific antibodies targeting *E. coli* adherence-associated proteins (Cook et al. 2007), orally administered pathogen-specific antibodies may alleviate enteric diseases.

7 Regulations Concerning Feed Additives in the European Union

Article 5 of Regulation (EC) No. 1831/2003 (EC 2003) states that a feed additive (1) shall not have an adverse effect on animal health, human health, or the environment, (2) shall not be presented in a manner that may mislead the user, and (3) shall not harm the consumer by impairing the distinctive features of animal products, or mislead the consumer with regard to the distinctive features of an animal product (Anadón et al. 2018).

According to the Regulation (EC) No. 1831/2003, a feed additive is a substance, microorganisms, or preparations, other than feed material and premixtures, that is intentionally added to feed or water to perform, in particular, one or more specific functions that are enumerated in Article 5(3) of the Regulation (EC) No. 1831/ 2003 (EC 2003): (1) favorably affect the characteristics of feed; (2) favorably affect the characteristics of animal products; (3) favorably affect the color of ornamental fish and birds; (4) satisfy the nutritional needs of animals; (5) favorably affect the environmental consequences of animal production; (6) favorably affect animal production, performance or welfare, particularly by affecting the gastrointestinal flora or digestibility of feedstuffs; or (7) have a coccidiostatic or histomonostatic effect; and shall be allocated to one or more of the following categories:

Technological Additives Technological additives are defined as any substance added to feed for a technological purpose, including the following functional groups. (1) Preservatives: substances or, when applicable, microorganisms which protect feed against deterioration caused by microorganisms or their metabolites; (2) antioxidants: substances prolonging the storage life of feedstuffs and feed materials by protecting them against deterioration caused by oxidation; (3) emulsifiers: substances that make it possible to form or maintain a homogeneous mixture of two or more immiscible phases in feedstuffs; (4) stabilizers: substances which make it possible to maintain the physicochemical state of feedstuffs; (5) thickeners: substances which increase the viscosity of feedstuffs; (6) gelling agents:

substances that give a feedstuff texture through the formation of a gel; (7) binders: substances that increase the tendency of particles of feedstuffs to adhere; (8) substances for control of radionuclide contamination: substances that suppress absorption of radionuclides or promote their excretion; (9) anti-caking agents: substances that reduce the tendency of individual particles of a feedstuff to adhere; (10) acidity regulators: substances that adjust the pH of feedstuffs; (11) silage additives: substances, including enzymes or microorganisms, intended to be incorporated into feed to improve the production of silage; (12) denaturants: substances that, when used for the manufacture of processed feeding stuffs, allow the identification of the origin of specific food or feed materials.

Sensory Additives Sensory additives are defined as any substance that improves or changes the organoleptic properties of the feed and/or the visual characteristics of food derived from an animal, and include the following functional groups: (1) colorants: substances that add or restore color in feeding stuffs; substances that, when fed to animals, add color to food of animal origin; and substances that favorably affect the color of ornamental fish or birds; and (2) flavoring compounds: substances when included in feedstuffs increase feed smell or palatability (Anadón et al. 2018).

Nutritional Additives Nutritional additives supply a specific nutrient required by the animal for optimal growth. The following functional groups are included: (1) vitamins, pro-vitamins, and chemically well-defined substances having similar effect; (2) compounds of trace elements; (3) amino acids, their salts, and analogues; and (4) urea and its derivatives (Anadón et al. 2018).

Zoo-technical Additives Zoo-technical additives are defined as any additive used to favorably affect the performance of animals in good health or to favorably affect the environment, including the following functional groups: (1) digestibility enhancers, substances that, when fed to animals, increase the digestibility of the diet, through action on target feed materials; (2) gut flora stabilizers, microorganisms or other chemically defined substances which, when fed to animals, have a positive effect on the gut flora; (3) substances that favorably affect the environment; and (4) other zoo-technical additives. Commission Regulation (EC) No. 429/2008 (EC 2008) on detailed rules for the implementation of Regulation (EC) No. 1831/2003 as regards the preparation and the presentation of applications and the assessment and the authorization of feed additives is in application.

This Regulation (EC) No. 429/2008 (EC 2008) includes general provisions for safety and efficacy studies to be carried out in support of the application of feed additives. Specific guidelines have been prepared for the FEEDAP Panel of EFSA for feed additives that are already authorized for use in food in minor species, as nutritional additives in pets, and for other non-food-producing animals as well as for sensory additives other than flavoring compounds, technological additives (silage additives), technological additives other than silage additives, zoo-technical additives (enzymes, microorganisms), zoo-technical additives other than enzymes and microorganisms, and coccidiostats and histomonostats, among others (Anadón et al. 2018). Regulation (EC) No. 1831/2003 will establish a community procedure for authorizing the placing on the market and use of feed additives and to lay down rules for the supervision and labelling of feed additives and pre-mixtures to provide a basis for the assurance of a high level of protection of human health, animal health and welfare, the environment, and users' and consumers' interests in relationship to feed additives, while ensuring the effective functioning of the internal market. This regulation will not apply to processing aids and veterinary medicinal products as defined in Directive 2001/82/EC (EC 2001) Directive 2004/28/EC (EC 2004) amending Directive 2001/82/EC on the Community code relating to veterinary medicinal products with the exception of coccidiostats and histomonostats used as feed additives Regulation (EU) 2019/6 of the European Parliament and of the council of 11 December 2018 on veterinary medicinal products and repealing Directive 2001/82/EC recentely published. However, prebiotics (inulin, fructo-mannan-oligosaccharides, yeast cell walls rich in β-glucans) or SCFAs and MCFAs are considered as feedstuffs and not as feed additives and so fall under the scope of Regulation (EC) No. 767/2009 (EC 2009) concerning the marketing and use of animal feeds. However, the classification of a product as either a feedstuff or a feed additive remains unclear because of differences in the relative impact of technological processing (Huyghebaert et al. 2011). Botanical or herbal extracts, flavours, and etheric oils now fall within the scope of Regulation (EC) No. 1831/2003 (EC 2003). In general, unprocessed herbs are regarded as feed materials and do not need authorisation. Notified plant extracts or components are included in the "Community Register of Feed Additives" established as laid down by Article 17 of Regulation (EC) No. 1831/2003 (EC 2003). The Commission has established this Community Register, which is regularly updated, and it makes reference/links to the relevant authorization. This Register is composed of two parts: the first part, Annex I, contains the list of modifications to the Register and the current authorizations, and the second part, Annex II, contains a list of additives that will be withdrawn in the short term. Also included are those for which a date is indicated in the column "Expiry date of Authorisation." Those additives are no longer allowed to be placed on the EU market if that date is expired. The Community Register of

Feed Additives has only informative purposes and does not replace community legal acts. The community legal acts concerning the authorization of each additive entered in the Register constitute the legal basis for the market placement and use of each additive concerned. Thus, before November 2010, a complete scientific dossier for each notified etheric oil or component shall be submitted to European Food Safety Authority (EFSA), which provides guidance on scientific data needed to carry out a safety assessment for botanical agents (EFSA 2009, 2014).

Unresolved Issues

A significant area of attention is enhancement of biosecurity employed in animal production, which improves the efficiency of animal production and animal welfare.

There is a strong drive by consumers, regulatory agencies, scientific committees, and governments to reduce the use of antibiotics in food-producing animals. Feed and pharmaceutical companies provide an array of nutraceuticals to use as feed additives or veterinary drugs, all offering to replace antibiotics, but not all of these can deliver on that promise. It is very important to evaluate each feed additive or veterinary drug on its claims. A description of mechanism of action, efficacy, and advantages and disadvantages of use must be available, and clear proof of the compatibility of different additives in drinking water and feed must be shown. Benefits must be visible in farms, herd, or flock conditions in animal production: only those that perform on scientific grounds are true alternatives. Replicable studies that demonstrate the effectiveness and safety of the nutraceuticals as alternatives to antibacterials in intensive production systems are required. Researchers continue to investigate and fine-tune nutritional solutions for animal husbandry to positively influence animal health and welfare and productivity and decrease use of antibiotics on intesive farms. Most of these compounds produce inconsistent results and are rarely equal to antibacterials in their effectiveness. There is still much to learn about which type or what level of fibre would be best in post-weaning diets, as investigation continues: also needed is avoiding dysbacteriosis, postweaning diarrhoea, poor hatchery environment, and mastitis among other pathological findings.

Phage therapy has promising results and also have some advantages over other technologies, but research is needed for phage use at a productive scale. The most important limitation to use of bacteriophages in producer farms is lack of approval and regulation for their use with animals, and acceptance of the therapy by the producer community, given that it is a relatively new technology. However, if such approval is to be achieved, research into the effectiveness of phages in the commercial conditions of factory farming is still required.

The use of phytogenics in farm practice is limited. The mode of action of the plant extracts is poorly investigated either because these were studied primarily with regard to mixtures or because only a few studies are available.

Essential oils and organic acids have been extensively used and studied in food animals to improve feed safety, growth rate and feed conversion, but further research is required to confirm possible improvement of productive parameters and animal health and welfare. Moreover, there is a need to validate reliable biomarkers to measure the efficacy and safety of phytogenics.

Studies on antimicrobial peptides and their applications in poultry have mostly focused on potential protection against diverse pathogens causing infectious diseases rather than growth-promoting activities. However, a few research trials have investigated the effect of antimicrobial peptides on poultry growth performance, intestinal morphology, and gut microbiology as potential AGP alternatives.

A legal basis must exist for the market placement and use of new nutraceuticals. Regulatory activity to take into account the new development and innovation of nutraceuticals to be used in food animals must be based on scientific data.

Acknowledgments This work was supported by Project S2013/ABI-2728 (ALIBIRD-CM Program) from Comunidad de Madrid, and by Project Ref. RTA2015-00010-C03-03 from Ministerio de Economía, Industria y Competitividad, Spain.

References

Aarestrup FM (2004) Monitoring of antimicrobial resistance among food animals: principles and limitations. J Vet Med B 51(8–9):380–388

Abd El-Motaal AM, Ahmed AMH, Bahakaim ASA et al (2008) Productive performance and immunocompetence of commercial laying hens given diets supplemented with eucalyptus. Int J Poult Sci 7 (5):445–449

Abedon ST, Kuhl SJ, Blasdel BG et al (2011) Phage treatment of human infections. Bacteriophage 1(2):66–85

Abt MC, Artis D (2013) The dynamic influence of commensal bacteria on the immune response to pathogens. Curr Opin Microbiol 16 (1):4–9

Adams C (1999) Nutricines: food components in health and nutrition. Nottingham University Press, Nottingham

Adil S, Banday T, Bhat GA et al (2011) Response of broiler chicken to dietary supplementation of organic acids. J Cent Eur Agric 12:498–508

Ahmad MS, Krishnan S, Ramakrishna BS et al (2000) Butyrate and glucose metabolism by colonocytes in experimental colitis in mice. Gut 46:493–499

Ali AHH (2014) Productive performance and immune response of broiler chicks as affected by dietary marjoram leaves powder. Egypt Poult Sci J 34:57–70

Amass SF, Clark LK (1999) Biosecurity considerations for pork production units. J Swine Health Prod 7:217–228

Anadón A (2006) The EU ban of antibiotics as feed additives. Alternatives and consumer safety. J Vet Pharmacol Ther 29(suppl 1):41–44

Anadón A, Martínez-Larrañaga MR, Ares I et al (2018) Regulatory aspects for the drugs and chemicals used in food producing animals. In: Gupta RC (ed) Veterinary toxicology. Basic and clinical

principles, 3rd edn. Elsevier/Academic Press, Amsterdam, pp 103–131
Atterbury RJ, Van Bergen MA, Ortiz F et al (2007) Bacteriophage therapy to reduce *Salmonella* colonization of broiler chickens. Appl Environ Microbiol 73(14):4543–4549
Barbosa LN, Rall VL, Fernandes AA et al (2009) Essential oils against foodborne pathogens and spoilage bacteria in minced meat. Foodborne Pathog Dis 6:725–728
Bardina C, Spricigo DA, Cortés P et al (2012) Significance of the bacteriophage treatment schedule in reducing *Salmonella* colonization of poultry. Appl Environ Microbiol 78:6600–6607
Boman HG (1991) Antibacterial peptides: key components needed in immunity. Cell 65(2):205–207
Bren L (2007) Bacteria-eating virus approved as food additive. FDA Consum 41(1):20–22
Brenes A, Roura E (2010) Essential oils in poultry nutrition: main effects and modes of action. Anim Feed Sci Technol 158(1–2):1–14
Canani RB, Di Costanzo M, Leone L et al (2011) Potential beneficial effects of butyrate in intestinal and extraintestinal diseases. World J Gastroenterol 17(12):1519–1528
Carvalho CM, Gannon BW, Halfhide DE et al (2010) The in vivo efficacy of two administration routes of a phage cocktail to reduce numbers of *Campylobacter coli* and *Campylobacter jejuni* in chickens. BMC Microbiol 10:232
Castillo M, Martín-Orúe SM, Roca M et al (2006) The response of gastrointestinal microbiota to avilamycin, butyrate, and plant extracts in early-weaned pigs. J Anim Sci 84(10):2725–2734
Cavaleri F, Bashar E (2018) Potential synergies of β-hydroxybutyrate and butyrate on the modulation of metabolism, inflammation, cognition, and general health. J Nutr Metab 2018:7195760
Chandar B, Poovitha S, Ilango K et al (2017) Inhibition of New Delhi metallo-β-lactamase 1 (NDM-1) producing *Escherichia coli* IR-6 by selected plant extracts and their synergistic actions with antibiotics. Front Microbiol 8:1580
Chaveerach P, Keuzenkamp DA, Lipman LJ et al (2004) Effect of organic acids in drinking water for young broilers on *Campylobacter* infection, volatile fatty acid production, gut microflora and histological cell changes. Poult Sci 83(3):330–334
Chen F, Wu W, Millman A et al (2014) Neutrophils prime a long-lived effector macrophage phenotype that mediates accelerated helminth expulsion. Nat Immunol 15(10):938–946
Cheng G, Hao H, Xie S et al (2014) Antibiotic alternatives: the substitution of antibiotics in animal husbandry? Front Microbiol 5:217
Chowdhury R, Islam KMS et al (2009) Effect of citric acid, avilamycin, and their combination on the performance, tibia ash, and immune status of broilers. Poult Sci 88:1616–1622
Clavijo V, Vives Flórez MJ (2017) The gastrointestinal microbiome and its association with the control of pathogens in broiler chicken production: a review. Poult Sci 97:1–16
Cogliani C, Goossens H, Greko C (2011) Restricting antimicrobial use in food animals: lessons from Europe. Microbe 6(6):274–279
Collineau L, Rojo-Gimeno C, Léger A et al (2017) Herd-specific interventions to reduce antimicrobial usage in pig production without jeopardising technical and economic performance. Prev Vet Med 144:167–178
Cook SR, Maiti PK, DeVinney R et al (2007) Avian- and mammalian-derived antibodies against adherence-associated proteins inhibit host cell colonization by *Escherichia coli* O157:H7. J Appl Microbiol 103(4):1206–1219
Cooper IR (2016) A review of current methods using bacteriophages in live animals, food and animal products intended for human consumption. J Microbiol Methods 130:38–47
Cosby DE, Cox NA, Harrison MA et al (2015) *Salmonella* and antimicrobial resistance in broilers: a review. J Appl Poult Res 24:408–426
Cotter PD, Hill C, Ross RP (2005) Bacteriocins: developing innate immunity for food. Nat Rev Microbiol 3(10):777–788
Dahiya JP, Wilkie DC, Van Kessel AG et al (2006) Potential strategies for controlling necrotic enteritis in broiler chickens in post-antibiotic era. Anim Feed Sci Technol 129(1–2):60–88
Davies G, Genini S, Bishop SC, Giuffra E (2009) An assessment of opportunities to dissect host genetic variation in resistance to infectious diseases in livestock. Animal 3(3):415–436
Diaz-Sanchez S, D'Souza D, Biswas D et al (2015) Botanical alternatives to antibiotics for use in organic poultry production. Poult Sci 94(6):1419–1430
Dibner JJ, Richards JD (2005) Antibiotic growth promoters in agriculture: history and mode of action. Poult Sci 84:634–643
Dierick NA, Decuyperea JA, Molly K et al (2002) The combined use of triacylglycerols (TAGs) containing medium chain fatty acids (MCFAs) and exogenous lipolytic enzymes as an alternative to nutritional antibiotics in piglet nutrition: II. In vivo release of MCFAs in gastric cannulated and slaughtered piglets by endogenous and exogenous lipases; effects on the luminal gut flora and growth performance. Livest Prod Sci 76(1–2):1–16
Dorman HJ, Deans SG (2000) Antimicrobial agents from plants: antibacterial activity of plant volatile oils. J Appl Microbiol 88 (2):308–316
Dorne JL, Heppner C, Kass GE et al (2013) Special issue: risk assessment of undesirable substances in feed. Toxicol Appl Pharmacol 270 (3):185–186
EC (2001) Directive 2001/82/EC of the European Parliament and of the Council of 6 November 2001 on the community code relating to veterinary medicinal products (OJ L 136, 30.4.2004)
EC (2003) Regulation (EC) No. 1831/2003 (EC, 2003) of the European Parliament and of the Council of 22 September on additives for use in animal nutrition (OJ L 268, 18.10.2003)
EC (2004) Directive 2004/28/EC of the European Parliament and of the Council of 31 March 2004 amending Directive 2001/82/EC on the Community code relating to veterinary medicinal products (OJ L 136, 30/04/2004)
EC (2008) Commission Regulation (EC) No 429/2008 of 25 April 2008 on detailed rules for the implementation of Regulation (EC) No 1831/2003 of the European Parliament and of the Council as regards the preparation and the presentation of applications and the assessment and the authorisation of feed additives (OJ No. L 133, 22.5.2008)
EC (2009) Regulation (EC) No 767/2009 of the European Parliament and of the Council of 13 July 2009 on the placing on the market and use of feed, amending European Parliament and Council Regulation (EC) No 1831/2003 and repealing Council Directive 79/373/EEC, Commission Directive 80/511/EEC, Council Directives 82/471/EEC, 83/228/EEC, 93/74/EEC, 93/113/EC and 96/25/EC and Commission Decision 2004/217/EC (OJ No. 229, 1.9.2009)
EC (2015) Commission notice. Guidelines for the prudent use of antimicrobials in veterinary medicine (2015/C 299/04) (OJ No. C 299, 11.09.2015)
EC (2018) DG Health and Food Safety. Overview report non-EU countries' national policies and measures on antimicrobial resistance. Publications Office of the European Union, Luxembourg. isbn:978-92-79-43534-8
EFSA (2009) Guidance on safety assessment of botanicals and botanical preparations intended for use as ingredients in food supplements. EFSA J 7(9):1249
EFSA (2014) Scientific opinion on a qualified presumption of safety (QPS) approach for the safety assessment of botanicals and botanical preparations. EFSA J 12(3):3593
EMA (2012) CVMP. Opinion following an Article 35 referral for all veterinary medicinal products containing systemically administered (parenteral and oral) 3rd and 4th generation cephalosporins intended for use in food producing species. http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/veterinary/referrals/Cephalosporins/vet_referral_000056.jsp&mid=WC0b01ac05805c5170

EMA (2016a) Opinion of the committee for medicinal products for veterinary use pursuant to Article 35 of Directive 2001/82/EC for veterinary medicinal products EMEA/V/A/118. Opinion 8 December 2016 EMA/CVMP/746319/2016

EMA (2016b) Updated advice on the use of colistin products in animals within the European Union: development of resistance and possible impact on human and animal health. 26 May 2016. EMA/231573/2016

EU (2017) A European One Health Action Plan against Antimicrobial Resistance (AMR). https://ec.europa.eu/health/amr/sites/amr/files/amr_action_plan_2017_en.pdf

Regulation (EU) 2019/6 of the European Parliament and of the council of 11 December 2018 on veterinary medicinal products and repealing Directive 2001/82/EC (OJ of EU L4/43, 7.1.2019)

FAWC (1993) Farm Animal Welfare Council: second report on priorities for research and development in farm animal welfare. Tolworth, MAFF, 1993

FDA (2013) Guidance for Industry #213: New animal drugs and new animal drug combination products administered in or on medicated feed or drinking water of food-producing animals: recommendations for drug sponsors for voluntarily aligning product use conditions withGFI#209. http://www.fda.gov/downloads/AnimalVeterinary/GuidanceComplianceEnforcement/GuidanceforIndustry/UCM299624.pdf

Feng Y, Zhang H, Wu Z et al (2014) *Streptococcus suis* infection: an emerging/reemerging challenge of bacterial infectious diseases? Virulence 5(4):477–497

Fischetti VA (2008) Bacteriophage lysins as effective antibacterials. Curr Opin Microbiol 11(5):393–400

Frese SA, Parker K, Calvert CC et al (2015) Diet shapes the gut microbiome of pigs during nursing and weaning. Microbiome 3:28

Fu Y, Zu Y, Chen L et al (2007) Antimicrobial activity of clove and rosemary oils alone and in combination. Phytother Res 21:989–994

Gadde U, Kim WH, Oh ST et al (2017) Alternatives to antibiotics for maximizing growth performance and feed efficiency in poultry: a review. Anim Health Res Rev 18(1):26–45

Geier MS, Torok VA, Guo P et al (2011) The effects of lactoferrin on the intestinal environment of broiler chickens. Br Poult Sci 52 (5):564–572

Gill PA, van Zelm MC, Muir JG et al (2017) Review article: short chain fatty acids as potential therapeutic agents in human gastrointestinal and inflammatory disorders. Aliment Pharmacol Ther 48:1–20

Gloaguen M, Le Floc'h N, Primot Y et al (2014) Performance of piglets in response to the standardized ileal digestible phenylalanine and tyrosine supply in low-protein diets. Animal 8(9):1412–1419

Grant A, Hashem F, Parveen S (2016) *Salmonella* and *Campylobacter*: antimicrobial resistance and bacteriophage control in poultry. Food Microbiol 53:104–109

Grashorn MA (2010) Use of phytobiotics in broiler nutrition—an alternative to infeed antibiotics? J Anim Feed Sci 19:338–347

Gresse R, Chaucheyras-Durand F, Fleury MA et al (2017) Gut microbiota dysbiosis in postweaning piglets: understanding the keys to health. Trends Microbiol 25(10):851–873

Guo FC, Williams BA, Kwakkel RP et al (2004) Effects of mushroom and herb polysaccharides, as alternatives for an antibiotic, on the cecal microbial ecosystem in broiler chickens. Poult Sci 83 (2):175–182

Gyles CL (2008) Antimicrobial resistance in selected bacteria from poultry. Anim Health Res Rev 9(2):149–158

Han Y-K, Hwan Hwang IL, Thacker PA (2011) Use of a microencapsulated eucalyptus-medium chain fatty acid product as an alternative to zinc oxide and antibiotics for weaned pigs. J Swine Health Prod 19(1):34–43

Hashemi SR, Davoodi H (2011) Herbal plants and their derivatives as growth and health promoters in animal nutrition. Vet Res Commun 35:169–180

Hodin R (2000) Maintaining gut homeostasis: the butyrate-NF-kappaB connection. Gastroenterology 118(4):798–801

Hong SM, Hwang JH, Kim IH (2012) Effect of medium-chain triglyceride (mct) on growth performance, nutrient digestibility, blood characteristics in weanling pigs. Asian-Aust J Anim Sci 25 (7):1003–1008

Hovi M, Sundrum A, Thamsborg SM (2003) Animal health and welfare in organic livestock production in Europe: current state and future challenges. Lives Prod Sci 80(1–2):41–53

Huyghebaert G, Ducatelle R, Van Immerseel F (2011) An update on alternatives to antimicrobial growth promoters for broilers. Vet J 187:182–188

IACG (2018) Antimicrobial resistance: invest in innovation and research, and boost R&D and access. International Coordination Group on Antimicrobial Resistance (IACG) discussion paper, June 2018

Isaacson R, Kim HB (2012) The intestinal microbiome of the pig. Anim Health Res Rev 13(1):100–109

Jamroz D, Wiliczkiewicz A, Wertelecki T et al (2005) Use of active substances of plant origin in chicken diets based on maize and locally grown cereals. Br Poult Sci 46(4):485–493

Józefiak D, Sip A, Rutkowski A et al (2012) Lyophilized *Carnobacterium divergens* AS7 bacteriocin preparation improves performance of broiler chickens challenged with *Clostridium perfringens*. Poult Sci 91(8):1899–1907

Józefiak D, Kierończyk B, Juśkiewicz J et al (2013) Dietary nisin modulates the gastrointestinal microbial ecology and enhances growth performance of the broiler chickens. PLoS One 8 (12):e85347

Kamada N, Seo SU, Chen GY et al (2013) Role of the gut microbiota in immunity and inflammatory disease. Nat Rev Immunol 13 (5):321–335

Khan SH, Iqbal J (2016) Recent advances in the role of organic acids in poultry nutrition. J Appl Anim Res 44:359–369

Kittler S, Fischer S, Abdulmawjood A et al (2013) Effect of bacteriophage application on *Campylobacter jejuni* loads in commercial broiler flocks. Appl Environ Microbiol 79:7525–7533

Laanen M, Persoons D, Ribbens S et al (2013) Relationship between biosecurity and production/antimicrobial treatment characteristics in pig herds. Vet J 198:508–512

Laanen M, Maes D, Hendriksen C et al (2014) Pig, cattle and poultry farmers with a known interest in research have comparable perspectives on disease prevention and on-farm biosecurity. Prev Vet Med 115:1–9

Li P, Piao X, Ru Y et al (2012) Effects of adding essential oil to the diet of weaned pigs on performance, nutrient utilization, immune response and intestinal health. Asian-Aust J Anim Sci 25 (11):1617–1626

Liu Y, Han F, Xie Y et al (2011) Comparative antimicrobial activity and mechanism of action of bovine lactoferricin-derived synthetic peptides. Biometals 24(6):1069–1078

Loc-Carrillo C, Abedon S (2011) Pros and cons of phage therapy. Bacteriophage 1:111–114

Mariadason JM, Barkla DH, Gibson PR (1997) Effect of short-chain fatty acids on paracellular permeability in Caco-2 intestinal epithelium model. Am J Phys 272:G705–G712

Marounek M, Skřivanová E, Rada V (2003) Susceptibility of *Escherichia coli* to C 2–C 18 fatty acids. Folia Microbiol 48 (6):731–735

Międzybrodzki R, Borysowski J, Weber-Dąbrowska B et al (2012) Clinical aspects of phage therapy. Adv Virus Res 83:73–121

Millet S, Maertens L (2011) The European ban on antibiotic growth promoters in animal feed: from challenges to opportunities. Vet J 187(2):143–144

Mitsch P, Zitterl-Eglseer K, Köhler B et al (2004) The effect of two different blends of essential oil components on the proliferation of

Clostridium perfringens in the intestines of broiler chickens. Poult Sci 83(4):669–675
Nilsson AS (2014) Phage therapy constraints and possibilities. Ups J Med Sci 119:192–198
O'Flaherty S, Ross RP, Coffey A (2009) Bacteriophage and their lysins for elimination of infectious bacteria: review article. FEMS Microbiol Rev 33:801–819
Oliveira H, Azeredo J, Lavigne R et al (2012) Bacteriophage endolysins as a response to emerging foodborne pathogens. Trends Food Sci Technol 28:103–115
Omonijo FA, Ni L, Gong J et al (2018) Essential oils as alternatives to antibiotics in swine production. Anim Nutr 4(2):126–136
Oostindjer M, Bolhuis JE, Mendl M et al (2011) Learning how to eat like a pig: effectiveness of mechanisms for vertical social learning in piglets. Anim Behav 82(3):503–511
Over K, Hettiarachchy N, Johnson M et al (2009) Effect of organic acids and plant extracts on *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella typhimurium* in broth culture model and chicken meat systems. J Food Sci 74:515–521
Panda AK, Rama Rao SV, Raju MVLN et al (2009) Effect of butyric acid on performance, gastrointestinal tract health and carcass characteristics in broiler chickens. Asian-Aust J Anim Sci 22:1026–1031
Patterson JA, Burkholder KM (2003) Applications of prebiotics and probiotics in poultry production. Poult Sci 82:627–631
Petri D, Hillb JE, Van Kessel AG (2010) Microbial succession in the gastrointestinal tract (GIT) of the preweaned pig. Lives Sci 133 (1–3):107–109
Postma M, Stärk KDC, Sjölund M et al (2015) Alternatives to the use of antimicrobial agents in pig production: a multi-country expert-ranking of perceived effectiveness, feasibility and return on investment. Prev Vet Med 118:457–466
Postma M, Backhans A, Collineau L et al (2016a) Evaluation of the relationship between the biosecurity status, production parameters, herd characteristics and antimicrobial usage in farrow-to-finish pig production in four EU countries. Porcine Health Manag 2:9
Postma M, Backhans A, Collineau L et al (2016b) The biosecurity status and its associations with production and management characteristics in farrow-to-finish pig herds. Animal 10(3):478–489
Pouillot F, Chomton M, Blois H et al (2012) Efficacy of bacteriophage therapy in experimental sepsis and meningitis caused by O25b:H4-ST131 *E. coli* strain producing CTX-M-15. Antimicrob Agents Chemother 56(7):3568–3575
Prescott JF (2008) Antimicrobial use in food and companion animals. Anim Health Res Rev 9(2):127–133
Rafacz-Livingston K, Parsons C, Jungk R (2005) The effects of various organic acids on phytate phosphorus utilization in chicks. Poult Sci 84:1356–1362
Ragland D, Stevenson D, Hill MA (2008) Oregano oil and multi-component carbohydrases as alternatives to antimicrobials in nursery diets. J Swine Health Prod 16(5):238–243
Ricke S (2003) Perspectives on the use of organic acids and short chain fatty acids as antimicrobials. Poult Sci 82:632–639
Rose E, Nunan C (2016) Alliance to save our antibiotics. Antibiotic use in the UK dairy sector. http://www.saveourantibiotics.org/media/1762/antibiotic-use-in-the-uk-dairy-sector.pdf
Rossi R, Pastorelli G, Cannata S et al (2010) Recent advances in the use of fatty acids as supplements in pig diets: a review. Anim Feed Sci Technol 162(1–2):1–11
Rutherford ST, Bassler BL (2012) Bacterial quorum sensing: its role in virulence and possibilities for its control. Cold Spring Harb Perspect Med 2(11):1–25
Samanta S, Haldar S, Ghosh TK (2008) Production and carcass traits in broiler chickens given diets supplemented with inorganic trivalent chromium and an organic acid blend. Br Poult Sci 49:155–163
Sang Y, Blecha F (2008) Antimicrobial peptides and bacteriocins: alternatives to traditional antibiotics. Anim Health Res Rev 9 (2):227–235
Sarica S, Ciftci A, Demir E et al (2005) Use of an antibiotic growth promoter and two herbal natural feed additives with and without exogenous enzymes in wheat based broiler diets. S Afr J Anim Sci 35(1):61–72
Schmelcher M, Shabarova T, Eugster MR et al (2010) Rapid multiplex detection and differentiation of *Listeria* cells by use of fluorescent phage endolysin cell wall binding domains. Appl Environ Microbiol 76(17):5745–5756
Schmelcher M, Donovan DM, Loessner MJ (2012a) Bacteriophage endolysins as novel antimicrobials. Future Microbiol 7:1147–1171
Schmelcher M, Powell AM, Becker SC et al (2012b) Chimeric phage lysins act synergistically with lysostaphin to kill mastitis-causing *Staphylococcus aureus* in murine mammary glands. Appl Environ Microbiol 78(7):2297–2305
Serafino A, Sinibaldi Vallebona P et al (2008) Stimulatory effect of *Eucalyptus* essential oil on innate cell-mediated immune response. BMC Immunol 9:17
Shin B, Park W (2018) Zoonotic diseases and phytochemical medicines for microbial infections in veterinary science: current state and future perspective. Front Vet Sci 5:166
Shin MS, Han SK, Ji AR, Kim KS, Lee WK (2008) Isolation and characterization of bacteriocin-producing bacteria from the gastrointestinal tract of broiler chickens for probiotic use. J Appl Microbiol 105(6):2203–2212
Simmons KJ, Chopra I, Fishwick CWG (2010) Structure-based discovery of antibacterial drugs. Nat Rev Microbiol 8:501–510
Skrivanová E, Marounek M, Dlouhá G et al (2005) Susceptibility of *Clostridium perfringens* to C-C fatty acids. Lett Appl Microbiol 41 (1):77–81
Skrzypek T, Valverde Piedra JL, Skrzypeka H et al (2007) Intestinal villi structure during the development of pig and wild boar crossbreed neonates. Livest Sci 109(1–3):38–41
Smith HW, Huggins MB, Shaw KW (1987) The control of experimental *Escherichia coli* diarrhea in calves by means of bacteriophages. J Gen Microbiol 133:1111–1126
Solis de los Santos F, Donoghue AM, Venkitanarayanan K et al (2008) Therapeutic supplementation of caprylic acid in feed reduces *Campylobacter jejuni* colonization in broiler chicks. Appl Environ Microbiol 74(14):4564–4566
Spiljar M, Merkler D, Trajkovski M (2017) The immune system bridges the gut microbiota with systemic energy homeostasis: focus on TLRs, mucosal barrier, and SCFAs. Front Immunol 8:1353
Stein HH, Kil DY (2006) Reduced use of antibiotic growth promoters in diets fed to weanling pigs: dietary tools, part 2. Anim Biotechnol 17 (2):217–231
Suchodolski JS, Ruaux CG, Steiner JM et al (2005) Assessment of the qualitative variation in bacterial microflora among compartments of the intestinal tract of dogs by use of a molecular fingerprinting technique. Am J Vet Res 66:1556–1562
Sulakvelidze A (2005) Phage therapy: an attractive option for dealing with antibiotic-resistant bacterial infections. Drug Discov Today 10 (12):807–809
Sulakvelidze A (2011) Safety by nature: potential bacteriophage applications. Microbe 6:122–126
Sulakvelidze A (2013) Using lytic bacteriophages to eliminate or significantly reduce contamination of food by foodborne bacterial pathogens. J Sci Food Agric 93:3137–3146
Summers WC (2012) The strange history of phage therapy. Bacteriophage 2(2):130–133
Tellez GI, Jaeger L, Dean CE et al (1993) Effect of prolonged administration of dietary capsaicin on *Salmonella enteritidis* infection in leghorn chicks. Avian Dis 37(1):143–148

Thacker PA (2013) Alternatives to antibiotics as growth promoters for use in swine production: a review. J Anim Sci Biotechnol 4(1):35

Timbermont L, Lanckriet A, Gholamiandehkordi AR et al (2009) Origin of *Clostridium perfringens* isolates determines the ability to induce necrotic enteritis in broilers. Comp Immunol Microbiol Infect Dis 32 (6):503–512

Timbermont L, Lanckriet A, Dewulf J et al (2010) Control of *Clostridium perfringens*-induced necrotic enteritis in broilers by target-released butyric acid, fatty acids and essential oils. Avian Pathol 39 (2):117–121

Tini M, Jewell UR, Camenisch G et al (2002) Generation and application of chicken egg-yolk antibodies. Comp Biochem Physiol A Mol Integr Physiol 131(3):569–574

Toro H, Price SB, McKee AS et al (2005) Use of bacteriophages in combination with competitive exclusion to reduce *Salmonella* from infected chickens. Avian Dis 49:118–124

Turner PV (2018) Improving animal production biosecurity to minimise global one health risks. IAHJ 5(4):28–30

Van Der Wielen PW, Biesterveld S, Notermans S et al (2000) Role of volatile fatty acids in development of the cecal microflora in broiler chickens during growth. Appl Environ Microbiol 66:2536–2540

Van Dijk A, Herrebout M, Tersteeg-Zijderveld MH et al (2012) *Campylobacter jejuni* is highly susceptible to killing by chicken host defense peptide cathelicidin-2 and suppresses intestinal cathelicidin-2 expression in young broilers. Vet Microbiol 160(3–4):347–354

Van Dijk A, Molhoek EM, Veldhuizen EJ, Tjeerdsma-van Bokhoven JL, Wagendorp E, Bikker F, Haagsman HP (2009) Identification of chicken cathelicidin-2 core elements involved in antibacterial and immunomodulatory activities. Mol Immunol 46(13):2465–2473

Van Dijk A, Molhoek EM, Bikker FJ, Yu PL, Veldhuizen EJA, Haagsman HP (2011) Avian cathelicidins: paradigms for the development of anti-infectives. Vet Microbiol 153(1–2):27–36

Van Immerseel F, Russell JB, Flythe MD et al (2006) The use of organic acids to combat *Salmonella* in poultry: a mechanistic explanation of the efficacy. Avian Pathol 35(3):182–188

Vanderhoof JA, Whitney DB, Antonson DL et al (1999) *Lactobacillus* GG in the prevention of antibiotic-associated diarrhea in children. J Pediatr 135(5):564–568

Vicente JL, Lopez C, Avila E et al (2007) Effect of dietary natural capsaicin on experimental *Salmonella enteritidis* infection and yolk pigmentation in laying hens. Int J Poult Sci 6:393–396

Von Borel E, Sørensen JT (2004) Organic livestock production in Europe: aims, rules and trends with special emphasis on animal health and welfare. Lives Prod Science 90(1):3–9

Walker WL, Epperson WB, Wittum TE et al (2012) Characteristics of dairy calf ranches: morbidity, mortality, antibiotic use practices, and biosecurity and biocontainment practices. J Dairy Sci 95 (4):2204–2214

Wang YZ, Shan TZ, Xu ZR et al (2007) Effects of the lactoferrin (LF) on the growth performance, intestinal microflora and morphology of weanling pigs. Anim Feed Sci Technol 135 (3–4):263–272

Wang HT, Li YH, Chou IP et al (2013) Albusin B modulates lipid metabolism and increases antioxidant defense in broiler chickens by a proteomic approach. J Sci Food Agric 93(2):284–292

Wernicki A, Nowaczek A, Urban-Chmiel R (2017) Bacteriophage therapy to combat bacterial infections in poultry. Virol J 14:179

Windisch W, Schedle K, Plitzner C et al (2008) Use of phytogenic products as feed additives for swine and poultry. J Anim Sci 86 (14 suppl):E140–E148

Wong CL, Sieo CC, Tan WS et al (2014) Evaluation of a lytic bacteriophage, Φ st1, for biocontrol of *Salmonella enterica* serovar *typhimurium* in chickens. Int J Food Microbiol 172:92–101

Yan H, Ajuwon KM (2017) Butyrate modifies intestinal barrier function in IPEC-J2 cells through a selective upregulation of tight junction proteins and activation of the Akt signalling pathways. PLoS One 12 (6):e0179586

Zasloff M (1987) Magainins, a class of antimicrobial peptides from *Xenopus* skin: isolation, characterization of two active forms, and partial cDNA sequence of a precursor. Proc Natl Acad Sci USA 84 (15):5449–5453

Zimmer M, Vukov N, Scherer S et al (2002) The murein hydrolase of the bacteriophage ϕ3626 dual lysis system is active against all tested *Clostridium perfringens* strains. Appl Environ Microbiol 68:5311–5317

Feed Additives in Animal Health

Amit Kumar Pandey, Prafulla Kumar, and M. J. Saxena

Abstract

Animal feed additives are used all over the world for various livestock including poultry for more reasons than one like to provide essential nutrients, increase palatability of the feed, improve their growth performance, as well as optimize the utilization of the feed. Animals with high growth performance need to maintain a high health status, and the use of proper additives is a predominant argument in such cases. With increasing industry standards and consumer awareness as well as demand for healthy food products of animal origin, there is an increased pressure on the industry for more natural and non-residual alternatives than the conventional feed additives used till recently as animal feed products. Consumer and animal welfare are primary concerns dictating the valuable alternatives of animal feed additives. Some of the alternatives seen for use as animal feed additives are probiotics, prebiotics, enzymes, and herbs. Such choice of feed additives is backed by scientific and empirical research on these alternatives as herbs and their extracts (botanicals) have been found to have a wide range of activity which cannot only stimulate feed intake but also stimulate endogenous secretions or have antimicrobial, coccidiostat, or anthelmintic activity.

Ban of antibiotic use as growth promoters, cost-effectiveness, and increased awareness about harmful residual effect cause herbal feed additive to gain importance in sustainable livestock production. Animal husbandry sector gets benefited by the use of number of feed additives such as ascorbic acid, prebiotic, probiotic, and herbal extracts. Medicinal properties of the herbs to improve antimicrobial, anti-inflammatory, antioxidant, digestibility, and immune-stimulant activity must be explored in the feeding of animals as well as safe food for human beings. Standardization of correct dosage regime of herbal feed additives for a particular function is the demand of situation so more research should be conducted in this direction.

Keywords

Feed additives · Phytogenic product · Probiotics · Prebiotics · Enzymes · Herbs · Plant extracts · Quality control

1 Introduction

One of the greatest challenges faced by farm managers, livestock rearers, animal scientists, as well as nutritionists involved in animal feed industry or research domain is designing the balanced ration practices of high yielding animals along with maintaining the cost-benefit ratio. Also, to be taken in consideration is the fact that meat, dairy, and animal by-product costs are not stable and vary for various reasons, and one among them is the feed cost involved (Thornton 2010). Feed costs represent the largest input cost in animal husbandry practices (estimated to be 35–50%). In general there is an opinion that animals ate plants, or grass or some other "food" natural to their ilk, but in reality, in today's farms, feeding livestock and poultry is a complicated endeavor fraught with controversy and split opinions. Feed additives have been considered as a group or class of feed ingredients which in a non-nutrient role can cause the desired animal response. Such responses may include a shift in pH, growth, or modifying the metabolic response of the animal (Hutjens 1991). According to the European Commission, feed additives are products used in animal nutrition for purposes of improving the quality of feed and the quality of food from animal origin or to improve the animals' performance and health, e.g., providing enhanced digestibility of the feed materials. Several feed additives may contain various

A. K. Pandey (✉) · P. Kumar · M. J. Saxena
Ayurvet, Ghaziabad, UP, India
e-mail: amit.pandey@ayurvet.com

R. C. Gupta et al. (eds.), *Nutraceuticals in Veterinary Medicine*, https://doi.org/10.1007/978-3-030-04624-8_23

nutrients such as sodium and protein which are part of sodium bicarbonate or yeast culture, respectively. Technically, feed additives are neither considered a requirement nor do guarantee high animal productivity or economic profitability in animal husbandry practices (Animal Feed Additives 2018, web source). There has been a rapid increase in the demand to be met from dairy and meat industry owing to increasing population pressure. Food security which is the second in the list of Sustainable Development Goals in form of "zero hunger" and 12th sustainable development goal which is "responsible consumption and production" are both sought after in animal feed industry up to a greater extent.

The importance of feed additives is gaining popularity day by day owing to the benefits that they can ascertain like growth promotion in animals, control over infectious diseases as well as enhancement of feed digestibility (Specialty Feed Additives Report 2016). There is a steadfast growth graph of animal feed additives market which is projected to grow in the future due to the rapid increase in demand for meat, meat products as well as dairy products around the globe (Animal Feed Additives Report 2014). The consensus of meat and dairy farmers has increased towards feed quality and certifications associated with them owing to frequent occurrences of epidemics such as bird flu, other diseases such as foot-and mouth-disease, and environmental concerns have led to increase in concern over animal health around the globe.

The top consumers of feed additives in the world are North America and Asia-Pacific. They account for more than 60% of the consumption of animal feeds in the world (Animal Feed Additives Report 2014). Asia-Pacific is estimated to be the fastest growing region in terms of revenue. Growth is particularly high in emerging economies such as India, China, and Brazil because of gradual increase in the income levels of population owing to increased industrialization and boom in service sector, and in the process it has given an uplift to the feed industry as well due to rising per capita meat consumption (Ruminant Feed Market Report 2018). Species wise the largest market coverage is of poultry feed additives which are followed by market share for feed additives for swine (Animal Feed Additives Report 2014). The main driving factors of the global market of animal feed additives can be classified as:

(a) Rise in global meat consumption
(b) Increasing awareness toward meat quality and safety
(c) Increasing mass production of meat
(d) Recent disease outbreaks in livestock

(Kearney 2010; Henchion et al. 2017).

The restraints of the market are increasing raw material cost and regulatory structure. However, increasing cost of natural feed products is creating an opportunity for feed additives as a cheap alternative. Leading manufacturers are focusing on the expansion of businesses across regions and setting up new plants for increasing their production capacity.

Though there are various definitions of feed additives, a comprehensive definition put forward by the European Commission is: "Feed additives are products used in animal nutrition for purposes of improving the quality of feed and the quality of food from animal origin, or to improve the animals' performance and health, e.g. providing enhanced digestibility of the feed materials."

With the tightening of the noose of regulatory authorities and stronger social media, there is hardly any scope to flout the norms set for industries. For any animal feed additives to be marketed, the process of scientific evaluation and validation to ascertain presence or absence of any harmful effects on human and animal health or environment needs to be performed in stringently (European Commission 2018). So, various domains such as health, environmental sustainability, regulatory requirements, and even climate change are a part of production, marketing, and post-utilization effects which are assimilated with the feed production industry.

2 Classification of Animal Feed Additives

Feed additives are classified into various categories according to different parameters. Feed additives can be of various types:

Based on European Commission regulations
Based on holistic approach
Based on their origin and function

Feed additives can be categorized as feed antioxidants, compound acidifiers, complex enzymes, mycotoxin adsorbent, mildew prevention, vitamins and electrolytes, L-carnitine hydrochloride, diluted chromium nicotinate, fattening agents, amino acids, antibiotics, binders, minerals, herbs, and premix. A widely recognized classification of animal feed additives based on European Commission regulations and guidelines is as below.

One additional class of feed additives used to include the technological interventions employed in feed additives is *Technological additives*.

2.1 Based on European Commission Regulations

2.1.1 Sensory Additives

This refers to a group of additives which improve the palatability (i.e., voluntary intake) of a diet by stimulating appetite, usually through the effect these products have on the flavor or color of the diet. For example, feed flavors or sweeteners such as vanilla extract may well encourage piglets to eat a ration.

2.1.2 Nutritional Additives

Additives provide specific nutrients for an animal for optimal growth. An example would be a vitamin, amino acid, or trace mineral. In most cases, such additives are simply concentrated forms of nutrients supplied in natural ingredients in the diet.

2.1.3 Zootechnical Additives

These additives improve the nutrient status and production of the livestock, not just by providing specific nutrients but also by assisting the more efficient use of the nutrients present in the diet. An example of such an additive would be an enzyme or direct-fed microbial product, both of which enhance the conditions of the intestinal tract, thus enabling more effective nutrient extraction from the diet. In this respect, they are often referred to as pro-nutrients, i.e., products which improve the nutritional value of a diet without necessarily providing nutrients directly. Other additives are used for environmental benefits that they provide to animal husbandry, and others are targeted for specific physiological functions.

2.1.4 Coccidiostats and Histomonostats

These additives control the health of poultry through direct effects. These compounds are used to control the intestinal health of poultry, and they directly act on the parasitic organisms inhabiting the intestines, and they are not classified as antibiotics (Feed additive classifications 2018).

2.1.5 Technological Additives

This classification refers to a group of additives which influences the technological aspects of the feed. These additives do not directly influence the nutritional value of the feed but may do so indirectly by improving its handling or hygiene characteristics. An example of such an additive would be an organic acid for the preservation of feed (Fig. 1).

2.2 Based on Holistic Approach

Apart from European classification, a more holistic classification provided by the Indian Council of Agricultural Research, Ministry of Agriculture, Government of India, is shown in Fig. 2.

Fig. 1 Classification of technological feed additives

2.2.1 Additives That Influence Feed Stability, Feed Manufacturing, and Properties of Feeds

(a) Antifungals
(b) Antioxidants
(c) Pellet binders

2.2.2 Additives That Modify Animal Growth, Feed Efficiency, Metabolism, and Performance

(a) Feed flavors
(b) Digestion modifiers
  I. Enzymes
  II. Prebiotics
  III. Buffers
  IV. Acidifiers
  V. Ionophores
  VI. Antibloat compound
  VII. Isoacids
  VIII. Salivation inducers
  IX. Probiotics
  X. Defaunating agents
(c) Metabolism modifiers
  I. Hormones
  II. Beta-adrenergic agents (repartitioning agents)
(d) Growth promotants
  I. Antibiotics

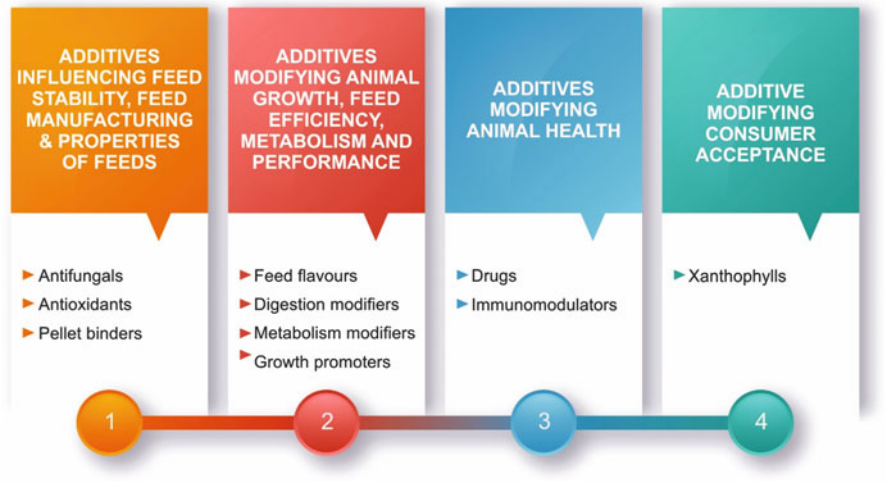


Fig. 2 Indian Council of Agricultural Research (ICAR)—classification of feed additives

II. Chemotherapeutic agents
III. Prebiotics and probiotics

2.2.3 Additives That Modify Animal Health

(e) Drugs
(f) Immunomodulators

2.2.4 Additives That Modify Consumer Acceptance

(a) Xanthophylls

Further to have a holistic classification to include all the types and subtypes of animal feed additives, including those included in the above classification based on the primary activity of function performed by a set class, we suggest the following detailed classification.

2.3 Based on Their Origin and Function (Fig. 3)

2.3.1 Additives That Enhance Feed Intake

Antioxidants

Antioxidants are compounds that prevent oxidative rancidity of polyunsaturated fats. Rancidity once develops may cause the destruction of vitamins A, D, and E and several of the B complex vitamins. Breakdown products of rancidity may react with lysine and thus affects the protein value of the ration. Ethoxyquin or BHT (butylated hydroxytoluene) can serve as an antioxidant in the feed.

Flavoring Agents

Flavoring agents are feed additives that are supposed to increase palatability and feed intake. There is a need for flavoring agents that will help to keep up feed intake.
When highly unpalatable medicants are being mixed:

- During attacks of diseases
- When animals are under stress
- When less palatable feedstuffs are being fed either as such or being incorporated in the ration

Fig. 3 Holistic classification of feed additives

Ruminants prefer sweet compounds. Additionally, cattle and goats respond positively to salts of volatile fatty acids. Horses will often refuse musty feed when there is so little mold that the owner fails to detect it.

2.3.2 Additives That Enhance the Quality and Acceptability of the Feed

Poultry owners will often enhance the yellow color by incorporating xanthophylls into broiler feed. Among various additives, arsanilic acid, sodium arsanilate, and roxarsone are added for the purpose.

Anticaking Agents

Anticaking agents are the anhydrous substance that can pick up moisture without themselves becoming wet. They are added to dry mixes to prevent the particles from clumping together and so keep the product free-flowing. They are either anhydrous salts or substance that hold water by surface adhesion yet themselves remain free-flowing:

- Salt or long chain fatty acids
- Calcium phosphate
- Potassium and sodium ferrocyanide
- Magnesium oxide
- Salts silicic acid—Al, Mg, Ca, and salt
- Sodium aluminum silicate
- Sodium calcium aluminum silicate
- Calcium aluminum silicate

Humectants

These are the substance which is required to keep the product moist, for example, bread and cakes. Anticaking agents immobilize moisture that was picked up. Humectants are not of much use in poultry feed.

Firming and Crisping Agents

These are substances that preserve the texture or vegetable tissues and, by maintaining the water pressure inside them, keep them turgid. It prevents a loss of water from the tissues.

Sequestrants

Certain elements—copper and iron—can act as prooxidant catalytic and therefore need to the immobilized. Sequestrants are compounds added to do this. These compounds should have an affinity to metal ions and should prevent the metal in becoming engaged in oxidative action. Most effective sequestrants ethylenediaminetetraacetic acid (EDTA) is a calcium salt of EDTA which works satisfactorily as a sequestrant without interfering with trace mineral metabolism.

Sweeteners

It is the common constitution of food but yet used as an additive, e.g., sugar. Some are poorly digestible and may cause digestive upsets. Saccharin is extensively used during World War I. It is a compound without any calorific value.

Additives such as humectants, firming and crisping agents, sweeteners, emulsifiers, stabilizers, acid, and buffers are not commonly used in poultry feeds.

2.3.3 Additives That Facilitate Digestion and Absorption

Grit

Poultry does not have teeth to grind any hard grain, most grinding takes place in the thick musculated gizzard. The more thoroughly feed is ground, the more surface area is created for digestion and subsequent absorption. Hence, when hard, coarse, or fibrous feeds are fed to poultry, grit is sometimes added to supply additional surface for grinding within gizzard. When mash or finely ground feeds are fed, the value of grit becomes less. Oyster shells, coquina shells, and limestone are used as grit.

Buffers and Neutralizers

During the maximum production stage ruminants are given high doses of concentrate feeds for meeting demands for extra energy and protein requirement of the animal. The condition, on the other hand, lowers the pH of the rumen. Since many of the rumen microbes cannot tolerate low pH environment, the normally heterogeneous balanced population of microbes become skewed, favoring the acidophilic (acid-loving) bacteria. The condition often leads to acidosis and thereby upsets normal digestion. The addition of feed buffers and neutralizers, such as carbonates, bicarbonates, hydroxides, oxides, salts of VFA, phosphate salts, ammonium chloride, and sodium sulfate, has been shown to have beneficial effects. Recently the use of baking soda ($NaHCO_3$) has been shown to increase average daily gain by about 10%, feed efficiency by 5–10%, and milk production by about 0.5 L per head per day.

Chelates

The word "chelates" is derived from the Greek word "chele" meaning "claw" which is a good descriptive term for the manner in which polyvalent cations are held by the metal-binding agents. Prior to union with the metal, these organic substances are termed as "ligands." Ligand + mineral = chelate element.

Organic chelates of mineral elements, which are cyclic compounds, are the most important factors controlling absorption of a number of mineral elements. A particular element in chelated form may be released in ionic form at the intestinal wall or might be readily absorbed as the intact chelate. Chelates may be of naturally occurring substances

such as chlorophyll, cytochromes, hemoglobin, vitamin B_{12}, some amino acids, etc. or may be of synthetic substances like ethylenediaminetetraacetic acid (EDTA).

Chelates as Feed Additives

Type I: Chelates that aid in transport and to store metal ions

Chelates of this group behave as a carrier for proper absorption, transportation in the circulatory system, and passing across cell membranes to deposit the metal ion at the site where needed. Among amino acids, cysteine and histidine are particularly effective metal-binding agents and may be of primary importance in the transport and storage of mineral elements throughout the animal body. Ethylenediaminetetraacetic acid (EDTA) and other similar synthetic ligands also may improve the availability of zinc and other minerals.

Type II: Chelates essential in metabolism

Many chelates of the animal body are holding metal ions in such a cyclic fashion which are absolutely necessary to be in that form to perform metabolic function. Vitamin B_{12}, cytochrome enzymes, and hemoglobin are some of the examples of this type. Hemoglobin molecule without its content of ferrous form of iron will be of no use in transporting oxygen.

Type III: Chelates which interfere with utilization of essential cations

There are some chelates found in the body which might have accidentally formed and are of no use to the subject. Rather, those chelates may be detrimental for the proper utilization of the element. Phytic acid-Zn chelate and oxalic acid calcium chelate are examples of this type.

2.3.4 Enzymes

Enzymes are the protein which has the property of catalyzing specific biochemical reactions. They are found in all plants and animals and are responsible for growth and the maintenance of health. Microorganisms also produce enzymes, and in recent years it has been possible to produce enzymes using microorganism on an industrial scale and extract and use these enzymes in a wide range of processes for the production of feed and natural products.

Poultry feeds are largely composed of plant and vegetable materials, and there are enzymes developed to degrade, modify, or extract the plant polymers found in some of the cereals and their by-products. The enzymes can be used to improve the feeding of poultry in the following ways:

- By improving the efficiency of the utilization of the feed
- By upgrading cereals by-products or feed components that are poorly digested
- By providing additional digestive enzymes to help poultry to withstand stress conditions, e.g., hot climates

Some of the cereals are compounds of polymers either of glucose (beta-glucan) or arabinose and xylose (pentosan or hemicellulose). These polymers are not well digested by poultry, and this can result in loss of energy in two ways. Energy may be lost because these polymers hinder the digestion of starch by coating starch granules and preventing the action of starch digesting enzymes in the intestine.

Energy may be lost because the animals own enzymes are not capable of degrading the polymers, and therefore they pass through the digestive system untouched. By adding microbial enzymes to the feed, these polymers can be degraded, and their energy value made available to the bird. The dual role of enzymes has been demonstrated in trials with barley-based feed supplemented with beta-glucanase, where the apparent increase in available energy was far in excess of that available in the beta-glucan of the barley. In this case not only was the problem of sticky dropping completely eliminated but the chicken's rate of growth was equivalent to that observed normally with feeds containing a higher energy density (e.g., wheat based).

Choice of Enzyme

- Because feed is normally composed of a single raw material of constant quality, it is important that the correct choice of enzyme product be made.
- Even in the case of a relatively well-defined problem such as that in barley, the use of multienzyme activity products has an advantage.
- The enzymes should fulfill the following criteria for practical application:
  - The enzymes must be active at the pH of the animals' digestive system and capable of surviving transit through the stomach.
  - They must be in a physical form in which they can be safely and easily mixed into all forms of animal feed.
  - The products should be of a high standardized activity that will remain stable both before and after incorporation into the feed or premix.

– The enzymes must be capable of surviving normal pelleting conditions.

2.3.5 Additives That Promote Growth and Production

Antibiotics

These are substances which are produced by living organisms (mold, bacteria, or green plants) and which in small concentration have bacteriostatic or bactericidal properties. They were originally developed for medical and veterinary purposes to control specific pathogenic organisms. Later, it was discovered that certain antibiotics could increase the rate of growth of young pigs and chicks when included in their diet in small amounts. Soon after this report, a wide range of antibiotics have been tested, and the following have been shown to have growth-promoting properties: penicillin, oxytetracycline (Terramycin), chlortetracycline, bacitracin, streptomycin, tyrothricin, gramicidin, neomycin, erythromycin, and flavomycin.

Increased weight gain is most evident during the period of rapid growth and then decreases. Differences between control and treated animals are greater when the diet is slightly deficient or marginal in protein, B vitamins, or certain mineral elements.

Mode of Action of Antibiotics

- Antibiotics "spare" protein, amino acids, and vitamin on diets containing 1–3% less protein, but balance experiments have often failed to show increased nitrogen retention. Growth stimulation has been greatest when the antibiotic penicillin supplement has been added to a ration containing no protein supplements of animal origin or to a ration low in vitamin B_{12}. Under hygienic conditions, growth increases are small.
- Intestinal wall of animals fed antibiotics is thinner than that of untreated animals which might explain the enhanced absorption of calcium shown for chicks.
- Reduce or eliminate the activity of pathogens causing "subclinical infection."
- Reduce the growth of microorganisms that compete with the host for supplies of nutrients.
- Antibiotics alter intestinal bacteria so that less urease is produced and thus less ammonia is formed. Ammonia is highly toxic and suppresses growth in nonruminants.
- Stimulate the growth of microorganisms that synthesize known or unidentified nutrients.

The following points should be kept in mind while using antibiotics for animal feeding:

- Antibiotics should be used only for:
  - Growing and fattening pigs for slaughter as pork or bacon
  - Growing chicks and turkey poults for killing as table poultry
- Antibiotics should not be used in the feed of ruminant animals (cattle, sheep, and goats), breeding pigs, and breeding and laying poultry stock.
- While adding antibiotics at the recommended level, care should be taken that they are thoroughly and evenly mixed with the feed.
- For best results, antibiotics should be used with properly balanced feeds. Also, the feeds containing antibiotics should be fed only to the type of stock for which they are intended.
- Antibiotics are not a substitute for good management and healthy living conditions or for properly balanced rations.

Probiotic and Prebiotic

The animal gut is composed of nearly a thousand different types of microorganisms, some of them are beneficial some are not. The gut microflora plays a very important role in health and disease condition of the living being. The healthy condition is due to the presence of beneficial bacteria which is also termed as probiotic or due to the intake of nutrients that stimulate the endogenous beneficial microbes (prebiotics).

A probiotic is defined classically as a viable microbial dietary supplement that beneficially affects the host through its effects in the intestinal tract (Sanchez and Rivas-Estilla 2006). Probiotics are live microbial feed supplements which are beneficial to the host animals and help to improve their intestinal microbial balance.

A prebiotic is defined as a nondigestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon (Gibson and Roberfroid 1995). Use of prebiotic causes change in the colonic microflora composition with dominance of a few of the potentially health-promoting bacteria, especially, but not exclusively, *Lactobacilli* and *Bifidobacterium* (Gibson and Roberfroid 1995). Prebiotics target animal-associated microbiota with the goal of improving health, whereas probiotics use live microorganisms.

Probiotics

The genera of bacteria which are most frequently used as prebiotics are *Lactobacilli* and *Bifidobacterium*, *Enterococcus faecium*, and spore-forming *Bacillus* spp., while some yeasts are also used such as *Saccharomyces*. There are several new uses of probiotics that were previously unthinkable. But the efficacy of these is not misunderstood and consider for the cure of everything. Probiotics are very specific and

strain dependent, and hence different strains are beneficial in different disorders. Some strains of probiotics may be detrimental to certain individuals, and it may worsen the condition of disease in certain individuals. Moreover, the investigation regarding the dose-dependence relationship is also very rare. Though numerous research have been conducted on probiotics in the past two decades, still there is much to be discovered.

They benefit the host by:

- Having a direct antagonistic effect against specific group of undesirable or harmful organism through production of antibacterial compounds, elementary, or minimizing their competition of nutrients
- Altering the pattern of microbial metabolism in the gastrointestinal tract
- Stimulation of immunity
- Neutralization of enterotoxins formed by pathogenic organism

Prebiotics

Prebiotics were recognized for their ability to manipulate host microbiota to the benefit of the host (Gibson and Roberfroid 1995). Prebiotics provide nutrients to favorable microorganisms raise by the host, including administered probiotic strains and indigenous (resident) microorganisms. Therefore, prebiotics are differing from most of the dietary fibers such as cellulose, xylans, and pectins which inspire the growth of a wide range of gut microorganisms. Currently, two main groups dominate the prebiotic category with their effects acting through enrichment of *Lactobacillus* and/or *Bifidobacterium* spp. thus resulting in increased growth rate and improved feed efficiency:

I. Fructans (fructooligosaccharides (FOS) and inulin)
II. Galactans (galactooligosaccharides or GOS)

2.3.6 Additives That Alter Metabolism

Hormones

These are chemicals released by a specific area of the body (ductless glands) and are transported to another region within the animal where they elicit a physiological response. Extensive use is being made of synthetic and purified estrogens, androgens, progestogens, growth hormones, and thyroxine or thyroprotein (iodinated casein) to stimulate the growth and fattening of meat-producing animals. There is concern, however, about possible harmful effects of any residues of these materials in the meat or milk for the consumers.

The whole question whether hormones should be used as growth promoters is still debatable, but it seems logical that with any feeding system, the economic advantages, however great, should never take precedence over any potential risk to human health. These substances may induce cancer in human beings if taken over a prolonged period through products of the treated animals. The use of such substances in poultry rearing has been prohibited by law in the USA.

Implant

Implants are hormone or hormone-like products that are designed to release slowly, but constantly, the active chemicals for absorption into the bloodstream. These are implanted subcutaneously in the ear [e.g., diethylstilbestrol (DES)].

2.3.7 Additives That Affect the Health Status of Livestock

Antibloat Compounds

Surfactants such as poloxalene are used as a preventive for pasture bloat, and several other products which have been shown to be highly effective to prevent bloat are also available in the market.

Antifungal Additives

Mold inhibitors are added to feed liable to be contaminated with various types of fungi such as *Aspergillus flavus*, *Penicillium cyclopium*, etc.

Before adding commercial inhibitors, all feedstuff should be dried below 10% moisture. Propionic, acetic acid, and sodium propionate are added in high-moisture grain to inhibit mold growth. Antifungals such as nystatin and copper sulfate preparations are also in use to concentrate feeds to prevent molds.

Anticoccidials

Various brands of anticoccidials are now available in the country to prevent the growth of coccidia which are protozoa and live inside the cells of the intestinal lining of livestock.

Anthelmintics

Under some practical feeding conditions, anthelmintics have also been used. The compounds act by reducing parasitic infections.

2.3.8 Phytogenic Feed Additive

Phytogenic feed additives are the products which are derived from plants to be used in animal feeding to improve the quality of feed, performance, and health of agricultural livestock and quality of food from animal origin. Since the last two decades, this group of feed additive gets immense interest among the farmers, especially for use in poultry and swine

farming. This increase in popularity is due to the increase in number of scientific publication in this field since 2000 which is also supported by the ban on the most of the antibiotic feed additives within the European Union (complete ban enforced in 2006), voluntarily withdrawal of the use of antibiotics as growth promoters by the USA, and growing discussion to restrict their use outside European Union. This ban and discussion is driven by the speculated risk for generating antibiotic resistance in pathogenic microorganisms.

Awareness among the people regarding the potential health hazards and environmental harm caused by the excessive use of synthetic pharmaceuticals including in-feed antibiotics as growth promoters and growth hormones and also public demand for organic foods have gradually changed the attitude toward these synthetic antibiotics (Greathead 2003; Rochfort et al. 2008).

Restrictions on the use of antibiotics as growth promoters have significantly increased the incidence of infection by pathogens, consequently having an inimical effect on the performance of livestock. This also intensifies the search for an alternative to the antibiotics as growth promoters and popularizes the phytogenic feed additive. Phytobiotics as a feed additive is a new member in the list of non-antibiotic growth promoters, such as probiotics, prebiotics, and organic acids, which are already well known in the field of livestock nutrition. The knowledge about their mode of action and aspect of the application is still rather limited, and it has a lot of potential in the coming time.

Phytogenic feed additive is a wide range of plant-derived products such as herbs, essential/aromatic oils, and oleoresins. They can be added to the diet of commercial animals to improve their productivity through enhancing feed properties, promoting animal's production performance, and improving the quality of products derived from these animals (Windisch et al. 2008).

Windisch et al. (2008) has also recommended some commonly used terms to classify different phytogenic compounds based on their origin and processing, including herbs (flowering, non-woody, and nonpersistent plants), spices (herbs with an intensive smell or taste commonly added to human food), essential oils (volatile lipophilic compounds), and oleoresins (extracts derived by nonaqueous solvents). The content of active substances and the chemical composition of phytogenic substance in the final products may vary widely depending on the plant parts used (seeds, leaves, etc.), geographical origins, and harvesting season (Burt 2004; Bakkali et al. 2008; Wendisch et al. 2008). Selection of particular part of a plant of particular species of a very particular geographical region is very important to get a specific needed effect. The active constituents of the same species of plant may vary depending on the different geographical region and climatic condition. These variations are the results of genetic and environmental interaction and a manifestation of biodiversity within the same plant species (Zhang et al. 2011).

Plenty of research studies have indicated the multiple roles of phytobiotics as a feed additive like growth promotion effects, antimicrobial activity, antioxidant activity, anti-inflammatory activity, etc. Based on the investigations, it seems that modulation of the gut environment and intestinal morphology in swine and poultry is the hypothesized mode of action of phytogenic feed additives (Stein and Kil 2006; Li et al. 2012) (Table 1).

Plant Secondary Metabolites (PSM)

The biological and therapeutic property of a medicinal plant is closely related to the phytochemicals in it. An extensive summary is required for a comprehensive overview of chemistry, biochemistry, and bioactivity of plant secondary metabolites because they are a very large group of compounds. Out of the more than 100,000 different compounds of natural origin that have been described, more than 80,000 are derived from plants (Hashemi and Davoodi 2010). These phytochemicals can further be classified into major groups such as alkaloids, tannins, saponins, steroids, essential oils, acids, etc.

Different classifications had been put forward to classify this broad range of phytogenic substances based on their source of origin, chemical composition, usage, mode of action, etc. Regarding classification of the phytogenic substances/phytobiotics with respect to biological origin, chemical composition, formulation, and purity, phytogenic substances comprise a very wide range and can further be classified into four groups as herbs (products from flowering, non-woody, and nonpersistent plants), botanicals (entire or processed part of a plant, e.g., root, leaves, and bark), essential oils (hydrodistilled extracts of volatile plant compound), and oleoresins (extracts based on nonaqueous solvents) (Windish and Kroismayr 2006) (Fig. 4).

Classification of Phytogenic Based on Their Properties

Antimicrobial, antioxidants and anti-inflammatory, growth promoters, palpability and gut function modulators, and immunomodulators

(I) **Antibacterial Activity of Phytogenic Solutions**

Herbs and spices are well known to exert antimicrobial actions in vitro against important pathogens including fungi (Windisch et al. 2008). A common feature of phytogenic compounds is that they are the very multifaceted mixture if bioactive component. Phytochemicals in phytogenic compounds are well known to have antimicrobial activity (Cowan 1999). Investigations on phytochemicals indicated that phenolic contents like carvacrol, thymol, phenylpropane, limonene, geraniol, and citronellal are the important active

Table 1 Commonly used plants as feed additive in animal healthcare and livestock production

Botanical name	Parts used	Important active constituents	Common uses	Reference
Asparagus racemosus	Root	Shatavarins I–IV, asparagamine	Galactogogue, antistress, immunostimulant	Ananthanarayana et al. (2002), Dahouda et al. (2009)
Acacia catechu	Stem wood extract, fruit	Catechin, quercetin, epicatechin	Antidiarrheal, anti-inflammatory, antioxidant	J.A. Duke (1992), Rastogi and Mehrotra (2005)
Allium sativum	Bulb	Allicin, allin, methyl allyl disulfide	Hypolipidemic, carminative, antiprotozoal, anti-inflammatory	Hussain et al. (1992), Sharma et al. (2000–2005)
Aloe barbadensis	Leaf	Aloin, barbaloin, emodin	Emmenagogue, anti-inflammatory, antibacterial	Blumenthal et al. (1998), Rastogi and Mehrotra (2005)
Balanites roxburghii	Fruit, seed, seed oil	Balanitisins A-E, marmesin, bergapten	Purgative, spasmolytic, anti-colic, anthelmintic	Bilore et al. (2004–2005), Hussain et al. (1992)
Cissus quadrangularis	Steam, root, leaves	Quadrangularins, piceatannol, pallidol	Fracture healing, useful in dyspepsia	Ananthanarayana et al. (2002), Rastogi and Mehrotra (2005)
Curcuma longa	Rhizome	Curcumin, turmerone, desmethoxycurcumin	Anti-inflammatory, carminative, spasmolytic, antioxidant, hepatoprotective	Blumenthal et al. (1998), J.A. Duke (1992), Hussain et al. (1992)
Eucalyptus globulus	Leaves, oils	Cineole, pinene, limonene, eucaglobulin	Anti-inflammatory, carminative, digestive, expectorant, antibacterial	Rastogi and Mehrotra (2005), Blumenthal et al. (1998), Hussain et al. (1992)
Glycyrrhiza glabra	Root	Glycyrrhizin, liquiritin, glabranins	Antihistaminic, expectorant, anti-inflammatory	J.A. Duke (1992), Blumenthal et al. (1998)
Leptadenia reticulata	Root	Hentriacontanol, stigmasterol, rutin	Galactogogue, stimulant, uterine cleanser	F. Mirzaei (2011), Hussain et al. (1992)
Ocimum sanctum	Whole plant, leave, oil	Eugenol, ursolic acid, carvacrol, luteolin, methylchavicol	Immunomodulator, anti-inflammatory, antitussive, antiprotozoal	Sharma et al. (2000–2005), D. Brown (1996), Rastogi and Mehrotra (2005)
Phyllanthus emblica	Fruit, leaves	Ascorbic acid, gallic acid, emblicanins A and B	Antioxidant, hepatoprotective, immunomodulator	Ananthanarayana et al. (2002), Rastogi and Mehrotra (2005)
Solanum nigrum	Whole plant	Solasodine, solasonine, solanine, solamargine	Hepatoprotective, antioxidant, mycotoxin inhibitor, diuretic	J.A. Duke (1992), Hussain et al. (1992)
Swertia chirata	Whole plant	Swertiamarin, swerchirin, gentianine	Hepatoprotective, anti-inflammatory, anthelmintic	Bilore et al. (2004–2005), Hussain et al. (1992)
Withania somnifera	Root	Withaferin-A, withanine, somniferin	Immunomodulator, antistress, antioxidant, adaptogenic	D. Brown (1996), J.A. Duke (1992)

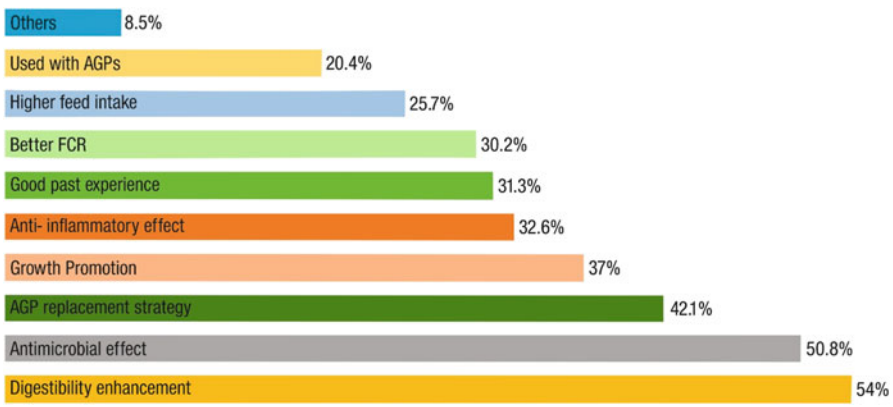


Fig. 4 Top reasons for use of phytogenic feed additives (in %)

compounds that have the antimicrobial function (Gheisar and Kim 2018). Limonene and compounds from *Sanguinaria canadensis* which are non-phenolic also show high antibacterial activity (Newton et al. 2002; Burt 2004). Yang et al. (2015) suggested that the antimicrobial action of PFAs varies by the location of their functional hydroxyl or alkyl groups. Investigations show that the presence of delocalized electrons and the hydroxyl group of phenolic terpenoids are

important elements for antimicrobial action of phytochemicals. Phytochemicals exert their antimicrobials activity through different mechanisms; tannins, for example, act by iron deprivation, hydrogen bonding, or non-specific interactions with vital proteins such as enzymes (Scalbert 1991). Tannic acid inhibits the growth of important intestinal bacteria such as *Bacteroides fragilis*, *Clostridium perfringens*, *E. coli*, and *Enterobacter cloacae* (Chung et al. 1993). Alkaloid is known to be a DNA intercalator and an inhibitor of DNA synthesis through topoisomerase inhibition (Karou et al. 2006). The main mechanism by which saponins display an antimicrobial activity is based on their ability to form a complex with sterols present in the membrane of microorganisms.

Essential oils have long been recognized for their antimicrobial activity (Lee et al. 2004), and they have gained much attention for their potential as an alternative to antibiotics in broiler chickens. Some studies with broiler demonstrated in vitro antimicrobial efficacy of essential oils against *E. coli* and *Clostridium perfringens* (Jamroz et al. 2003; Mistsch et al. 2004). Essential oils act on pathogenic bacteria by blocking quorum sensing (anti-quorum sensing) activity of the pathogenic bacteria. Extracts of plants of different species like *Chamaesyce hypericifolia*, *Conocarpus erectus*, and *Quercus virginiana* show quorum sensing inhibiting property and guard the growth of pathogenic bacteria (Adonizio et al. 2006). Moreover, structural properties such as the presence of functional groups (Farag et al. 1989) and aromaticity (Bowels and Miller 1993) are also responsible for the antibacterial activity of essential oils. It was postulated regarding the terpenoids that terpenoids and phenylpropanoids can penetrate the membrane of the bacteria and reach the inner part of the cell because of their lipophilicity (Helander et al. 1998). The important benefits of antimicrobial action of phytogenic products are that they can improve the microbial hygiene of carcasses. Aksit et al. (2006) illustrate that the addition of essential oil reduces the load of total viable bacteria or pathogens (e.g., *Salmonella*) on broiler carcasses.

(II) Antioxidant and Anti-inflammatory Action

Antioxidant activity is one of the important properties of phytogenic products which contribute to the reason for use as feed additives in humans as well as animals. Their ability to scavenge free radicals may play an important role in preventing some diseases caused by free radicals, such as cancer and heart diseases (Miguel 2010). The previous investigation has suggested that the antioxidant activity is due to their ability to donate hydrogen or an electron to free radicals and also delocalize the unpaired electron within the aromatic structure which are the main mechanisms of protecting other biological molecules against oxidation (Fernandez-Panchon et al. 2008; Giannenas et al. 2013). Brenes and Roura (2010) have reported that a wide range of herbs and their extracts have potential antioxidant functions, especially those products derived from the plant family Labiatae such as rosemary, oregano, and thyme. Blending of the phytogenic products like thyme in the feed of the ducks causes the reduction in thiobarbituric acid reactive substances (TBARS) value of breast meat significantly (Mohammadi et al. 2015a). Cherian et al. (2013) reported that feeding broiler chickens with PFA (*Artemisia annua*) resulted in a significant reduction in TBARS value in breast and thigh meat. The reduction in TBARS value might be due to an individual or shared antioxidant properties of polyphenolic compounds or vitamin E in *Artemisia annua*. The antioxidant activity of the phytogenic products is due to both phenolic and non-phenolic content (Cuppett and Hall 1998). Placha et al. (2014) have demonstrated that supplementing the diet of broiler chickens with thymol can reduce the oxidation of fatty acids indicated by the lower malondialdehyde level in duodenal mucosa. Franz et al. (2010) have advocated that phytobiotics can beneficially affect some antioxidant enzymes such as glutathione peroxidase and superoxide dismutase, consequently affecting lipid metabolism in animals. Other plant species such as coriander, curcuma, ginger, anise, and plants that are rich in flavonoids or anthocyanins also have antioxidant activities (Nakatani 2000; Wei and Shibamoto 2007). The active compounds of phytogenic compounds may have protective roles for feed lipids against oxidative damage, similar to antioxidants such as a-tocopheryl acetate or butylated hydroxytoluene that is usually added to diets (Gheisar and Kim 2018).

Inflammation is the normal protective phenomenon induced due to injury to the tissue or infection to counter invaders in the body (pathogens) and to remove dead or damaged host cells (Stevenson and Hurst 2007). Miguel (2010) stated that some essential oils have the ability to scavenge free radicals. In addition, they can also act as anti-inflammatory agents because one of the inflammatory responses is oxidative burst in diverse cells. Essential oils (eucalyptus, rosemary, lavender, millefolia) and other plants (pine, clove, and myrrh) are also used in mixed formulations as anti-inflammatory agents.

(III) Growth-Promoting Property

Regarding the growth-promoting activity of the phytogenic compounds, lots of investigations have been conducted during the last two decades. The growth-promoting activity of the herbal feed additive has been reported in swine (Wenk 2003; Kim et al. 2010; Mohammadi et al. 2015b). Li et al. (2012) compared the performance of pigs fed with the diets supplemented with essential oils and reported weight gain and digestibility of dry matter, and

crude protein were improved by 10.3%, 2.9%, and 5.9%, respectively. They suggested that improved performance of pigs was the result of improvement of the intestinal morphology and consequently improvement of nutrients digestibility. Yan et al. (2011) have reported that adding an herb extract blend (containing buckwheat, thyme, curcuma, black pepper, and ginger) to the diet of growing pigs resulted in increases in average daily feed intake (ADFI) and final body weight (BW). Feeding broiler chickens with the diet containing 0.075% of a phytogenic blend led to 3.9% and 3.4% improvement in BWG and FCR, respectively (Mohammadi et al. 2015a). According to the researcher proposal, phytogenic compounds act differently to elicit its growth-promoting activity. Improving palatability and flavor of feed, increasing feed intake, stimulating the secretion of digestive enzymes, and increasing antimicrobial activity are some of the main modes of action that might have led to the improved growth performance of animals (Jang et al. 2004; Czech et al. 2009). Different investigations have suggested that removal of in-feed antibiotics has resulted in the significantly negative effect on the performance of pig and poultry. This negative effect can be alleviated by incorporation of phytogenic product as growth promoters in the feed of pig and poultry (Yakhkeshi et al. 2011).

(IV) **Influence on Palatability and Gut Function**

It is claimed that phytogenic products are positively effective on the palatability and flavor of feed, thus promoting feed utilization and enhancing the production performance (Windisch et al. 2008). Some investigators had found a decrease in the feed intake due to incorporation of phytogenic products as growth promoters in feed (Maass et al. 2005; Roth-Maier et al. 2005), while some are claiming phytogenic solutions cause increase in the feed intake and palatability of feed (Kyriakis et al. 1998; Kroismayr et al. 2008). Chrubasik et al. (2005) have reported that a wide range of phytobiotics (including herbal plants and their extracts) are known to have beneficial impacts on the digestive tract (such as laxative and spasmolytic effects). In addition, they can prevent flatulence. Furthermore, Patel and Srinivasan (2004) have suggested that phytogenic substances can stimulate digestive secretions such as saliva and bile. They reported that improving enzyme activity is the main mode of nutritional action of phytogenic feed additive (PFA). Rao et al. (2003) have reported that the in vitro activities of rat pancreatic lipase and amylase are significantly enhanced when they were in contact with various spices and spice extracts. There is increase in enzymatic activities in pancreatic homogenate and pronounced bile acid flow in rats fed with phytogenic feed additive (Patel and Srinivasan 2000). Similar reports of enhancing the activities of digestive enzymes such as pancreatic amylase, trypsin, and maltase by essential oils in the diets of broiler chicken were given by researchers (Lee et al. 2003; Jang et al. 2004, 2007). Jamroz et al. (2006) suggested that feeding broilers with a diet supplemented with PFA resulted in stimulating the secretion of mucus in the intestine of broilers. This effect was assumed to reduce the adhesion of pathogens, thus stabilizing microbial eubiosis in the gut of animals.

(V) **Immunomodulators**

Phytogenic additives are having potential effect on the immune system of living organisms. β-glucans (oligosaccharide) is found in plant components mainly in aleuronic layer of barley and oat bran is an important immunomodulator. Bamboo leaf extract appears a new and promising source of β-glucans. Ohtsuka et al. (2014) evaluated the effect of an extract of β-glucans obtained from bamboo leaves (*Sasa sensanensis*) in cattle and report the increase in the activity of $CD8^{+}$T lymphocytes.

3 Quality Standards for Animal Feed Additive Industry

Faced with the problem of increasing nutritive value of the feed with limited resources of land and capital, soon it was discovered that unwanted chemicals and adulterants were being used in animal feeds and it helped to have a high analytical grading for the feed. So, came in place quality standards which laid down guidelines for the manufacturing to marketing of the animal feed and set up regulations which made it mandatory for feed manufacturers to abide by the set standards.

One of the premier authorities in the world with respect to animal feed industry—the American Feed Industry Association (AFIA)—has defined feed quality-control programs as: "All actions directed towards ensuring the product meets the specifications established by the manufacturer." AFIA is one of the most premier organizations (Maurya 2017). Any standard feed quality-control program must contain four necessary components (Pal and McSpadden Gardener 2006):

- Ingredient quality
- Process control
- Finished feed quality
- Control of toxic substances, including pathogenic microorganisms

3.1 The Importance of Quality Assurance

Quality assurance is one of the most important criteria in maintaining the industry standards and also to apply for various certifications related to product manufacturing

practices and standards. It creates a market value for the product and greater consumer acceptability. It needs to be understood that feed safety is not the only element that determines the safety of food of animal origin but that the use of other products, such as drugs and growth promoters (hormones and beta-agonists), also has an impact.

3.2 Quality Control of Feed

The process of conversion of high-quality ingredients into high-quality feeds involves three important components within the feed mill: personnel, machinery, and procedures. In event of any compromise in any of these three components, the consistent production of high-quality feeds is unlikely.

However, it is equally important to ensure the blending of personnel, machinery, and procedures together toward the common goal of efficient production of high-quality feeds (Jones 2006). Quality control in feed plant is of utmost importance for overall success and profitability of animal enterprises. The most critical factors affecting nutrition and high performance of animals are feed quality control and ration consistency. The degree of quality is the consistency in which feed is formulated, processed, produced, and delivered as compared to what is expected.

Quality has been defined as "Degree to which a set of inherent characteristics fulfills requirements" (Garg et al. 2013). This clearly indicates that achieving quality means fulfilling requirements. The requirements may come from customers and in some cases from regulatory authorities. Usually quality is verified by comparison with a known standard. However, a relative value of quality over time is extremely valuable and useful in many situations.

Animal performance is directly affected by feed quality, and this relationship is important as it not only encompasses the quantitative amounts of all feed components but also the digestibility and metabolism of those components. Thus, the main challenge that lies for animal science researchers as well as for nutritionists and other stakeholders involved in feed production is the consistent monitoring of all the aspects of feed production system and measurement of those variables that are good indicators of quality control.

In some cases, post-marketing quality checks are done to keep a tab on shelf life as well as customer satisfaction as it can have an enormous impact on quality as perceived by them. In view of this, monitoring of quality control at different points has been classified as under:

- Quality control of raw materials and finished products
- Quality control during storage
- Quality control during production

3.3 Necessity for Quality Control

The objective of quality control of animal feed and feed additives is to ensure that consumer obtains feeds that are unadulterated and true to their nature and produce desired results. Quality control is, therefore, defined as the maintenance of quality at levels and tolerances acceptable to the buyer while minimizing the cost of processing.

The Bureau of Indian Standards is the nodal organization responsible for laying down the quality control specifications of various feed ingredients and compound feeds to ensure maintenance of the minimum contract specifications, suitable for inclusion in the compounded feeds, and indicating the maximum proportions of inclusion of feedstuffs (Uppal et al. 2004).

3.4 Evaluation of Feed for Quality

The feeds are usually subject to the following three types of tests: physical, chemical, and biological.

3.4.1 Physical Evaluation

Physical evaluation must be carried out by highly trained personnel to identify the changes in the nature of the raw material/feeds. The main attributes checked in a physical evaluation are color, size, homogeneity, smell, taste, touch, and sound.

Physical Methods to Detect Adulteration or Contamination

The common contaminant or adulterant is husk or sand. The best method to detect husk in the feedstuff is winnowing while sieving is performed for differentiating contaminants based on particle size. Sand is detected in feed using a traditional yet effective method where a weighed quantity of the grain is soaked in water and then by sieving with hand the grains can be separated. The remaining water is decanted, and the settled sand is weighed to assess the level of contamination.

3.4.2 Chemical Evaluation

For chemical evaluation of animal feed, the first and foremost requirement is that of an analytical laboratory for precise estimation of nutrient contents and contaminants. Proximate principles of feed are analyzed using chemical evaluation.

3.4.3 Ingredient Specifications

Ingredient specifications of animal feeds and feed additives are crucial in a feed quality assurance program. Ingredient specifications serve as the basis on which agreements are written, feed/rations are formulated, and ingredient inspections are performed. Description of feed ingredients and general nutritional specifications may be found in BIS

specifications for feeds and feed ingredients in India. It is done both for quantitative and qualitative specifications and standards.

3.5 Quality Control Legislations in Indian Feed Industry

In the organized sector, animal feed production is a competitive domain, and feed producers therefore attempt to produce feed of the highest possible quality (Dhobi and Malla 2015). Regular analysis of proximate principles is done for keep a check on the quality of feed. The animal feeds and feed additives are analyzed for amino acids, aflatoxin, ochratoxin, castor, tannins, and urease activity compulsorily among others. Raw materials employed in manufacturing of animal feed and finished products are subjected to microbial examinations such as microbial counts, *Salmonella* and *Escherichia coli* testing and mold count as well. Industry also employs latest technologies and modern equipment such as High-Performance Liquid Chromatography (HPLC) and near-infrared (NIR) analyzers for spectroscopic examination. Current analytical techniques are engaged to estimate vitamins, minerals, and other feed additives. Most of industries are promoting HACCP—Hazard Analysis and Critical Control Points— measures to guarantee safe feeds. Now, India is also following international standards of animal feed industry to upgrade its manufacturing standards and compete with the world market by indigenous manufacturing of animal feed and feed additives to reduce the cost as well.

In India the national quality control standards for manufacture and storage conditions of animal feeds and feed additives are regulated by a statuary body, the Bureau of Indian Standards (BIS). It was established under BIS Act, 1986. Before 1986, Indian standards Institute was regulating the quality control of several feed commodities. The objectives of BIS are as follows:

I. Harmonious development of the activities for standardization of various commodities
II. Marking
III. Quality certification of goods
IV. Attending to the connected methods

The Bureau has set up subcommittees for the standardization of different types of commodities. A subcommittee on animal feeds called Animal Feeds Sectional Committee has been specifically set up to check the quality of animal feeds and feed additives ingredients. The members of Animal Feeds Sectional Committee are a panel of eminent nutritionists and comprise of expert members from the following bodies:

I. Indian Council of Agricultural Research (ICAR) institutes
II. State agricultural universities
III. Feed industry
IV. Government departments having specialization in animal nutrition
V. Feed technologist concerned with animal husbandry activities

The Government of India is empowered with registration act on the Agricultural produce (Grading and Marketing), known as 'AGMARK' standards to fix quality standards and prescribe terms and conditions for using the seal, 'AGMARK'.

Government is also bound to ensure following of control measures by legislation to ensure quality and safe feeds and feed additives at controlled cost. Many regulations have been put forward:

I. The Prevention of Black Marketing and Maintenance of Supplies of Essential Commodities Act, 1980
II. The Standards of Weights and Measures (Packaged Commodities) Rules, 1977
III. The Consumer Protection Act, 1986
IV. Schedule of Tariff Values of the Articles Liable to Cess for 2006–2007
V. Agricultural Produce Cess Act, 1940
VI. Edible Oils Packaging (Regulation) Order, 1998
VII. The Prevention of Food Adulteration Rules, 1955

The latest legislation in this respect is Cattle Feed (Regulation of Manufacture and Sale) Order, 2009. However, in spite of all legal presence of these measures and legislations, feed quality checks in India leaves much to be desired. All said and done, not enough is done to keep a cap on feed quality in this country. This is very much factual for small-scale feed manufacturer, where adulteration is barricade.

The nodal organization involved in India toward developing interlinkages between industry academia and other sectors toward feed manufacturing practices and setting quality norms is the Compound Livestock Feed Manufacturers' Association (CLFMA).

3.6 Latest Developments in Feed Quality Assurance Sector in the World

Safe animal feeds can only be produced with safe ingredients. In order to combine the experience of existing feed ingredient assurance programs into one program that can operate across the world with one set of standards, the International Feed Safety Alliance (IFSA) as a joint project is initiated by the standard owners (IFSA Feed Ingredients Standard 2007).

To comply with the standards of IFSA, the participant countries need to apply the principles of Hazard Analysis and Critical Control Points (HACCP) and Good Manufacturing Practice (GMP). Participants certified will have to demonstrate that there are controls at each step of the supply chain that guarantee the safety of the feed constituents supplied.

3.6.1 GMP: Good Manufacturing/Managing Practice

A key point for attention in the quality control programs for animal husbandry is the safety of animal feed. GMP standard is one of the main elements for any animal feed quality program (Coelho and de Toledo 2017).

3.6.2 Risk Management

Risk assessment forms the basis for determining control measures. Risk assessment is a component of risk management, resulting in:

- Determination of control measures for eliminating or reducing these risks and controlling them at an acceptable level, including tracking and tracing of products
- Determination of product standards and action values for undesirable contaminants in feeds
- Implementation of a measuring strategy (monitoring and verification) for checking whether or not the control measures are effective

In an HACCP methodology, a well-considered and sensible balance has to be established between preventive procedures and monitoring of feed constituents for the presence of risks. Nevertheless, where there are uncertainties about the ability to get a hold on the risks, precautionary measures such as eluding use of a specific product must be taken. Several control measures and product standards have already been combined in legislation, but they are moderately fragmented with many limitations. To address this shortcoming, the GMP+ standard for animal feed offers a coherent framework.

The GMP+ standard currently comprises the following elements:

- General requirements for a company's quality system, comparable with and based on the ISO 9002 standard, in order to render quality assurance demonstrable.
- Criteria for risk assessment based on HACCP principles.
- Several additional sub-codes including generic control measures in the production process allied to the use of additives, drugs, undesirable substances, and hygiene (*Salmonella*). These additional control measures have been specified for the production and supply of compound feedstuffs, premixes, straight feedingstuffs and feed ingredients, and feed fats (in addition to those for feed ingredients), for storage and transshipment of feeds, and for transport.
- Minimum requirements for in-company inspections, such as quality assurance for laboratory analyses, sampling frequency, etc.

A set of product standards, comprising of European Union legal standards, additional national legal standards, and several supra-legal standards settled with the partners in the chain.

3.6.3 Early Warning System

An early warning system (EWS) is intended to be a safety net, as a supplement to quality management systems like GMP, ISO 9002, and HACCP. The goal is to identify, communicate, and eliminate possible or potential hazards which may occur despite all preventive measures taken (Stark and Jones Frank 2009).

No quality system is able to avoid totally all problems which may be caused by incidental factors (human error, natural events) or criminal acts. A proactive approach must be adopted to prevent potential hazards manifesting themselves. Key elements in such a system include speed, care, confidentiality, accountability, and responsibility.

3.6.4 International Legislations Associated with Feed Safety

Food hazards associated with feeds form an important part of public health importance. Hazard is defined as "A biological, chemical or physical agent in, or condition of, food/feed with the potential to cause adverse health effect" (Sareen 2010).

Under WTO, SPS Article 3 which deals with harmonization encourages use of international standards for food safety and animal and plant health, i.e., Codex. Important Codex work on feed safety includes:

- Classification of foods and animal feeds (CAC/Misc 4-93)
- Codex General Standard for Contaminants and Toxins in Foods and Feeds (CODEX STAN 193-1995)
- MRLs for pesticides (CAC/MRL 1-2009), veterinary drug (2-2009), extraneous MRLs (CAC/MRL 3-2001)
- Code of Practice for the Reduction of Dioxin and Dioxin-like PCB Contamination in Foods and Feeds (CAC/RCP 62-2006)
- Code of Practice for the Reduction of Aflatoxin B1 in Raw Materials and Supplemental Feedingstuffs for Milk-Producing Animals (CAC/RCP 45-1997)
- Code of Practice on Good Animal Feeding (CAC/RCP 54-2004)

Other Codex Standards: Applicable to Feeds

Traceability: Principles for traceability/product tracing as a tool within a food inspection and certification system (CAC/GL 60-2006)

Risk Analysis:

- Working principles for risk analysis for application in framework of Codex Alimentarius
- Principles and GL for the conduct of microbiological risk management
- GL for conduct of food safety assessments of foods derived from recombinant-DNA animals
- Principles for the risk analysis of foods derived from modern biotechnology

HACCP: Recommended International Code of Practice—general principles of food hygiene (4 rev 2003) and Annex on HACCP systems and GL for its application

- Emergency situation—principles and guidelines for exchange of information in food safety emergency situations (CAC/GL 19-2004)
- Inspection & certification—principles (CAC/GL 20-1995); GL for design, operation, assessment & accreditation of food import & export inspection & certification systems (CAC/GL 26-1997)

References

Adonizio AL, Downum K, Bennett BC et al (2006) Anti-quorum sensing activity of medicinal plants in Southern Florida. J Ethnopharmacol 105(3):427–435

Aksit M, Goksoy E, Kok F et al (2006) The impacts of organic acid and essential oil supplementations to diets on the microbiological quality of chicken car-casses. Archiv Fur Geflugelk 70:168–173

Ananthanarayana DB, Brindavanam NB, Dobriyal RM et al (2002) Major herbs of ayurveda. Elsevier Science

Animal Feed Additives (2014) Market by types (antibiotics, vitamins, antioxidants, amino acids, feed enzymes), livestock (swine, poultry, cattle, aquaculture, others) and geography – trends & forecasts: 2011–2018. Markets and Markets Report 1715

Animal Feed Additives (2018.) http://www.primaryinfo.com/industry/animal-feed-additives.htm. Accessed 20 June 2018

Bakkali F, Averbeck S, Averbeck D et al (2008) Biological effects of essential oils—a review. Food Chem Toxicol 46:446–475

Bilore KB, Yelne MB, Dennis TJ et al (2004–2005) Database on medicinal plants used in ayurveda, vols 6–7. CCRAS, New Delhi

Biomin Phytogenic feed additive survey (2018.) https://info.biomin.net/acton/attachment/14109/f-09d6/1/-/-/-/-/MAG_SciSol_PFA%202018_EN.pdf?sid=TV2:AQ3kflqii

Blumenthal M, Busse WR, Goldberg A et al (eds) (1998) Therapeutic guide to herbal medicines, 1st edn (trans: Klein S, Rister RS). American Botanical Council/Integrative Medicine Communication, Austin, TX/Boston

Bowels BL, Miller AJ (1993) Antibouulinal properties of selected aromatic and aliphatic aldehydes. J Food Prod 56:788–794

Brenes A, Roura E (2010) Essential oils in poultry nutrition: main effects and modes of action. Anim Feed Sci Technol 158:1–14

Brown D (1996) Encyclopedia of herbs and their uses, The Herbal Society of America. Dorling Kindersley, New York

Burt S (2004) Essential oils: their antibacterial properties and potential applications in foods—a review. Int J Food Microbiol 94:223–253

Cherian G, Orr A, Burke IC, Pan W (2013) Feeding Artemisia annua alters digesta pH and muscle lipid oxidation products in broiler chickens. Poult Sci 92:1085–1090

Chrubasik S, Pittler MH, Roufogalis BD (2005) Zingiberis rhizoma: a comprehensive review on the ginger effect and efficacy profiles. Phytomedicine 12:684–701

Chung KT, Stevens SE, Lin WF Jr et al (1993) Growth inhibition of selected food borne bacteria by tannic acid, propyl gallate and related compounds. Lett Appl Microbial 17:29–32

Coelho RP, de Toledo JC (2017) Safety programs for the feed industry: characterization and perceived benefits of the implementation. Gest Prod São Carlos 24(4):704–718

Cowan MM (1999) Plant products as antimicrobial agents. Clin Microbiol Rev 12:564–582

Cuppett SL, Hall CA (1998) Antioxidant activity of the Labiatae. Adv Food Nutr Res 42:245–271

Czech A, Kowalczuk E, Grela ER (2009) The effect of an herbal extract used in pig fattening on the animals performance and blood components. Ann Univ Mariae Curie Sklodowska 27:25–33

Dahouda M, Toleba SS, Youssao AK et al (2009) Int J Poult Sci 8 (9):882–889

Dhobi IA, Malla BA (2015) Indian feed industry: past perspective and future challenges. Think Green Think Feed, Benison Media, pp 1–3

Duke JA (1992) Handbook of phytochemical constituents of GRAS herbs and other economic plants. CRC Press, Boca Raton, FL

European Commission (2018.) https://ec.europa.eu/food/safety/animal-feed/feed-additives_en. Accessed 20 June 2018

Farag RS, Dawz ZY, Hewedi FM et al (1989) Antimicrobial activity of some Egyptian Spice essential oils. J Food Prot 52:665–667

Feed additive classifications based on Europe regulation and guideline (2018) Bevenovo Co Limited

Fernandez-Panchon MS, Villano D, Troncoso AM et al (2008) Antioxidant activity of phenolic compounds: from in vitro results to in vivo evidence. Crit Rev Food Sci Nutr 48:649–667

Franz C, Baser KHC, Windisch W (2010) Essential oils and aromatic plants in animal feeding – a European perspective. A review. Flavour Frag J 25:327–340

Garg MR, Sherasia PL, Bhanderi BM (2013) Quality control manual for the cattle feed plants, Animal Nutrition Group. NDDB, Anand, Gujarat

Gheisar MM, Kim IH (2018) Phytobiotics in poultry and swine nutrition – a review. Ital J Anim Sci 17(1):92–99

Giannenas I, Bonos E, Christaki E et al (2013) Essential oils and their applications in animal nutrition. Med Aromatic Plants 2:1–12

Gibson GR, Roberfroid MB (1995) Dietary modulation of the human colonic microflora: introducing the concept of prebiotics. J Nutr 125:1401–1412

Greathead H (2003) Plants and plant extracts for improving animal productivity. Proc Nutr Soc 62:279–290

Hashemi HR, Davoodi H (2010) Phytogenics as new class of feed additives in poultry industry. J Anim Vet 9(17):2295–2304

Helander IM, Alakomi HL, Latva-kala K et al (1998) Characterization of the action of selected essential oil components on gram-negative bacteria. J Agric Food Chem 46:3590–3595

Henchion M, Hayes M, Mullen AM et al (2017) Future protein supply and demand: strategies and factors influencing a sustainable equilibrium. Foods 6:53. https://doi.org/10.3390/foods6070053

Hussain A, Virmani OP, Popli SP et al (1992) Dictionary of Indian medicinal plants. CIMAP, Lucknow

Hutjens MF (1991) Feed additives. Vet Clinics North Am Food Anim Pract 7(2):525

IFSA Feed Ingredients Standard, April 2007
Jamroz D, Orda J, Kamel C et al (2003) The influence of phytogenetic extracts on performance, nutrient digestibility, carcass characteristics and gut microbial status in broiler chickens. J Anim Sci 17:394–400
Jamroz D, Wertelecki T, Houszka M et al (2006) Influence of diet type on the inclusion of plant origin active substances on morphological and histochemical characteristics of the stomach and jejunum walls in chicken. J Anim Physiol Anim Nutr 90:255–268
Jang IS, Ko YH, Yang HY et al (2004) Influence of essential oil components on growth performance and the functional activity of the pancreas and small intestine in broiler chickens. Asian-Australas J Anim Sci 17:394–400
Jang IS, Ko YH, Kang SY, Lee CY (2007) Effect of commercial essential oils on growth performance, digestive enzyme activity and intestinal microflora population in broiler chickens. Anim Feed Sci Technol 134:304–315
Jones FT (2006) Quality control in feed manufacturing. The poultry site. http://www.thepoultrysite.com/articles/526/quality-control-in-feed-manufacturing. Accessed 20 June 2018
Karou D, Savadogo A, Canini A et al (2006) Antibacterial activity of alkaloids from *Sida acuta*. Afr J Biotechnol 5:195–200
Kearney J (2010) Food consumption trends and drivers. Philos Trans R Soc Lond B Biol Sci 365(1554):2793–2807
Kim JD, Sherwin JA, Shim KS (2010) Effects of feed additive as an alternative for antibiotics on growth performance and feed cost in growing-finishing pigs. Korean J Org Agric 18:233–244
Kroismayr A, Sehm J, Pfaffl M et al (2008) Effects of essential oils or Avilamycin on gut microbiology and blood parameters of weaned piglets. J Land Manage Food Environ 59:111–120
Kyriakis SC, Sarris K, Lekkas S, et al (1998) Control of post weaning diarrhea syndrome of piglets by in-feed application of Origanum essential oils. In: Done S, Thomson J, Varley M, (eds) Proceedings of the 15th IPVS Congress, July 5–9, Birmingham, UK. Nottingham University Press, Nottingham, 218 p
Lee KW, Everts H, Kappert HJ et al (2003) Effects of dietary essential oil components on growth performance, digestive enzymes and lipid metabolism in female broiler chickens. Br Poult Sci 44:450–457
Lee KW, Everts H, Kappert HJ et al (2004) Growth performance of broiler chickens fed a carboxymethyl cellulose containing diet with supplemental carvacrol and/or cinnamaldehyde. Int J Poult Sci 3:619–612
Li PF, Piao XS, Ru YJ et al (2012) Effects of adding essential oil to the diet of weaned pigs on performance, nutrient utilization, immune response and intestinal health. Asian-Australas J Anim Sci 25:1617–1626
Maass N, Bauer J, Paulicks BR et al (2005) Efficiency of Echinacea purpurea on performance and immune status in pigs. J Anim Physiol Anim Nutr 89:244–252
Maurya P (2017) Quality standards in animal feed industry. Think Green Think Feed, Benison Media, pp 1–4.
Miguel MG (2010) Antioxidant and anti-inflammatory activities of essential oils: a short review. Molecules 15:9252–9287
Mirzaei F (2011) Rep opinion 3(10):18–36
Mistsch P, Zitterl-Eglseer K, Kohler B et al (2004) The effect of two different blends of essential oils components on the proliferation of *Clostridium perfringens* in the intestines of broiler chickens
Mohammadi Gheisar M, Hosseindoust A et al (2015a) Evaluating the effect of microencapsulated blends of organic acids and essential oils in broiler chickens diet. J Appl Poult Res 24:511–519
Mohammadi Gheisar M, Im YM, Lee HH et al (2015b) Inclusion of phytogenic blends in different nutrient density diets of meat-type ducks. Poult Sci 94:2952–2958
Nakatani N (2000) Phenolic antioxidants from herbs and spices. Biofactors 13:141–146
Newton SM, Lau C, Gurcha SS et al (2002) The evaluation of forty-three plant species for in vitro anti-mycobacterial activities; isolation of active constituents from *Psoralea corylifolia* and *Sanguinaria canadensis*. J Ethnopharmacol 79:57–67
Ohtsuka H, Fujiwara H, Nishio A et al (2014) Effect of oral supplementation of bamboo grass leaves extract on cellular immune function in dairy cows. Acta Veterinaria Brno 83:213–218
Pal KK, McSpadden Gardener B (2006) Biological control of plant pathogens. The Plant Health Instructor. https://doi.org/10.1094/PHI-A-2006-1117-02
Patel K, Srinivasan K (2000) Stimulatory influence of select spices on bile secretion in rats. Nutr Res 20:1493–1503
Patel K, Srinivasan K (2004) Digestive stimulant action of spices: a myth or reality? Ind J Med Res 119:167–179
Placha I, Takacova J, Ryzner M et al (2014) Effect of thyme essential oil and selenium on intestine integrity and anti-oxidant status of broilers. Br Poult Sci 55:105–114
Rao RR, Platel K, Srinivasan K (2003) In vitro influence of spices and spice-active principles on digestive enzymes of rat pancreas and small intestine. Nahrung 47:408–412
Rastogi RP, Mehrotra BN (2005) Compendium of Indian medicinal plants, vol 1–5. CDCRI/NISCAIR, Lucknow/New Delhi
Rochfort S, Parker AJ, Dunshea FR (2008) Plant bioactives for ruminant health and productivity. Phytochemistry 69:299–322
Roth-Maier DA, Bohmer BM, Maass N et al (2005) Efficiency of Echinacea purpurea on performance of broilers and layers. Archiv Fur Geflugelkunde 69:123–127
Ruminant Feed Market (2018) Segmented by ingredient, supplement, and geography – growth, trends, and forecast: 2018–2023. Mordor Intelligence, India
Sanchez ARR, Rivas-Estilla AM (2006) Role of probiotics in hepatic ischemia-reperfusion injury. J Gastroenterol Hepatol 21:647–656
Sareen S (2010) Feed Safety: Importance, Codex Standards & FAO Initiatives for First OIE/FAO APHCA Regional Workshop on Feed Safety – Feed borne Disease Prevention, Tokyo. FAO Regional Office for the Asia & the Pacific
Scalbert A (1991) Antimicrobial properties of tannins. Phytochemistry 30:3875–3883
Sharma PC, Yelne MB, Dennis TJ (2005) Database on medicinal plants used in ayurveda, vol 1–5. CCRAS, New Delhi
Specialty Feed Additives (2016) Market by type (flavors & sweeteners, minerals, binders, vitamins, acidifiers, antioxidants), livestock (swine, ruminants, poultry, aquatic animals), function, form, and region global forecast to 2022. Research and Markets report 173, ID: 3897708
Stark CR, Jones Frank T (2009) Quality assurance program in feed manufacturing. Feedstuffs 16:60–65
Stein HH, Kil DY (2006) Reduced use of antibiotic growth promoters in diets fed to weanling pigs: dietary tools, Part 2. Anim Biotechnol 17:217–231
Stevenson DE, Hurst RD (2007) Polyphenolic phytochemicals—just antioxidants or much more? A review. Cell Mol Life Sci 64:2900–2916
Thornton PK (2010) Livestock production: recent trends, future prospects. Philos Trans R Soc Lond B Biol Sci 365 (1554):2853–2867
Uppal DS, Ilyas SM, Sikka SS (2004) Quality and safety of animal feeds in India. Central Institute of Post-Harvest Engineering & Technology, (ICAR) Ludhiana, Punjab Agricultural University, Ludhiana (India)
Wei A, Shibamoto T (2007) Antioxidant activities and volatile constituents of various essential oils. J Agric Food Chem 55:1737–1742
Wenk C (2003) Herbs and botanicals as feed additives in monogastric animals. Asian-Australas J Anim Sci 16:282–289
Windisch W, Kroismayr A (2006) The effects of phytobiotics on performance and gut function in monogastrics. http://en.engormix.com/

MA-feed-machinery/articles/the-effect-phytobiotics-performance_285.htm

Windisch W, Schedle K, Plitzner C et al (2008) Use of phytogenic products as feed additives for swine and poultry. J Anim Sci 86:140–148

Yakhkeshi S, Rahimi S, Gharib Naseri K (2011) The effects of comparison of herbal extracts, antibiotic, probiotic and organic acid on serum lipids, immune response, GIT microbial population, intestinal morphology and performance of broilers. J Med Plants 10:80–95

Yan L, Meng QW, Kim IH (2011) The effects of dietary *Houttuynia cordata* and *Taraxacum officinale* extract powder on growth performance, nutrient digestibility, blood characteristics and meat quality in finishing pigs. Livestock Sci 141:188–193

Yang C, Kabir-Chowdhury MA, Hou Y, Gong J (2015) Phytogenic compounds as alternatives to in-feed antibiotics: potentials and challenges in application. Pathogens 4:137–156

Zhang XB, Zhou T, Guo LP et al (2011) Volatile oil contents correlate with geographical distribution patterns of the miaoethnic herb *Fructus cinnamomi*. Acta Ecol Sinic 31(18):5299–5306

Part III

Nutraceuticals in Organ- and System-Disorders

Nutraceuticals in Arthritis

Ramesh C. Gupta, Robin B. Doss, Rajiv Lall, Ajay Srivastava, and Anita Sinha

Abstract

Currently, in the United States, every fifth adult dog or horse suffers from arthritis. The two most common types of arthritis are osteoarthritis (OA) and rheumatoid arthritis (RA). OA occurs with greater frequency than RA. OA is an inflammatory heterogeneous chronic degenerative joint disease (DJD) characterized by chronic and progressive degradation of the articular cartilage, osteophyte formation, thickening and sclerosis of the subchondral bone, bone marrow lesions, hypertrophy of bone at the margin, synovitis, synovial fluid effusion, and fibrosis. Common clinical signs and symptoms associated with OA in dogs and horses include limping, immobility, stiffness of joints, crepitus, periarticular swelling, palpable effusion, and pain upon manipulation of the joint and limb. The pathophysiology of OA is very complex because there are multiple etiologies for this disease, and as a result, treatment is complicated. Pain and inflammation associated with OA are often managed by pharmacological suppression or surgery among a few other modalities. NSAIDs are known to have severe side effects, and surgery is very expensive, so the use of nutraceuticals appears to be a viable alternative for prevention and treatment of OA. This chapter describes various nutraceuticals that have the potential to exert antioxidative, anti-inflammatory, antinociceptive, and chondroprotective effects in osteoarthritis.

Keywords

Arthritis · Osteoarthritis · Nutraceuticals · Glucosamine · Chondroitin · Green-lipped mussel · Hyaluronan · Type II collagen · Shilajit · Devil's claw

R. C. Gupta (✉) · R. B. Doss
Murray State University, Toxicology Department, Breathitt Veterinary Center, Hopkinsville, KY, USA
e-mail: rgupta@murraystate.edu

R. Lall · A. Srivastava · A. Sinha
Vets Plus Inc., Menomonie, WI, USA

1 Introduction

Arthritis is a chronic debilitating disease that commonly afflicts a large number of humans and animals around the world. Currently, in the United States, every fifth adult dog or horse suffers from arthritis. The two most common types of arthritis are osteoarthritis (OA) and rheumatoid arthritis (RA). OA is an inflammatory heterogeneous chronic degenerative joint disease (DJD) characterized by chronic and progressive degradation of the articular cartilage, osteophyte formation, thickening and sclerosis of the subchondral bone, bone marrow lesions, hypertrophy of bone at the margin, synovitis, synovial fluid effusion, and fibrosis. Eventually, an animal's quality of life is compromised due to decreased stability, decreased mobility, loading, stiffness of joints, lameness, and pain, and in advanced stages, animals are unable to walk. RA is a chronic disease characterized by inflammation, pain, swelling, and stiffness of multiple joints. Among all animal species, dogs and horses suffer more with arthritis, and OA occurs with a greater frequency. The etiology of OA is multifactorial involving age, injury, lack of exercise/excessive exercise, nutritional deficiency, metabolism, obesity, genetic predisposition, infection, environment, etc. (Van Meurs 2017; Gupta et al. 2019). Although any breed of dog can develop OA, large breed dogs (such as German Shepherds, Labrador Retrievers, Newfoundland, Rottweilers, Siberian Huskies, and others) are genetically predisposed for OA and are >45% more likely to develop this disease. Due to multiple etiologies in OA, its pathophysiology is very complex involving several cellular and biochemical mechanisms and molecular pathways in degradation or loss of cartilage.

Common signs and symptoms associated with OA in dogs and horses include limping, immobility, stiffness of joints, crepitus, periarticular swelling, palpable effusion, pain upon manipulation of the joint, and lameness (Gupta et al. 2009, 2012; Lawley et al. 2013; Fleck et al. 2014; May et al. 2015; Murdock et al. 2016). Nonsteroidal anti-inflammatory drugs

R. C. Gupta et al. (eds.), *Nutraceuticals in Veterinary Medicine*, https://doi.org/10.1007/978-3-030-04624-8_24

(NSAIDs), which are potent cyclooxygenase-2 (COX-2) inhibitors, have been the choice of therapeutic drugs for OA for a long time. Other pharmaceuticals, such as grapiprant, diacerein, and tramadol, are also used to minimize OA-associated pain (Permuy et al. 2015; Rausch-Derra et al. 2016; Guedes et al. 2018). Due to the side effects of NSAIDs on the cardiac, hepatic, renal, and GI systems, they are currently being replaced by nutraceuticals. This chapter describes the pathophysiology of OA, biomarkers of disease detection and progression, and a number of nutraceuticals that are currently used or have a potential to manage the signs and symptoms of OA and to improve the quality of an animal's life.

2 Pathophysiology of OA

OA is a heterogeneous chronic degenerative joint disease (DJD) of the entire joint affecting cartilage, bone, adipose, and skeletal muscle, thereby causing remodeling and failure of the joint (Ramírez-Flores et al. 2017; Gupta 2016; Svala et al. 2017; Gupta et al. 2019). Degradation of cartilage is a primary pathological feature of OA, which occurs in two phases, anabolic and catabolic. In the anabolic phase, the chondrocytes attempt to repair the damaged extracellular matrix (ECM), and in the catabolic phase, enzymes produced by chondrocytes and other cells digest the ECM. In the latter phase, inhibition of ECM synthesis also occurs, leading to accelerated erosion of the cartilage (Castrogiovanni et al. 2016; Svala et al. 2017).

In OA, change in the shape of the joint occurs as a result of a loss of articular cartilage, osteophyte formation, subchondral sclerosis, bone marrow lesions (BMLs), and synovial proliferation, and these alterations subsequently lead to decreased stability, mobility, and loading. The normal joint cartilage consists of 5% chondrocytes and 95% ECM. In OA cartilage, decreases in the number of chondrocytes and in their ability to regenerate the ECM in response to stress have been described (Portal-Núñez et al. 2016). OA chondrocytes show a senescence secretory phenotype (SSP) exhibiting overproduction of cytokines (IL-1 and IL-6), matrix metalloproteinases (e.g., MMP-1, MMP-3, MMP-10, and MMP-13), and growth factors (e.g., epidermal growth factor). During cartilage degeneration, the inflammatory processes cause excess production of ROS, RNS, oxygen, and PGE_2 levels, and as a result, their increased levels are found within the joint (Bakker et al. 2017; Wan and Zhao 2017; Chin and Ima-Nirwana 2018). The release of inflammatory mediators such as PGE_2 and NO triggers chronic inflammation and apoptosis (Amin et al. 1997; Attur et al. 2008). Modulation of *N*-methyl-*D*-aspartate (NMDA) receptors and ATP-citrate lyase is also reported to be involved in chondrocyte metabolism alteration and articular cartilage degeneration (Chen et al. 2018a; Kalev-Zylinska et al. 2018).

In the early stages of OA, progressive depletion of the cartilage proteoglycan leads to a net loss of matrix from the cartilage. OA is characterized by the degradation of cartilage matrix components, including cartilage-specific type II collagen and proteoglycan, ultimately resulting in the loss of cartilage structure and function. Breakdown and deterioration of the cartilage have been correlated with increased activities of certain enzymes, including MMPs. In the early and middle stages of OA, bone attrition (decrease or loss of bone height and contour) is observed. In late-stage OA, thickening of subchondral bone occurs, which is accompanied by decreased mineralization. Subchondral bone attrition is also associated with the severity and loss of cartilage in adjacent regions and areas of BMLs, indicating an increased load on the bone-cartilage biomechanical unit. BMLs are indicators of OA progression and are considered an important risk factor for structural deterioration. Radiographic evidence of OA may show cartilage degeneration, presence of its remnants, and osteophyte formation in the OA joint.

Activity of pro-inflammatory cytokine IL-1β can be detected in synovial fluid from OA joints where it induces gene expression of matrix-degrading enzymes in the chondrocytes. In synovial fluid of OA patients, another pro-inflammatory molecule from natural killer cells is expressed, i.e., protease Granzyme A, which may contribute to chronic articular inflammation. NF-κB appears to be a key transcription factor that drives the expression of OA inducer genes in response to the activation of toll-like receptors and an interleukin receptor by ECM fragments and IL-1β. Pro-inflammatory cytokines, such as IL-1β, are produced in the OA-stimulated mitogen-activated protein kinase (MAPK) pathways through the extracellular signal-regulated kinases (ERK)½, p38 kinase, and c-June N-terminal kinase (JNK). Elevated MAPK phosphorylation results in activation of transcription factors, which in turn upregulates the production of several molecules, such as MMPs and aggrecanases, which are responsible for matrix degradation. In OA, IL-1β induces activation of the NF-κB signaling pathway in human chondrocytes, thereby causing low-grade pain (Wan and Zhao 2017). Sluzalska et al. (2017) demonstrated that NF-κB, p38 MAPK, and JNK signaling pathways are all involved in IL-1β-induced phospholipids (PLs) biosynthesis. Recently, McAllister et al. (2018) demonstrated that the nucleotide-binding and oligomerization domain-like receptor containing protein 3 (NLRP3) inflammasome is implicated in the pathogenesis of OA by producing IL-1β, TNF-α, and MMP-3, which drive cartilage degeneration and synovial inflammation. During OA progression, fibroblast-like synoviocytes undergo alterations in their PL composition to adapt to the new diseased environment (Reviewed in Gupta 2016; Gupta et al. 2019). Additionally, multiple studies have implicated PKCδ as the rate limiting factor in which PKCδ is situated at the convergent point of multiple signaling inputs, including PGF-2, substance P, TNF-α, IL-1, and fibronectin

fragment. PKCδ activation can lead to NFκB activation in addition to MAPK activation, and these pathways work in concert to inhibit anabolic signaling and stimulate ECM degeneration (Lee et al. 2013).

There are some additional signaling pathways involved in OA-related cartilage degradation (Wang et al. 2017; Chen et al. 2018b; Deshmukh et al. 2018). Wnt/β-catenin plays a critical modulatory role in maintaining the bone-cartilage biochemical unit. Increased expression of Wnt-induced signaling protein 1 (WISP-1), Wnt-16, and Wnt-28 have been reported in OA cartilage and may contribute to cartilage degradation by upregulating MMPs and aggrecanases (Blom et al. 2009). Wnt signaling affects the pathogenesis of OA by modulating both the differentiation of osteoblasts and chondrocytes and through the production of catabolic proteases (Deshmukh et al. 2018). A bone-cartilage modulatory pathway, transforming growth factor β (TGFβ), is required for the maintenance of metabolic homeostasis and structural integrity of healthy cartilage. This factor is highly expressed in normal cartilage but nearly absent in OA cartilage. Recently, Wang et al. (2017) described that TGFβ/ALK5 signaling maintains articular cartilage homeostasis, in part, by upregulating proteoglycan 4 (PRG4) expression through the PKA-CREB signaling pathway in articular chondrocytes. Loss/interruption of TGFβ signaling in cartilage results in loss of proteoglycans and cartilage degeneration. Thus, inhibition of endogenous TGFβ leads to increased damage to the cartilage.

Recently, Lu et al. (2014) and Carpio and Westendorf (2016) suggested that the initiation and progression of OA are contributed to expression patterns and enhanced activation of histone deacetylases (HDACs). Overexpression of HDAC4 promotes matrix-degrading enzymes and enhances catabolic activity of chondrocytes in OA cartilage (Lu et al. 2014). Both HDAC1 and HDAC2 suppress the expression of genes encoding ECM genes, such as type II collagen (Cil2a1), aggrecan (ACAN), and cartilage oligomeric protein (COMP). Interestingly, Pujol et al. (2018) reported that the reduction in nerve fiber density in synovia occurs with advanced cartilage degeneration, suggesting that peripheral neuropathy is associated with OA in the horse. Whether this link is associated with neuropathic pain remains to be elucidated. The pathophysiology of OA has been discussed in detail elsewhere (Gupta 2016; Gupta et al. 2019).

3 Diagnosis of OA

In clinical veterinary settings, diagnosis of OA is often based on observational (Gupta et al. 2009, 2012; Fleck et al. 2014; Lawley et al. 2013; Murdock et al. 2016) and radiographic findings (Ramírez-Flores et al. 2017). Dogs, cats, and horses all exhibit OA-associated pain due to inflammation in the joint(s). Biochemical biomarkers have become exceptionally useful in diagnosis and treatment of OA.

3.1 Pain Measurement

On a monthly basis, dogs and horses are evaluated for overall pain, pain upon limb manipulation, and exercise-associated lameness using the Glasgow scoring system for a study period of at least 4–5 months (Gupta et al. 2009, 2012, 2019; May et al. 2015; Murdock et al. 2016). In dogs, overall pain is graded on a scale of 0–10: 0, no pain; 5, moderate pain; and 10, severe and constant pain. Pain upon limb manipulation is evaluated during the extension and flexion of all four limbs for a period of several minutes. Pain level is graded on a scale of 0–4; 0, no pain; 1, mild; 2, moderate; 3, severe; and 4, severe and constant. Pain and lameness are measured after physical exercise and graded on a scale of 0–4: 0, no pain; 1, mild; 2, moderate; 3, severe; and 4, severe and constant. In horses, pain is measured using similar criteria and scales. Additionally, flexibility and range of motion in the affected joints are measured using a goniometer (May et al. 2015).

In dogs, a ground force plate (Kistler Instrument, Amherst, NY, USA) is utilized to quantitatively measure the lameness-associated pain in each leg of every dog (Gupta et al. 2012). The Kistler's ground force plate (GFP) system consists of plates, lasers, and a computer (Fig. 1). The GFP measures two major parameters: (1) peak vertical force or g force (Newton/kg body weight) and (2) impulse area (Newton sec/kg body weight).

3.2 Observation of Ortolani and Cranial Tibial Drawer Examination

Ortolani and cranial tibial drawer examination is performed to evaluate pain, gait, and lameness in dogs. The Ortolani Maneuver is a common test performed on canines that are predisposed to hip dysplasia such as German Shepherds or larger breed canines. The Ortolani is performed on the hip joint by flexing the knee and hip to 90 degrees, placing the index finger on the greater trochanters and abducting the hip (Ortolani's sign 2007; Fleck et al. 2014). As the hip is abducted, or moved away from the body, a positive Ortolani will present with a "clunk" sound or feeling as the femoral head relocates anteriorly to the acetabulum, or hip socket. In moderate arthritis, dogs may exhibit a negative Ortolani sign (Fleck et al. 2014; Gupta et al. 2019). Cranial tibial drawer is another test that can be performed upon physical examination to indicate arthritic changes and to diagnose the rupture of the cranial cruciate ligament (CCL). A positive tibial drawer is elicited with the ability to move the tibia cranially or forward

Fig. 1 Pain evaluation using ground force plate

in respect to the fixed femur. For a detailed procedure, refer to Devine (1993) and Gupta et al. (2019).

3.3 Biochemical Biomarkers

There are several biochemical biomarkers related to inflammation and pain (cytokines, PGE_2, c-reactive protein, TNF-α, TSG-6, etc.) and cartilage damage and loss (COMP, MMPs, fractalkine, type II cartilage fragments, bone sialoprotein, c-telopeptide of type I collagen, hyaluronan, aggrecan, ghrelin, lubricin, follistatin-like protein 1, etc.). Some of these biomarkers can be measured in synovial fluid (Trumble et al. 2004; Venable et al. 2008; Kamm et al. 2010; de Bakker et al. 2017; Heikkilä et al. 2017; Shahid et al. 2018), while others are measured in blood or urine (Bay-Jensen et al. 2016; Singh et al. 2015; Gupta et al. 2019). PGE_2 is produced by the action of COX enzymes in various cells, and its increased concentration in synovial fluid correlates positively with OA pain in dogs (Trumble 2005) and horses (Van Loon et al. 2010). It has also been reported that substance P enhances the release of PGE_2 from chondrocytes, and the concentrations of substance P and PGE_2 correlate with each other in the synovial fluid of OA horses (Kirker-Head et al. 2000). Recently, microRNAs (miRNAs) that are more sensitive and specific to OA have been identified in circulation, and they aid in early disease diagnosis and progression. Some of these have potential for therapy. For details on biomarkers of OA, see recent publications (Gupta 2016; Gupta et al. 2019).

4 Nutraceuticals in the Management of OA

There are a number of nutraceuticals that are currently used or have potential to manage the signs and symptoms of OA in dogs and horses (Gupta et al. 2009, 2012; Gupta 2016; Comblain et al. 2015; Bhathal et al. 2017). Furthermore, multicomponent nutraceuticals are popular for treatment or adjunctive therapies in veterinary medicine despite the lack of evidence of efficacy for many products (Martinez et al. 2015). The objectives in managing OA include minimizing joint pain by reducing the inflammation and slowing the progression of the cartilage damage, thereby increasing joint flexibility and quality of life. Some of the nutraceuticals are discussed here in brief.

4.1 Glucosamine and Chondroitin

Glucosamine (glucosamine sulfate, *GS*, or glucosamine hydrochloride, *GH*) and chondroitin sulfate (CS) are components of the ECM of articular cartilage. The structural formulas of glucosamine and chondroitin are shown in Fig. 2. Glucosamine (2-amino-2-deoxy-*D*-glucose) is a constituent of glycosaminoglycan, which plays a role in the normal growth and repair of articular cartilage. It is commonly considered a building block of cartilage.

Glucosamine is commonly obtained from crab, lobster, and shrimp shells. After oral administration of glucosamine (GS or GH), 90% of GS is absorbed from the small intestine. Due to an extensive first-pass metabolism, its bioavailability is only 25% (Sentikar and Rovati 2001). Adebowale et al. (2002) reported the bioavailability of GH at 12%. Excretion of glucosamine is mainly through the kidneys with only a trace amount of unmodified glucosamine eliminated through the stool (Persiani et al. 2005; Block et al. 2010). CS is a sulfated glycosaminoglycan (GAG) composed of a chain of alternating sugars, *N*-acetyl-*D*-galactosamine and *D*-glucuronic acid. CS is also a normal constituent of articular cartilage, and it is usually obtained from animal cartilage (such as the trachea and shark cartilage).

Approximately 30% of CS is absorbed, with a 12–13% bioavailability (Deal and Moskowitz 1999). Adebowale et al. (2002) found the bioavailability of CS to be 4.8–5.0%. For detailed pharmacokinetics of CS, readers are referred to Adebowale et al. (2002), Jackson et al. (2010), and Jerosch

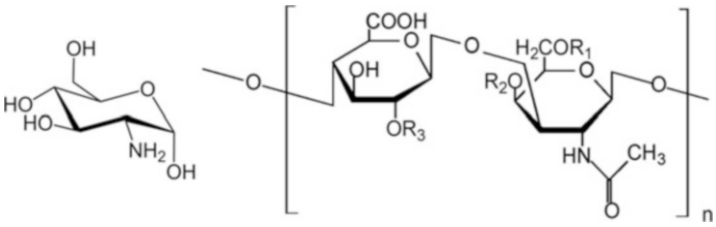


Fig. 2 Structural formula of glucosamine (left) and chondroitin (right)

(2011). Comblain et al. (2015) reported that the oral bioavailability and pharmacokinetics of GS and CS play an important role in optimizing OA management.

Glucosamine and chondroitin provide anti-inflammatory, anti-arthritic, and cartilage repair effects by multiple mechanisms. Some of these mechanisms include (1) reduced expression of MMPs (MMP-1, MMP-3, and MMP-13) and inhibited c-jun amino-terminal kinase and p38 phosphorylation and consequently c-jun binding activity (D'Abusco et al. 2007), (2) enhanced proteoglycan synthesis (Hooper 2001), and (3) suppression of IL-1-induced gene expression of iNOS, COX-2, mPGEs, and NF-κB in cartilage explants. CS also inhibits NF-κB nuclear translocation and phosphorylation of p38 MAPK, ERK½, and JNK (Jomphe et al. 2008; Stabler et al. 2017). As a result, reduced production of NO and PGE_2, two mediators responsible for the cell death of chondrocytes and inflammatory reactions, may occur (Chan et al. 2005).

Frech and Clegg (2007) and Hochberg et al. (2008) reported that CS exerts its anti-OA effects by producing hydration that helps create osmotic pressure within the ECM to maintain the compressive resistance of cartilage and improves function/mobility of the joint. CS stimulates chondrocyte metabolism, leading to the synthesis of collagen and proteoglycan (Jerosch 2011). It may also protect existing cartilage from premature breakdown. CS may reduce inflammation, inhibit synthesis of MMPs, increase synthesis of ECM constituents, and reduce apoptosis of articular chondrocytes (Monfort et al. 2008; Vangness et al. 2009). Glucosamine and chondroitin, when given in combination, offer synergistic effects, such as anti-inflammatory, antioxidative, and chondroprotective (Dechant et al. 2005; Neil et al. 2005; Trumble 2005; Valvason et al. 2008; Gupta 2016).

The doses, route, and duration of glucosamine and chondroitin treatment in dogs and horses are summarized in Table 1. In a double-blind study, McCarthy et al. (2007) evaluated the efficacy of Synoquin SA, a product of Vets Plus Inc. (475 mg GH and 350 mg CS per 1 g), in OA dogs for a period of 70 days. Dogs received Synoquin SA according to their body weight (1 g for 5–19.9 kg; 1.5 g for 20–40 kg; and 2 g for >40 kg) twice daily for 42 days. After 42 days, the daily dose of GH/CS was reduced by one-third for the subsequent 28 days. The treatment significantly ameliorated the signs and symptoms of OA in the study dogs. In a number of studies, GH and CS produced no side effects and were found to be well tolerated (Gupta et al. 2009, 2012). However, in few studies, glucosamine was found to be unsafe at higher doses (Giaccari et al. 1995; Breese McCoy and Bryson 2003; Lafontaine-Lacasse et al. (2011).

4.2 Hyaluronan

Hyaluronic acid (HA), commonly called hyaluronate or hyaluronan, is a nonsulfated glycosaminoglycan (GAG), produced by chondrocytes and synovial fibroblasts. HA is an important component of articular cartilage ECM that coats each chondrocyte. It is also present in synovial fluid. Its chemical formula is shown in Fig. 3. Within the joint cavity, HA molecules are predominately synthesized by type B synoviocytes. HA (a polymer of disaccharides) can be 25,000 disaccharide repeats in length, with a molecular weight (Mol Wt) of 5000–20,000,000 Da. HA is synthesized by hyaluronan synthases (HAS), of which vertebrates have three types (HAS1, HAS2, and HAS3). These enzymes lengthen HA by repeatedly adding glucuronic acid and *N*-acetylglucosamine to the nascent polysaccharide. HA is catabolized by hyaluronidases, and the MW of HA in cartilage decreases with age, but the amount increases (Holmes et al. 1988; Gupta 2016).

Intra-articular (IA) treatment with HA has been investigated in a number of studies, and it has been used for decades as OA therapy in dogs and horses (Armstrong et al. 1994; Kuroki et al. 2002; Frisbie et al. 2009; Jerosch 2011; Carapeba et al. 2016). In a recent study, HA was also administered intravenously in horses (Frisbie et al. 2016). There is controversy over the efficacy of orally administered HA. Pharmacokinetic data revealed that orally administered high-molecular weight HA also reached the joint (Balogh et al. 2008), which provides a rationale for the oral supplementation of HA. In addition to its contribution to the structural properties of the ECM, HA may have a role in regulating the synthesis of proteoglycans during maturation of articular cartilage and in repair processes (Kuroki et al. 2002).

Philip (1989) and Jerosch (2011) reported that HAs of higher MW were more effective than those of lower MW. Ghosh and Guidolin (2002) demonstrated that HAs within the Mol Wt range of 0.5×10^6–1.0×10^6 Da partially restored synovial fluid rheological properties and synovial

Table 1 Nutraceuticals in osteoarthritis

Nutraceuticals	Dog	Horse	References
Glucosamine + chondroitin	2 g glucosamine HCl + 1.6 g chondroitin sulfate, daily	5.4 g glucosamine HCl + 1.8 g chondroitin sulfate, twice daily for the first month, and once daily thereafter	D'Altilio et al. (2007), Gupta et al. (2009, 2012)
	1 g Synoquin SA (475 mg GH: 350 mg CS) for 5–19.9 kg; 1.5 g for 20–40 kg; and 2 g for >40 kg) b.i.d for 42 days. The daily dose was reduced by one-third for the subsequent 28 days. For details, see text	–	McCarthy et al. (2007)
Hyaluronic acid (HA)	5 mg for ≤10 kg; and 10 mg for ≥11 kg, IA	40 mg, IA	Auer et al. (1980), Carapeba et al. (2016)
Curcumin	4 mg/kg, twice daily for 20 days	–	Colitti et al. (2012)
Omega-3 fatty acids	69 mg EPA + DHA/kg/day for 84 days	15 g EPA + 19.8 g DHA/day for 90 days	Manhart et al. (2009), Mehler et al. (2016)
Vitamin E	400 IU/dog, once a day	–	Rhouma et al. (2013)
Shilajit (purified)	500 mg twice daily for 5 months	–	Lawley et al. (2013)
Type II collagen (active)	10 mg daily	160 mg daily	DeParle et al. (2005), D'Altilio et al. (2007), Peal et al. (2007), Gupta et al. (2009, 2012)
Bioactive collagen peptide (BCP) (Petagile®)		25 g or 50 g/day for 12 weeks	Dobenecker et al. (2017)
Avocado/soybean unsaponifiables (ASU)	10 mg/kg/day for 8 weeks or 300 mg every 3 day for 15 weeks	Avocado/soybean (1:2 ratio) unsaponifiable oil in 6 mL molasses/day for 70 days	Kawcak et al. (2007), Boileau et al. (2009), Altinel et al. (2011)
Green-lipped mussel (GLM) (*Perna canaliculus*)	GLM 0.3% of dry food diet or Lyproflex®	25 mg/kg body wt/day, po for 56 days	Bui and Bierer (2003), Cayzer et al. (2012), Rialland et al. (2013), Hielm-Björkman et al. (2009)
	450 mg GLM for <25 kg; 750 mg for 25–34 kg; and 1 g GLM for >34 kg/day	–	Bierer and Bui (2002)
Crominex® -3+	25 mg Crominex® -3+ (500 μg trivalent chromium; 7.5 mg amla extract; and 7.5 mg purified shilajit), twice daily	500 mg Crominex® -3+ (10 mg trivalent chromium; 150 mg amla extract; and 150 mg purified shilajit), twice daily	Fleck et al. (2014), May et al. (2015)
Boswellia serrata extract (acetyl-keto-beta-boswellic acid, AKBA)	150 mg daily	–	No publication available
Terminalia chebula extract (TCE)	500 mg TCE, twice daily	–	Murdock et al. (2016)

IA intra-articularly

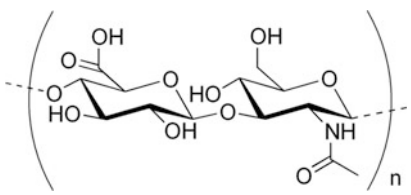


Fig. 3 Structural formula of hyaluronic acid (HA)

fibroblast metabolism in animal models. These authors also described the interaction of HA with pain receptors and analgesic effects. HA exerts pharmacological actions by mitigating the activities of pro-inflammatory mediators and pain-producing neuropeptides released by activated synovial cells (Kuroki et al. 2002; Gupta 2016). Moreland (2003) reported that HA can reduce nerve impulses and nerve sensitivity associated with the pain of OA. HA can also reduce OA-associated pain by decreasing PGE_2 and bradykinin synthesis, as well as substance P (Reviewed in Gupta 2016).

In regard to cartilage repair, Kuroki et al. (2002) suggested that intra-articular administration of HA has a direct effect on chondrocytes or synoviocytes and the production of transforming growth factor (TGF)-β, basic fibroblast-derived growth factor (FGF), and insulin-like growth factor (IGF)-1. Histological evidence suggests that HA prevents the degradation of cartilage and may promote its regeneration. Ghosh and Guidolin (2002) also provided evidence that HA treatment mitigated synovial hypertrophy and increased the

numbers of synovial fibroblast-like cells while decreasing macrophages, lymphocytes, mast cells, and adipocytes. HA appears to provide cartilage protection by the downregulation of cytokine; free radicals, such as NO; and proteolytic activities in the synovial fluid.

Like glucosamine and CS, HA is a slow-acting anti-OA agent that may be used prophylactically or therapeutically as an anti-inflammatory disease-modifying agent in OA (Kuroki et al. 2002; Venable et al. 2008; Aubry-Rozier 2012; Carapeba et al. 2016). Marshall et al. (2000) reported that a series of three weekly injections of 0.5 ml (4 mg) of Ha (Mol Wt, 6×10^6 Da) ameliorated the severity of OA in dogs. The use of intra-articular (IA) HA has been prescribed for synovitis or OA in the horse since 1975. HA is commonly used in the treatment of articular disorders (carpal and fetlock joints) in horses, especially those involved in racing competition and heavy work. HA can be injected directly into an affected joint. Auer et al. (1980) reported that in naturally occurring and experimentally induced OA in horses, IA injection of HA (40 mg) significantly reduced lameness and increased weight bearing on the treated limb, measured using the ground force plate. HA is especially indicated for mild to moderate levels of synovitis associated with equine OA. However, it has a limitation in treating severe synovitis or OA.

During the last decade, the use of intravenous HA has become common, especially for less localized disorders. However, there is only one licensed product made by Bayer (Legend® in the United States, Hyonate® elsewhere). It has been suggested that when used in combination, HA not only provides improved beneficial effects of corticosteroids but it can placate the side effects of certain corticosteroids. According to Canadian regulations, HA in HY-5- preparation should not be administered to horses that are to be slaughtered for meat. However, in Europe the same preparation is not considered to have any such effect. HA is known to produce some side effects, such as muscle pain, cramping, pain in the injected knee, and swelling in the arms and legs making movement difficult (Rutjes et al. 2012).

4.3 Curcumin

Curcumin is an active ingredient of the yellow Indian spice turmeric, which is obtained from the roots of *Curcuma longa*. The nutraceutical value of curcumin has been recognized in a number of diseases (Javeri and Chand 2016; Shome et al. 2016; Du et al. 2017: Kurien et al. 2017; Shen et al. 2017), including OA (Henroitin et al. 2014; Comblain et al. 2015; Zhang et al. 2016) and cancer (Jayakumar et al. 2017). In in vitro studies, curcumin has been shown to decrease the synthesis of iNOS, NO, PGE_2, Il-6, IL-9, COX-2, MMP-3, and MMP-9 by inhibiting NF-κB translocation and TNF-α signaling pathways in the chondrocytes, thereby providing a chondroprotective effect (reviewed in Comblain et al. 2015). In a mouse model of OA, Zhang et al. (2016) reported that both curcumin and the nanoparticles encapsulating curcumin suppressed mRNA expression of pro-inflammatory mediators IL-1β and TNF-α; MMPs 1, 3, and 13; and aggrecanase ADAMTS5 and upregulated the chondroprotective transcriptional regulator CITED2 in primary cultured chondrocytes in the absence or presence of IL-1β. Oral administration of curcumin significantly reduced OA disease progression but showed no significant effect on OA pain relief.

Colitti et al. (2012) treated dogs with curcumin (4 mg/kg, bid) for 20 days and found that curcumin targets I-κB upregulation in the TNRF1 signaling pathway and IL-18 downregulation in the role of cytokines in mediating communication between immune cells. Curcumin was also found to inhibit macrophage proliferation by downregulating TNF-α and activating fibrinolysis. In essence, curcumin exerts antioxidative, anti-inflammatory, and chondroprotective activities. Additionally, curcumin increases synthesis of type II collagen. Following its oral administration, the bioavailability of curcumin is poor as is its effectiveness against OA. For that reason, intense research efforts are underway to enhance its bioavailability by using various formulations (including bioenhancers) and delivery systems. Recent studies suggest that by conjugating curcumin to metal oxide nanoparticles or encapsulating it in lipid nanoparticles, dendrimers, nanogels, and polymeric nanoparticles, the water solubility and bioavailability of curcumin can be improved and thus increase its pharmacological effectiveness (Shome et al. 2016).

In several studies, curcumin has been investigated in combination with other nutraceuticals, such as boswellic acid, type II collagen, green tea, resveratrol, and others to achieve greater anti-OA effects than curcumin alone (reviewed in Comblain et al. 2015; Haroyan et al. 2018; Zhang et al. 2016). For further details on curcumin, readers are referred to Chap. 1 in this book.

4.4 Methylsulfonylmethane

Methylsulfonylmethane (MSM), also known as dimethyl sulfone (Fig. 4), is a naturally occurring organosulfur compound present in some plants. MSM supplements, available in both tablet and liquid form, are marketed with a variety of claims. MSM is often given in combination with glucosamine and/or chondroitin to prevent or treat OA.

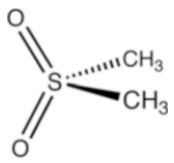


Fig. 4 Structural formula of methylsulfonylmethane (MSM)

MSM, by having high sulfur content, is used by the body to maintain normal connective tissue. It may provide anti-arthritic effects via its antioxidative, anti-inflammatory, and analgesic activities (Kim et al. 2006; also reviewed in Gupta 2016). In OA, MSM serves the same purpose as the NSAIDs, but MSM has none of the negative outcomes that are associated with NSAIDs.

In a clinical trial, Kim et al. (2006) found that compared to placebo, 3 g of MSM twice a day (6 g/day total) for 12 weeks produced significant decreases in WOMAC pain and physical function impairment. MSM ameliorated symptoms of pain and improved physical function during the intervention without major adverse events. In another clinical trial, Debbi et al. (2011) reported that patients with OA of the knee taking MSM 3 × daily (1.125 g) for 12 weeks showed a decrease in pain and improvement in physical function. Usha and Naidu (2004) treated patients with knee OA with either 1.5 g MSM (500 mg tid), 1.5 g glucosamine sulfate (GS), or MSM plus GS, or placebo for 12 weeks. Significant decreases in the Lequesne Index were reported with MSM, GS, and their combination ($P < 0.05$). The authors reported a 33% decrease in pain in the MSM group, joint mobility, swelling, and global evaluation, and walking time was also improved.

The recommended dose of MSM in dogs is 250–500 mg/day, and in horses it is 10–15 g/day. Dogs receiving MSM should be checked regularly for liver and kidney functions, and the lens in their eyes for opacity. Some dogs may show mild GI upset with vomiting and diarrhea, while others may show allergic reactions such as rash, hives, and difficulty breathing. MSM is not recommended for use in pregnant animals and those that are allergic to MSM or have urinary tract or bladder cancer.

4.5 Collagen

Type II collagen is the main collagen component of the hyaline cartilage, which is destroyed in OA by MMP 13 (Xu et al. 2007). Glycosylated undenatured type II collagen (hereafter referred to as type II collagen) has been shown to be effective in ameliorating pain associated with arthritis in humans (Trentham et al. 1993; Crowley et al. 2009), dogs (Gupta et al. 2012), and horses (Gupta et al. 2009). In clinical trials conducted on moderately arthritic dogs, type II collagen at 10 mg daily for a period of 120–150 days significantly reduced pain associated with OA (DeParle et al. 2005; D'Altilio et al. 2007; Peal et al. 2007; Gupta et al. 2012). In horses also, daily administration of type II collagen (160 mg) was found to be effective in reducing OA pain (Gupta et al. 2009). These supplements were well tolerated as no side effects were observed. For details on mechanism of action and safety of type II collagen, readers are referred to Marone et al. (2010) and Gupta (2016).

Collagen peptides are reported to stimulate the biosynthesis of extracellular matrix molecules in cartilage tissue and diminish degenerative processes by modulating the expression of collagenases and proteoglycanases (Schunck et al. 2007a, b, 2017). In a clinical trial, Dobenecker et al. (2017) supplemented OA horses with 25 g or 50 g bioactive collagen peptides (BCP, PETAGILE®) for 12 weeks. An orthopedic examination revealed that in a dose-dependent manner, BCP provided significant improvement in OA-associated lameness and pain in horses.

4.6 Avocado/Soybean Unsaponifiables

Avocado and soybean unsaponifiables (ASU) have been used in the treatment of OA for decades. The active ingredients of this extract are a sterol-rich hydrolyzed lipid fraction (Castrogiovanni et al. 2016) and synergism between the avocado and soya components. Their relative ratios (one-third avocado oil and two-third soybean oil) also appear to be important (Reviewed in Gupta 2016). ASU appears to stimulate ECM synthesis. Boumediene et al. (1999) and Altinel et al. (2007) also reported that ASU can stimulate the expression of TGF-β1 and TGF-β2 genes in cultured bovine chondrocytes and in canine joint fluid. Furthermore, Altinel et al. (2011) demonstrated that dogs treated with ASU (300 mg every 3 days) for 15 weeks showed significantly more collagen and chondral tissue content by increasing TGF-β in the tissues. Synthesis of plasminogen activator inhibitor 1 (PAI-1) at both the mRNA and protein levels has been an additional contributing factor. Henrotin et al. (1998) demonstrated that ASU can inhibit the action of IL-1β on MMP 3, IL-6, IL-8, and PGE_2 production and IL-1-β-stimulated collagenase synthesis by articular chondrocytes.

In another study, Boileau et al. (2009) demonstrated that treatment with ASU (10 mg/kg/day) could reduce the development of early OA cartilage and subchondral bone lesions in the anterior cruciate ligament of dogs. This effect appears to be mediated through the inhibition of iNOS and MMP-13, which are key mediators of the structural changes that take place in OA. In essence, ASU can be useful in preventing or blocking erosion of the tissue in OA and slow down joint space narrowing and repair of cartilage (Maheu et al. 1995, 1998; Lequesne et al. 2002). ASU extract did not decrease clinical signs of pain in horses with experimentally induced OA and there did appear to be a disease-modifying effect of treatment compared to placebo.

4.7 The New Zealand Green-Lipped Mussel

Perna canaliculus (green-lipped mussel, GLM) is a rich source of glycosaminoglycans (GAGs), omega-3 fatty acids, and eicosatetraenoic acid (ETA). GAGs exert anti-inflammatory activity and lubricate joints in OA patients. ETA appears to act as a dual inhibitor of arachidonic acid oxygenation by both the cyclooxygenase (COX) and lipoxygenase pathways. Unlike many NSAIDs, GLM is gastroprotective and does not affect platelet aggregation, suggesting that ETA may selectively block the pro-inflammatory COX-2 pathway rather than the physiologically important COX-1 pathway (Rainsford and Whitehouse 1980; Bierer and Bui 2002).

Bui and Bierer (2003) evaluated the efficacy of GLM powder (0.3% of a dry diet) for alleviating clinical signs of OA in dogs. Joints were evaluated for degree of pain, swelling, crepitus, and reduction in range of motion. By the end of 6 weeks, GLM-treated dogs showed significant improvement in arthritic score, joint pain, and swelling, without significant improvement in crepitus and range of joint movement. In another study, these investigators (Bierer and Bui 2002) treated OA dogs with GLM-containing PEDIGREE® JointCare™ Treats (450 mg for <25 kg, 1 Treat; 750 mg for 25–34 kg, 1.5 Treat; and 1 g for >34 kg, 2 Treats) for 8 weeks. The findings revealed reduction in lameness and pain associated with OA in dogs. Rialland et al. (2013) evaluated the effect of a GLM-supplemented diet on pain behavior and functioning in dogs. These investigators observed that dogs receiving a GLM-rich diet showed an increase in concentrations of plasma omega-3 fatty acids (EPA and DHA), improvement of peak vertical force using ground force plate, and reduced OA signs. In a double-blind clinical trial, Hielm-Björkman et al. (2009) evaluated efficacy of freeze-dried green-lipped mussel preparation (LYPROFLEX®) in OA dogs for a period of 8 weeks. The findings revealed that GLM was more effective than placebo and less effective than the positive control dogs receiving carprofen, without any side effects. The standard dose of GLM is 77 mg/kg/day in OA dogs.

Lyophilized products from green-lipped mussel are also used to treat horses with OA (Cayzer et al. 2012). In a randomized, double-blind, placebo-controlled study, horses were dosed orally with 25 mg/kg body wt/day for 56 days. The findings revealed that the treatment significantly alleviated the severity of lameness and joint pain and improved response to joint flexion in horses with lameness attributed to OA in the fetlock (Cayzer et al. 2012).

4.8 Naturally Preferred Holistic Frozen Dog Treats

Broderick et al. (2013) conducted a clinical trial on client-owned moderately arthritic dogs to evaluate Naturally Preferred Holistic Frozen Dog Treats (a product of Henry Schein Animal Health) for anti-arthritic efficacy, safety, and tolerability. In Group-I (<50 pounds each), each dog received a half treat, while those in Group-II (>50 pounds each) received a full treat daily for a period of 4 weeks. At weekly intervals, dogs were evaluated for OA-associated pain (overall pain, pain upon limb manipulation, and pain after physical exertion) and physical parameters (body weight, heart rate, and body temperature), serum analysis for hepatic (bilirubin, ALT, and AST), renal (BUN and creatinine), heart and skeletal muscle (creatine kinase) biomarkers, and hematological parameters. Marked reduction in arthritic pain was noted in dogs of both groups within a week with significant and maximum effects after 4 weeks. The product, Naturally Preferred Holistic Frozen Dog Treats, provided amazing results in reducing OA-associated pain and was found to be safe and well tolerated. All ingredients of this product are natural, and the mechanism of action for anti-arthritic effects remains proprietarily confidential at this time. For further details, refer to Broderick et al. (2013).

4.9 Omega-3 Fatty Acids

Omega-3 fatty acids (also called ω-3 fatty acids or *n*-3 fatty acids) are polyunsaturated fatty acids (PUFAs). The three types of omega-3 fatty acids involved in mammalian systems are α-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA), and they are commonly found in walnuts, flaxseed, fish oils, etc. *n*-3 fatty acids act via reducing IL-1β, PGE_2, ADAMTS-4, COX-2, IL-1α, iNOS, and TNF-α, MMP-3, and MMP-13 (Kremer et al. 1987; Zainal et al. 2009; Adler et al. 2018), aggrecanase and collagenase activities, and increasing collagen synthesis (Kremer et al. 1987). EPA is the most effective, followed by DHA, and ALA. PUFAs can modulate mammalian targets of rapamycin (mTOR) signaling, leading to a protective activation of autophagy in the chondrocytes (Villalvilla et al. 2013).

In several studies, it has been shown that dietary supplementation with marine *n*-3 fatty acids is beneficial for the treatment of canine OA (Fritsch et al. 2010; Roush et al. 2010a, b; Moreau et al. 2013; Mehler et al. 2016). In dogs, cats, and horses with naturally occurring OA, omega-3 fatty

acids supplemented diets could improve mobility and activity (Moreau et al. 2013; Corbee et al. 2012; Gupta 2016). In a 12-week clinical study, Fritsch et al. (2010) found that the required dose of carprofen to improve lameness decreased significantly faster in dogs supplemented with omega-3 fatty acids compared to control (3.5% and 0.1%, respectively). Findings of Roush et al. (2010a, b) suggested that ingestion of fish oil omega-3 fatty acids (3.5% in food) for 90 days increased the EPA concentration in serum 15-fold and improved clinical signs in dogs with OA based on orthopedic evaluations and force plate analysis. In a recent study, Mehler et al. (2016) determined the effects of EPA + DHA (69 mg/kg/day for 84 days) on the clinical signs and erythrocyte PUFA concentrations in OA dogs. The findings revealed that dogs receiving EPA + DHA showed a significant decrease in arachidonic acid (17%) and an increase in EPA (13.3-fold) and DHA (3.3-fold) in the blood, which correlated well with improvement in OA signs, without any major side effect. Horses supplemented with omega-3 fatty acids (EPA, 15 g/day; and DHA, 19.8 g/day) for 90 days showed significant decreases in plasma concentrations of PGE_2 and synovial fluid white blood cell counts, but force plate analysis indicated no significant change. For details on omega-3 fatty acids, readers are referred to a chapter on omega fatty acids in this book.

4.10 *Boswellia serrata* Extract

The beneficial health effects of *B. serrata* (often called Indian Frankincense, a plant native to India) extract are attributed to boswellic acids (alpha-boswellic acid and beta-boswellic acid). The other chemical constituents include volatile oil, terpinols, arabinose, xylose, uronic acid, β-sitosterol, and phlobaphenes (Goyal et al. 2011; Patel et al. 2013). Both boswellic acids have a hydroxyl group, and they differ only in their triterpene structure. The chemical structure of alpha-boswellic acid is shown in Fig. 5. One of the six boswellic acids, acetyl-keto-beta-boswellic acid (AKBA) is present at 2–3% of the total extract and is the most important for anti-OA effects. Acetyl-boswellic acids exhibit anti-inflammatory and anti-arthritic properties by inhibiting leukotriene synthesis (Ammon et al. 1993; Upaganlawar and Ghule 2009).

5-Loxin® (a patent product of PLT Health Solutions, Inc.), having 30% AKBA, provides improvement in joint mobility and comfort within a week. It inhibits 5-lipoxygenase, NF-κB, and MMP-3. 5-Loxin® positively impacts biomarkers of inflammation and arthritis, such as TNFα, CRP, and IL-6. Currently, the product Nutraquin+ is also on the market for OA in dogs, cats, and horses.

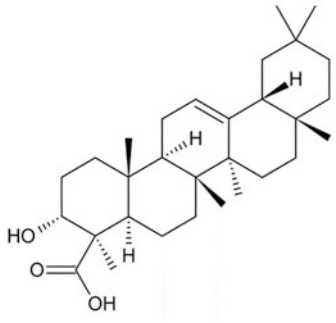


Fig. 5 Structural formula of alpha-boswellic acid

4.11 Shilajit

Shilajit is a blackish-brown exudate from the sedimentary rocks of the Himalayan Mountains, which contains many herbo-mineral constituents, primarily dibenzo-α-pyrones (DBPs), DBP-chromoproteins, and fulvic acids with a DBP core. Purified shilajit is prepared from this exudate by a proprietary extraction process (Natreon Inc., New Brunswick, NJ). Shilajit is known to exert several biological and pharmacological actions, including antioxidative, anti-inflammatory, anti-arthritic, immunomodulatory, and energetic properties (Lawley et al. 2013; Gupta 2016). In a 5-month clinical trial, administration (bid) of purified shilajit alone (500 mg) or shilajit (7.5 mg) in combination with trivalent chromium (500 μg) and *Phyllanthus emblica* (amla) extract (7.5 mg) significantly reduced arthritic pain in dogs (Lawley et al. 2013; Fleck et al. 2014). It seems that shilajit alone or in combination with chromium and amla extract attenuated arthritic pain by antioxidative, anti-inflammatory, and immunomodulatory mechanisms. Shilajit has been reported to be safe in long-term studies (Lawley et al. 2013; Fleck et al. 2014; Stoh 2014; Gupta 2016; Velmurugan et al. 2012).

4.12 Devil's Claw

Devil's claw (*Harpagophytum procumbens* and *Harpagophytum zeyheri*) is a small desert plant native to Southern Africa. It is also known as the wood spider or grapple plant. The plant/root extract has iridoid glycosides, such as harpagoside, harpagide, and β-sitosterol, which are active ingredients (Georgiev et al. 2013). Harpagogenin has also been suggested as an active principle of these plants. The structural formula of harpagoside and harpagide is shown in Fig. 6. The plant extract or its active ingredient(s) are

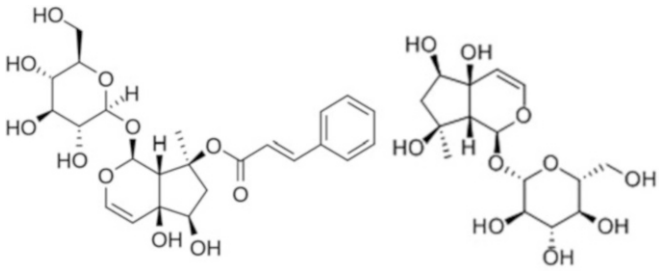


Fig. 6 Structural formula of harpagoside (left) and harpagide (right)

commonly used to treat pain and inflammation in joints associated with OA in humans, dogs, and horses, thereby improving their flexibility and mobility. The plant/root extract is available in powder or liquid form. In humans, Devil's claw extract has been found as effective as NSAIDs or diacerhein in reducing back pain or OA pain (Leblan et al. 2000; Wegener and Lupke 2003; Oltean et al. 2006). Lim et al. (2014) reported that harpagides can reduce the production of inflammatory cytokines (IL-1β, IL-6, and TNF-α) in macrophage cells. In another study, this plant extract has been shown to reduce inflammation by suppressing the inflammatory gene expression pathway by blocking a transcription factor activation protein-1 (AP-1) (Fiebich et al. 2012). In addition, harpagoside inhibits COX-I, COX-II, iNOS, and NF-κB activities (Huang et al. 2006).

In humans, a dose of 500 mg two to four times daily has been shown to exert anti-inflammatory, analgesic, and anti-arthritic effects. The recommended dose for standard Devil's claw extract is 250 mg capsule (small), 500 mg (medium), and 1 g for a large dog for the treatment of OA. The extract is also recommended for horses and ponies (0.5–10 mg/kg body wt/day) that suffer from OA and other joint-related illnesses. Devil's claw extract can be given for a long period of time without producing side effects. It is not recommended in pregnant animals.

4.13 Sauchinone

Sauchinone is one of the lignans isolated from *Saururus chinensis*, which has antioxidative, anti-inflammatory, hepatoprotective, and anti-osteoarthritic properties. Recently, Gao et al. (2017) investigated the anti-inflammatory effects of sauchinone in IL-1β-stimulated chondrocytes. Results revealed that sauchinone significantly attenuated activation of NF-κB, production of NO and PGE_2, as well as inhibited MMP-3 and MMP-13 release in human OA chondrocytes, most likely by inhibiting the activation of NF-κB signaling pathway. Gao et al. (2017) suggested that sauchinone may be a potential agent in the treatment of OA.

5 Novel Nutraceuticals

5.1 C-Phycocyanin

In an in vitro model of canine OA, Martinez et al. (2015) evaluated a commercially available c-phycocyanin-based nutraceutical (PHYCOX, which contains the active ingredient blue-green algae extract, a product of Dechra Pharmaceuticals, Northwich, UK) and some other selected ingredients (glucosamine hydrochloride, methylsulfonylmethane, turmeric, eicosapentaenoic acid, docosahexaenoic acid, vit C, vit E, selenium, etc.) for antioxidative, anti-inflammatory, and chondroprotective activities. Biomarkers for inflammation and catabolism of the ECM included PGE_2, TNF-α, IL-6, MMP-3, NO, and sulfated glycosaminoglycans. The antioxidant capacity and inhibitory activities of nutraceuticals on COX-1, COX-2, and lipoxygenase (LOX) were also evaluated. The findings revealed that PHYCOX and select ingredients mediate antioxidative, anti-inflammatory, and chondroprotective activities and may be useful in OA management in dogs and other animals after in vivo investigations and clinical trials.

5.2 Resveratrol

Resveratrol (trans-3,4′,5-trihydroxystilbene) is a natural polyphenol compound present in the skin of red grapes, cranberries, and peanuts. The compound is proven to exert antioxidative, anti-inflammatory, cardioprotective, and antitumor properties. In a number of in vitro and animal studies, resveratrol provided significant protective effects against articular cartilage degradation (Elmali et al. 2005; Lee et al. 2013). In human chondrocytes, Csaki et al. (2008) and Shakibaei et al. (2008) demonstrated that both

anti-apoptotic and anti-inflammatory regulatory mechanisms are mediated by resveratrol (reviewed in Lee et al. 2013). Recently, resveratrol has also been found to alleviate RA by inhibiting ROS, inflammation, MAPK signaling pathways, and angiogenesis (Yang et al. 2018). Based on these findings, resveratrol appears to be a novel nutraceutical that should be evaluated for anti-arthritic properties and safety in dogs, cats, and horses.

5.3 Bovine Lactoferricin

Bovine lactoferricin is a 25-amino acid cationic peptide with an amphipathic, anti-parallel β-sheet structure that is obtained by acid-pepsin hydrolysis of the N-terminal region of lactoferrin found in cow's milk (Lee et al. 2013). In addition to antiviral, antibacterial, and anticancer properties, this peptide exerts antioxidative, anti-inflammatory, and anti-pain activities (Guillen et al. 2000; Hayashida et al. 2004). Lee et al. (2013) also reported that bovine lactoferricin reverses the catabolic effects of PGE_2 and IL-1 on matrix-degrading enzyme production, proteoglycan accumulation, and expression of factors associated with oxidative stress and inflammation. By exhibiting these properties, lactoferricin seems to be a promising nutraceutical for the treatment of OA in animals.

5.4 Botulinum Toxin

Botulinum toxin A (BoNT A, IA) has been shown to reduce joint pain in OA dogs. In a recent investigation, Heikkilä et al. (2017) injected BoNT A intra-articularly (30 IU, IA) in the OA joint of dogs. The findings revealed that synovial fluid PGE_2, but not serum PGE_2, could be a biomarker for chronic OA and pain in dogs. However, substance P in neither synovial fluid nor in serum seems to be a good biomarker of OA pain in dogs. For detailed biology, pharmacology, toxicology, and safety of BoNTs, see recent publications elsewhere (Pirazzini et al. 2017; Cope 2018).

5.5 Eggshell Membrane

Eggshell membrane, which contains glucosamine, chondroitin, collagen, and hyaluronic acid, has been indicated in the treatment of OA (Ruff et al. 2009). The product NEM® (natural eggshell membrane; ESM Technologies, LLC, Carthage, MO, USA) contains glucosamine, 1%; chondroitin sulfate, 1%; hyaluronic acid, 2%; and collagen type I, 5% (Ruff and DeVore 2014). In a series of in vitro and in vivo studies in rats, Ruff et al. (2009) and Ruff and DeVore (2014) found that NEM® can influence early phase pro-inflammatory cytokines such as IL-1β and TNF-α. This product can also influence later-phase pro-inflammatory cytokines like monocyte chemotactic protein 1 (MCP-1), macrophage inflammatory proteins (MIP-1α and MIP-1β), regulated upon activation of normal T-cell expressed and secreted (RANTES), and vascular endothelial growth factor (VEGF). Another product Movoflex™ Soft Chew (Virbac Corp., Fort Worth, TX, USA), which contains eggshell membrane, is also on the market for OA in dogs, although no published data are available.

5.6 microRNAs (miRNAs)

The importance of miRNAs has recently been recognized in veterinary medicine (Hollis and Starkey 2018). The "Omics" technology has helped in identifying miRNAs that not only serve as novel biomarkers to predict the early onset of OA and its progression but they also have potential for new modalities in OA treatment. In a number of investigations, miRNAs have been shown to be involved in the pathogenesis of OA and degradation of the ECM and cartilage (iNOS expression, inflammation, autophagy, apoptosis, chondrocyte metabolism, differentiation, and homeostasis) (reviewed in Gupta et al. 2019). In an OA rat model, Wang et al. (2016) demonstrated that miRNA-98 expression is reduced in the cartilage cells by apoptosis, and the overexpression of miRNA-98 inhibits cartilage cell apoptosis. In a similar investigation, miRNA-140 levels were significantly reduced in human OA cartilage-derived chondrocytes and synovial fluid compared with normal chondrocytes and synovial fluid. Overexpression of miRNA-140 in primary human chondrocytes promoted collagen II synthesis and inhibited MMP-13 and ADAMST-5 expression (Si et al. 2017). These investigators demonstrated that intra-articular (IA) injection of miRNA-140 can alleviate OA progression by modulating ECM homeostasis in rat and may have potential as a new therapy for OA in dogs, horses, and humans. Currently, a large number of miRNAs are under investigation to identify their roles in pathogenesis, diagnosis, and treatment of OA (Gupta et al. 2019).

5.7 Spermidine

Spermidine, a natural polyamine involved in a wide range of cellular processes, is widely recognized to induce autophagy and to reduce oxidative stress. Silvestri et al. (2018) evaluated the ability of spermidine to protect cultured articular chondrocytes against hydrogen peroxide (H_2O_2)-induced oxidative stress by modulating the autophagic process. The findings revealed that autophagy promotion participates in the protection afforded by spermidine against oxidative stress. Jeong et al. (2018) demonstrated that spermidine

significantly inhibited the production of pro-inflammatory mediators such as NO and PGE_2 and cytokines including TNF-α and IL-1β in RAW 264.7 macrophages without any significant cytotoxicity. Spermidine significantly decreased the elevation of NO and ROS levels in an LPS-stimulated zebra fish model and reduced the inflammation-associated migration of immune cells such as neutrophils and macrophages. Spermidine, via autophagy, antioxidative, and anti-inflammatory properties, appears to be a promising candidate as a new treatment for OA. In addition, spermidine has emerged with antiaging properties, such as reducing the age-related oxidative protein damage, free radical-scavenging activities, and the overproduction of ROS (Reviewed in Jeong et al. 2018).

6 Concluding Remarks and Future Directions

Osteoarthritis (OA) is a chronic degenerative joint disease (DJD) that commonly afflicts dogs and horses due to many predisposing factors. The pathophysiology of OA is very complex because of multiple etiologies and underlying mechanisms. Biomarkers of early disease detection and progression and disease reversal during treatment are well defined, and many of them are validated. Inflammation and pain associated with OA are major complaints. Signs and symptoms are often managed by using NSAIDs, therapeutic drugs, and sometimes surgery. Currently, nutraceuticals have replaced other treatment modalities because of their efficacy and safety. This chapter describes etiologies and pathophysiologies of OA and a number of nutraceuticals that are commonly used to prevent or ameliorate OA in dogs and horses. Also described are some nutraceuticals that have the potential for treating OA in animals.

References

Adebowale A, Du J, Leslie JL et al (2002) The bioavailability and pharmacokinetics of glucosamine hydrochloride and low molecular weight chondroitin sulfate after single and multiple doses to beagle dogs. Biopharm Drugs Dispos 23(6):217–225

Adler N, Schoeniger A, Fuhrmann H (2018) Polyunsaturated fatty acids influence inflammatory markers in a cellular model for canine osteoarthritis. J Anim Physiol Anim Nutr 102:e623–e632

Altinel L, Saritas ZK, Kose KC et al (2007) Treatment with unsaponifiables extracts of avocado and soybean increases TGF-β1 and TGF-β2 levels in canine joint fluid. Tohuku J Exp Med 211:181–186

Altinel L, Şahin Ö, Köse KÇ et al (2011) Healing of osteochondral defects in canine knee with avocado/soybean unsaponifiables: a morphometric comparative analysis. Eklem Hastalik Cerrhisi 22 (1):48–53

Amin AR, Attur M, Patel RN et al (1997) Superinduction of cyclooxygenase-2 activity in human osteoarthritis-affected cartilage. Influence of nitric oxide. J Clin Invest 99:1231–1237

Ammon HP, Safayi H, Mack T et al (1993) Mechanism of anti-inflammatory actions of curcumin and boswellic acids. J Ethnopharmacol 38:113–119

Armstrong S, Read R, Ghosh P (1994) The effect of intra-articular hyaluronan on cartilage and subchondral bone changes in an ovine model of early osteoarthritis. J Rheumatol 21:680–687

Attur M, Al-Mussawir HE, Patel J et al (2008) Prostaglandin E_2 exerts catabolic effects in osteoarthritis cartilage: evidence for signaling via the EP4 receptor. J Immunol 181:5082–5088

Aubry-Rozier B (2012) Role of slow-acting anti-arthritic agents in osteoarthritis (chondroitin sulfate, glucosamine, hyaluronic acid). Rev Med Suisse 14:571–572

Auer JA, Fackelman GE, Gingerich DA et al (1980) Effect of hyaluronic acid in naturally occurring and experimentally induced osteoarthritis. Am J Vet Res 41(4):568–574

Bakker B, Eijkel GB, Heeren RMA et al (2017) Oxygen-dependent lipid profiles of three-dimensional cultured human chondrocytes revealed by MALDI-MSI. Anal Chem 89:9438–9444

Balogh L, Polyak A, Mathe D et al (2008) Absorption, uptake and tissue affinity of high-molecular-weight hyaluronan after oral administration in rats and dogs. J Agr Food Chem 56:10582–10593

Bay-Jensen AC, Reker D, Kjelgaard-Petersen CF et al (2016) Osteoarthritis year in review 2015: soluble biomarkers and the BIPED criteria. Osteoarthr Cart 24:9–20

Bhathal S, Spryszak M, Louizos C et al (2017) Glucosamine and chondroitin use in canines for osteoarthritis: a review. Open Vet J 7(1):36–49

Bierer TL, Bui LM (2002) Improvement of arthritic signs in dogs fed green-lipped mussel (*Perna canaliculus*). J Nutr 132:1634s–1636s

Block JA, Oegema TR, Sandy JD et al (2010) The effects of oral glucosamine on joint health: is a change in research approach needed? Osteoarthr Cartil 18:5–11

Blom AB, Brockbank SM, van Lent PL et al (2009) Involvement of the Wnt signaling pathway in experimental and human osteoarthritis: prominent role of Wnt-induced signaling protein 1. Arthr Rheum 60:501–512

Boileau C, Martel-Pelletier J, Caron J et al (2009) Protective effects of total fraction of avocado/soybean unsaponifiables on the structural changes in experimental dog osteoarthritis: inhibition of nitric oxide synthase and matrix mettaloproteinase-13. Arthr Res Ther 11:R41

Boumediene K, Felisaz N, Bogdanowicz P et al (1999) Avocado/soy unsaponifiables enhance the expression of transforming growth factor β1 and β2 in cultured articular chondrocytes. Arthr Rheumat 42:148–156

Breese McCoy SJ, Bryson JC (2003) High-dose glucosamine associated with polyuria and polydipsia in a dog. J Am Vet Med Assoc 222:431–432

Broderick BA, Miller J, Goad JT, Gupta RC (2013) Efficacy and safety of naturally preferred holistic frozen dog treats in moderately arthritic dogs. In: Proc Ann Meet Ohio Valley Chapt Soc Toxicol., Louisville, KY, USA, p 20

Bui LM, Bierer TL (2003) Influence of green lipped mussels (*Perna canaliculus*) in alleviating signs of arthritis in dogs. Vet Ther 4 (4):397–407

Carapeba GOL, Cavaleti P, Nicácio GM et al (2016) Intra-articular hyaluronic acid compared to traditional conservative treatment in dogs with osteoarthritis associated with hip dysplasia. Evid-Based Compl Altern Med 2016:20726921

Carpio LR, Westendorf JJ (2016) Histone deacetylases in cartilage homeostasis and osteoarthritis. Curr Rheumatol 18:52

Castrogiovanni P, Trovato FM, Loreto C et al (2016) Nutraceutical supplements in the management and prevention of osteoarthritis. Int J Mol Sci 17:2042

Cayzer J, Hedderley D, Gray S (2012) A randomized, double-blinded, placebo-controlled study on the efficacy of a unique extract of green-lipped mussel (*Perna canaliculus*) in horses with chronic fetlock lameness attributed to osteoarthritis. Equine Vet J 44:393–398

Chan PS, Caron JP, Rosa GJ et al (2005) Glucosamine and chondroitin sulfate regulate gene expression and synthesis of nitric oxide and prostaglandin E_2 in articular cartilage explants. Osteoarthr Cartil 13:387–394

Chen L-Y, Lotz M, Terkeltaub R et al (2018a) Modulation of matrix metabolism by ATP-citrate lyase in articular chondrocytes. J Biol Chem 293(31):12259–12270

Chen L-Y, Wang Y, Terkeltaub R et al (2018b) Activation of AMPK-SIRT3 signaling is chondroprotective by preserving mitochondrial DNA integrity and function. Osteoarthr Cartil 26:1539–1550

Chin K-Y, Ima-Nirwana S (2018) The role of Vitamin E in preventing and treating osteoarthritis – a review of the current evidence. Front Pharmacol 9:946. https://doi.org/10.3389/phar.2018.00946

Colitti M, Gaspardo B, Della Pria A et al (2012) Transcriptome modification of white blood cells after dietary administration of curcumin and non-steroidal anti-inflammatory drug in osteoarthritic affected dogs. Vet Immunol Immunopathol 147:136–146

Comblain F, Serisier S, Barthelemy N et al (2015) Review of dietary supplements for the management of osteoarthritis in dogs in studies from 2004–2014. J Vet Pharmacol Ther 39(1):1–15

Cope RB (2018) Botulinum neurotoxins. In: Gupta RC (ed) *Veterinary toxicology: basic and clinical principles*. Academic, Amsterdam, pp 743–757

Corbee RJ, Barnier MMC, van de Lest CHA et al (2012) The effect of dietary long-chain omega-3 fatty acid supplementation on owner's perception of behavior and locomotion in cats with naturally occurring osteoarthritis. J Anim Physiol Anim Nutr 97:846–853

Crowley DC, Lau FC, Sharma P et al (2009) Safety and efficacy of undenatured type II collagen in the treatment of osteoarthritis of the knee: a clinical trial. Int J Med Sci 6:312–321

Csaki C, Keshishzadeh N, Fischer K et al (2008) Regulation of inflammation signaling by resveratrol in human chondrocytes *in vitro*. Biochem Pharmacol 75(3):677–687

D'Abusco AS, Calamia V, Cicione C et al (2007) Glucosamine affects intracellular signaling through inhibition of mitogen-activated protein kinase phosphorylation in human chondrocytes. Arthr Res Ther 9:R104

D'Altilio M, Peal A, Alvey M et al (2007) Therapeutic efficacy and safety of undenatured type II collagen singly or in combination with glucosamine and chondroitin in arthritic dogs. Toxicol Mech Meth 17:189–196

de Bakker E, Stroobants V, VanDael F et al (2017) Canine synovial fluid biomarkers for early detection and monitoring of osteoarthritis. Vet Rec 180:328–329

Deal CL, Moskowitz RW (1999) Nutraceuticals as therapeutic agents in osteoarthritis. The role of glucosamine, chondroitin sulfate, and collagen hydrolysate. Rheum Dis Clin North Am 25(2):379–395

Debbi EM, Agar G, Fichman G et al (2011) Efficacy of methylsulfonylmethane supplementation on osteoarthritis of the knee: a randomized controlled study. BMC Complem Altern Med 11:50

Dechant JE, Baxter GM, Frisble DD et al (2005) Effects of glucosamine hydrochloride and chondroitin sulfate, alone and in combination, on normal and interleukin-1 conditioned equine cartilage explants metabolism. Equine Vet J 37:227–231

DeParle LA, Gupta RC, Canerdy TD et al (2005) Efficacy and safety of glycosylated undenatured type-II collagen (UC-II) in therapy of arthritic dogs. J Vet Pharmacol Ther 28:385–390

Deshmukh V, Hu H, Barroga C et al (2018) A small-molecule inhibitor of the Wnt pathway (SM04690) as a potential disease modifying agent for the treatment of osteoarthritis of the knee. Ostoarthr Cart 26 (1):18–27

Devine SB (1993) Cranial tibial thrust: a primary force in the canine stifle. J Am Vet Med Ass 183(4):456–459

Dobenecker B, Reese S, Jahn W et al (2017) Specific bioactive collagen peptides (Petagile®) as supplement for horses with osteoarthritis: a two-centered study. J Anim Physiol Anim Nutr 102(Suppl.1): 16–23

Du T, Shi Y, Xiao S et al (2017) Curcumin is a promising inhibitor of genotype 2 porcine reproductive and respiratory syndrome virus infection. BMC Vet Res 13:298

Elmali N, Esenkaya I, Harma A et al (2005) Effect of resveratrol in experimental osteoarthritis in rabbits. Infl Res 54(4):158–162

Fiebich BL, Muñoz E, Rose T et al (2012) Molecular targets of the anti-inflammatory *Harpagophytum procumbens* (devil's claw): inhibition of TNFα and COX-2 gene expression by preventing activation of AP-1. Phytother Res 26(6):806–811

Fleck A, Gupta RC, Goad JT et al (2014) Anti-arthritic efficacy and safety of Chrominex 3+ (trivalent chromium, *Phyllanthus emblica* extract, and shilajit) in moderately arthritic dogs. J Vet Sci Anim Husb 1(4e):1–6

Frech TM, Clegg DO (2007) The utility of nutraceuticals in the treatment of osteoarthritis. Curr Rheumatol Rep 9:25–30

Frisbie DD, Kawcak CE, Werpy NM et al (2009) Evaluation of polysulfated glycosaminoglycan or sodium hyaluronan administered intra-articularly for treatment of horses with experimentally induced osteoarthritis. Am J Vet Res 70:203–209

Frisbie DD, McIlwraith CW, Kawcak CE et al (2016) Efficacy of intravenous administration of hyaluronan, sodium chondroitin sulfate, and *N*-acetyl-D-glucosamine for prevention or treatment of osteoarthritis in horses. Am J Vet Res 77(10):1064–1070

Fritsch DA, Allen TA, Dodd CE et al (2010) A multicenter study of the effect of dietary supplementation with fish oil omega-3 fatty acids on carprofen dosage in dogs with osteoarthritis. J Am Vet Med Assoc 236:535–539

Gao Y, Zhao H, Li Y (2017) Sauchinone prevents IL-1β-induced inflammatory response in human chondrocytes. J Biochem Mol Toxicol 32:e22033

Georgiev MI, Ivanovska N, Alipieva K et al (2013) Harpagoside: from Kalahari Desert to pharmacy shelf. Phytochemistry 92:8–16

Ghosh P, Guidolin D (2002) Potential mechanism of action of intra-articular hyaluronan therapy in osteoarthritis; are the effects molecular weight dependent? Sem Arthr Rheum 32:10–37

Giaccari A, Morviducci L, Zorretta D et al (1995) *In vivo* effects of glucosamine on insulin secretion and insulin sensitivity in the rat: possible relevance to the maladaptive responses to chronic hyperglycemia. Diabetologia 38:518–524

Goyal S, Sharma P, Ramchandani U et al (2011) Novel anti-inflammatory topical herbal gels containing *Withania somnifera* and *Boswellia serrata*. Int J Pharm Biol Arch 2(4):1087–1094

Guedes AGP, Meadows JM, Pypendop BH et al (2018) Evaluation of tramadol for treatment of osteoarthritis in geriatric cats. J Am Vet Med Assoc 252(5):565–571

Guillen C, McInnes IB, Vaighan D et al (2000) The effects of local administration of lactoferrin on inflammation in murine autoimmune and infectious arthritis. Arthr Rheum 43(9):2073–2080

Gupta RC (2016) Nutraceuticals in arthritis. In: Gupta RC (ed) Nutraceuticals: efficacy, safety and toxicity. Academic, Amsterdam, pp 161–176

Gupta RC, Canerdy TD, Scaggs P et al (2009) Therapeutic efficacy of type-II collagen (UC-II) in comparison of glucosamine and chondroitin in arthritic horses. J Vet Pharmacol Therap 32:577–584

Gupta RC, Canerdy TD, Lindley J et al (2012) Comparative therapeutic efficacy and safety of type-II collagen (UC-II), glucosamine and chondroitin in arthritic dogs: pain evaluation by ground force plate. J Anim Physiol Anim Nutr 96:770–777

Gupta RC, Srivastava A, Lall R, Sinha A (2019) Osteoarthritis biomarkers. In: Gupta RC (ed) Biomarkers in toxicology, 2nd edn. Academic, Amsterdam, pp 929–943

Haroyan A, Mkuchyan V, Mkrtchyan N et al (2018) Efficacy and safety of curcumin and its combination with boswellic acid in osteoarthritis: a comparative, randomized, double-blind, placebo-controlled study. BMC Compl Altern Med 18:7

Hayashida K-I, Kaneko T, Takeuchi T et al (2004) Oral administration of lactoferrin inhibits inflammation and nociception in rat adjuvant-induced arthritis. J Vet Med 66(2):149–154

Heikkilä HM, Hielm-Björkman AK, Innes JF et al (2017) The effect of intra-articular botulinum toxin A on substance P, prostaglandin E_2, and tumor necrosis factor alpha in the canine osteoarthritic joint. BMC Vet Res 13:74

Henroitin YE, Gharbi M, Dierckxsens Y et al (2014) Decrease of a specific biomarker of collagen degradation in osteoarthritis, Coll2-1, by treatment with highly bioavailable curcumin during an exploratory clinical trial. BMC Compl Altern Med 14:159

Henrotin YE, Labasse AH, Jaspar JM et al (1998) Effects of three avocado/soybean unsaponifiables mixtures on metalloproteinases, cytokines and prostaglandin E_2 production by human articular chondrocytes. Clin Rheumatol 17:31–39

Hielm-Björkman A, Tulamo R-M, Salonen H et al (2009) Evaluating complementary therapies for canine osteoarthritis part I: green-lipped mussel (*Perna canaliculus*). eCAM 6(3):365–373

Hochberg MC, Zhan M, Langenberg P (2008) The rate of decline of joint space width in patients with osteoarthritis of the knee: a systematic review and meta-analysis of randomized placebo-controlled trials of chondroitin sulfate. Curr Med Res Opin 4:3029–3035

Hollis AR, Starkey MP (2018) MicroRNAs in equine veterinary medicine. Equine Vet J 50:721–726

Holmes MWA, Bayliss MT, Muir H (1988) Hyaluronic acid in human articular cartilage. Biochem J 250:435–441

Hooper M (2001) Is glucosamine an effective treatment for osteoarthritic pain? Cleveland Clin J Med 68:494–495

Huang THV, Tran VH, Duke RK et al (2006) Harpagoside suppresses lipopolysaccharide-induced iNOS and COX-2 expression through inhibition of NF-κB activation. J Ethnopharmacol 104(1–2): 149–155

Jackson CG, Plaas AH, Sandy JD et al (2010) The human pharmacokinetics of oral ingestion of glucosamine and chondroitin sulfate taken separately or in combination. Osteoarthr Carti 19:297–302

Javeri I, Chand N (2016) Curcumin. In: Gupta RC (ed) Nutraceuticals: efficacy, safety and toxicity. Academic, Amsterdam, pp 435–445

Jayakumar S, Patwardhan RS, Pal D et al (2017) Mitochondrial targeted curcumin exhibits anticancer effects through disruption of mitochondrial redox and modulation of TrxR2 activity. Free rad Biol Med 113:530–538

Jeong J-W, Cha H-J, Han MH et al (2018) Spermidine protects against oxidative stress in inflammation models using macrophages and zebrafish. Biomol Ther 26(2):146–156

Jerosch J (2011) Effects of glucosamine and chondroitin sulfate on cartilage metabolism in OA: outlook on other nutrient partners especially omega-3 fatty acids. Int J Rheumatol 2011:1–17

Jomphe CR, Gabriac M, Hale TM et al (2008) Chondroitin sulfate inhibits the nuclear translocation of nuclear factor-kappa B in interleukin-1beta-stimulated chondrocytes. Basic Clin Pharmacol Toxicol 102:59–65

Kalev-Zylinska ML, Hearn JI, Rong J et al (2018) Altered *N*-methyl D-receptor subunit expression causes changes to the circadian clock and cell phenotype in osteoarthritic chondrocytes. Osteoarthr Cartil 26:1518–1530

Kamm JL, Nixon AJ, Witte TH (2010) Cytokine and catabolic enzyme expression in synovium, synovial fluid and articular cartilage of naturally osteoarthritic equine carpi. Equine Vet J 42:693–699

Kawcak CE, Frisbie DD, McIllwraith CW et al (2007) Evaluation of avocado and soybean unsaponifiable extracts for treatment of horses with experimentally induced osteoarthritis. Am J Vet Res. 68 (6):598–604

Kim LS, Axelrod LJ, Howard P et al (2006) Efficacy of methylsulfonylmethane (MSM) in osteoarthritis pain of the knee: a pilot clinical trial. Osteoarthr Cart 14:286–294

Kirker-Head CA, Chandna V, Agrawal R et al (2000) Concentrations of substance P and prostaglandin E_2 in synovial fluid of normal and abnormal joints of horses. Am J Vet Res 61:714–718

Kremer JM, Jubiz W, Michalek A et al (1987) Fish-oil fatty acid supplementation in active rheumatoid arthritis. A double-blinded, controlled, crossover study. Ann Intl Med 106:497–503

Kurien BT, Matsumoto H, Scofield RH (2017) Nutraceutical value of pure curcumin. Pharmacogn Mag 13(Suppl 1):S161–S163

Kuroki K, Cook JL, Kreeger JM (2002) Mechanisms of action and potential uses of hyaluronan in dogs with osteoarthritis. J Am Vet Med Assoc 221(7):944–950

Lafontaine-Lacasse M, Dore M, Picard F et al (2011) Hexosamine stimulate apoptosis by altering Sirt1 action and levels in pancreatic β-cells. J Endocr 208:41–49

Lawley S, Gupta RC, Goad JT et al (2013) Anti-inflammatory and anti-arthritic efficacy and safety of purified shilajit in moderately arthritic dogs. J Vet Sci Anim Husb 1(3e):1–6

Leblan D, Chantre P, Fournié B (2000) *Harpagophytum procumbens* in the treatment of knee and hip osteoarthritis. Four-month results of a prospective, multicenter, double-blind trial versus diacerhein. Joint Bone Spine 67(5):462–467

Lee A, Ellman MB, Yan D et al (2013) A current review of molecular mechanisms regarding osteoarthritis and pain. Gene 527(2):440–447

Lequesne M, Maheu E, Cadet C et al (2002) Structural effects of avocado/soybean unsaponifiables on joint space loss in osteoarthritis of the hip. Arthr Rheumatol 47:50–58

Lim DW, Kim JG, Han D et al (2014) Analgesic effect of *Harpagophytum procumbens* on postoperative and neuropathic pain in rats. Molecules 19(1):1060–1068

Lu J, Sun Y, Ge Q et al (2014) Histone deacetylase 4 alters cartilage homeostasis in human osteoarthritis. BMC Musculoskelet Disord 15:438

Maheu E, Le Loet X, Loyau G (1995) 6-Month symptomatic efficacy of avocado/soya unsaponifiables in osteoarthritis (OA) at the lower limb. Rev Rhumat 60:667–673

Maheu E, Mazières B, Valat J-P et al (1998) Symptomatic efficacy of avocado/soybean unsaponifiables in the treatment of osteoarthritis of the knee and hip. A prospective, randomized, double-blind, placebo-controlled, multicenter clinical trial with six-month treatment period and two-month follow-up demonstrating a persistent effect. Arthr Rheumat 41:81–91

Manhart DR, Scott BD, Gibbs PG et al (2009) Markers of inflammation in arthritic horses fed omega-3 fatty acids. Profess Anim Scient 25 (2):155–160

Marone PA, Lau FC, Gupta RC et al (2010) Safety and toxicological evaluation of undenatured type II collagen. Toxicol Mechan Meth 20:175–189

Marshall KW, Manolopoulos V, Mancer K et al (2000) Amelioration of disease severity by intraarticular hylan therapy in bilateral canine osteoarthritis. J Orthop Res 18:416–425

Martinez SE, Chen Y, Ho EA et al (2015) Pharmacological effects of a c-phycocyanin-based multicomponent nutraceutical in an *in vitro* canine chondrocyte model of osteoarthritis. Can J Vet res 79:241–249

May K, Gupta RC, Miller J et al (2015) Therapeutic efficacy and safety evaluation of a novel chromium supplement (Crominex® +3-) in moderately arthritic horses. Jacobs J Vet Sci Res 2(1):014

McAllister MJ, Chemaly M, Eakin AJ et al (2018) NLRP3 as a potentially novel biomarker for the management of osteoarthritis. Osteoarthr Cartil 26(5):612–619

McCarthy G, O'Donovan J, Jones B et al (2007) Randomized double-blind, positive-controlled trial to assess the efficacy of glucosamine/chondroitin sulfate for the treatment of dogs with osteoarthritis. Vet J 174:54–61

Mehler SJ, May LR, King C et al (2016) A prospective, randomized, double-blind, placebo-controlled evaluation of the effects of eicosapentaenoic acid and docosahexaenoic acid on the clinical signs and erythrocyte membrane polyunsaturated fatty acid concentrations in dogs with osteoarthritis. Prostaglandins Leukot Essent Fatty Acids 109:1–7

Monfort J, Pelletier JP, Garcia-Giralt N et al (2008) Biochemical basis of the effect of chondroitin sulfate on osteoarthritis articular tissues. Ann Rheum Dis 67:735–740

Moreau M, Troncy E, del Castillo JRE et al (2013) Effects of feeding a high omega-3 fatty acids diet in dogs with naturally occurring osteoarthritis. J Anim Physiol Anim Nutr 97:830–837

Moreland LW (2003) Intra-articular hyaluronan (hyaluronic acid) and hylans for the treatment of osteoarthritis: mechanisms of action. Arthr Res Ther 5:54–67

Murdock N, Gupta RC, Vega N et al (2016) Evaluation of *Terminalia chebula* extract for anti-arthritic efficacy and safety in osteoarthritic dogs. J Vet Sci Technol 7:1

Neil KM, Orth MW, Coussens PM et al (2005) Effects of glucosamine and chondroitin sulfate on mediators of osteoarthritis in cultured equine chondrocytes stimulated by use of recombinant equine interleukin-1 beta. Am J Vet Res 66:1861–1869

Oltean H, Robbins C, van Tulder MW et al (2006) Herbal medicine for low back pain. Cochrane Database Syst Rev 19(2):CD004504

Ortolani's Sign (2007) In Saunders comprehensive veterinary dictionary, 3rd edn. Elsevier, St Louis, MO. Retrieved from http://medical-dictionary.thefreedictionary.com/Ortolani'ssign

Patel D, Kaur G, Sawant MG et al (2013) Herbal medicine—a natural cure to arthritis. Indian J Nat Prod Resour 4(1):27–35

Peal A, D'Altilio M, Simms C et al (2007) Therapeutic efficacy and safety of undenatured type-II collagen (UC-II) alone or in combination with (-)-hydroxycitric acid and chromemate in arthritic dogs. J Vet Pharmacol Ther 30:275–278

Permuy M, Guede D, López-Peña M et al (2015) Effects of diacerein on cartilage and subchondral bone in early stages of osteoarthritis in a rabbit model. BMC Vet Res 11:143

Persiani S, Roda E, Rovati LC et al (2005) Glucosamine oral bioavailability and plasma pharmacokinetics after increasing doses of crystalline glucosamine sulfate in man. Osteoarthr Cart 13:1041–1049

Philip MW (1989) Clinical trial comparison of intra-articular sodium hyaluronan products in horses. J Equine Vet Sci. 9:39–40

Pirazzini M, Rossetto O, Eleophra R et al (2017) Botulinum neurotoxins: biology, pharmacology, and toxicology. Pharmacol Rev 69:200–235

Portal-Núñez S, Esbrit P, Alcaraz MJ et al (2016) Oxidative stress, autophagy, epigenetic changes and regulation by miRNA as potential therapeutic targets in osteoarthritis. Biochem Pharmacol 108:1–10

Pujol R, Girard CA, Richard H et al (2018) Synovial nerve fiber density decreases with naturally-occurring osteoarthritis in horses. Osteoarthr Cartil 26:1379–1388

Rainsford KD, Whitehouse MW (1980) Gastroprotective and anti-inflammatory properties of green-lipped mussel (*Perna canaliculus*) preparation. Arzneimittel Forschung 30:2128–2132

Ramírez-Flores GI, Angel-Caraza JD, Quijano-Hernández IA et al (2017) Correlation between osteoarthritic changes in the stifle joint in dogs and the results of orthopedic, radiographic, ultrasonographic and arthroscopic examinations. Vet Res Commun 41:129–137

Rausch-Derra LC, Rhodes L, Freshwater L et al (2016) Pharmacokinetic comparison of oral tablet and suspension formulations of grapiprant, a novel therapeutic for the pain and inflammation of osteoarthritis in dogs. J Vet Pharmacol Ther 39(6):566–571

Rhouma M, de Oliveira El WA, Troncy E et al (2013) Anti-inflammatory response of dietary vitamin E and its effects on pain and joint structures during early stages of surgically induced osteoarthritis in dogs. Can J Vet Res. 77:191–198

Rialland P, Bichot S, Lussier B et al (2013) Effect of a diet enriched with green-lipped mussel on pain behavior and functioning in dogs with clinical osteoarthritis. Can J Vet Res 77:66–74

Roush JK, Chadwick ED, Fritsch DA et al (2010a) Multicenter veterinary practice assessment of the effects of omega-3 fatty acids on osteoarthritis in dogs. J Am Vet Med Assoc 236 (1):59–66

Roush JK, Cross AR, Renberg WC et al (2010b) Evaluation of the effects of dietary supplementation with fish oil omega-3 fatty acids on weight bearing in dogs with osteoarthritis. J Am Vet Med Assoc 236(1):67–73

Ruff KJ, DeVore DP (2014) Reduction of pro-inflammatory cytokines in rats following 7-day oral supplementation with a proprietary eggshell membrane-derived product. Mod Res Inflam 3 (1):19–25

Ruff KJ, Winkler A, Jackson RW et al (2009) Eggshell membrane in the treatment of pain and stiffness from osteoarthritis of the knee: a randomized, multicenter, double-blind, placebo-controlled clinical study. Clin Rheumatol 28:907–914

Rutjes AW, Jüni P, de Costa BR (2012) Viscosupplementation for osteoarthritis of the knee: a systematic review and meta-analysis. Ann Int Med 157:180–191

Schunck M, Schulze CH, Oesser S (2007a) Orally administered collagen hydrolysate halts the progression of osteoarthritis in STR/ort mice. Osteoarthr Cartil 15:94–95

Schunck M, Schulze CH, Oesser S (2007b) Collagen peptide supplementation stimulates proteoglycan biosynthesis and aggrecan expression of articular chondrocytes. Osteoarthr Caritl 17:261

Schunck M, Louton H, Oesser S (2017) The effectiveness of specific collagen peptides on osteoarthritis in dogs-impact on metabolic processes in canine chondrocytes. Open J Anim Sci 7:254. https://doi.org/10.4236/ojas.2017.73020

Sentikar J, Rovati LC (2001) Absorption, distribution, metabolism and excretion of glucosamine sulfate. A review. Arzneimittelforschung 51:699–725

Shahid M, Manchi G, Brunnberg L et al (2018) Use of proteomic analysis to determine the protein constituents of synovial fluid samples from the stifle joints of dogs with and without osteoarthritis secondary to cranial cruciate ligament rupture. Am J Vet res 79 (4):397403

Shakibaei M, Csaki C, Nebrich S et al (2008) Resveratrol suppresses interleukin-1beta- induced inflammatory signaling and apoptosis in human articular chondrocytes: potential for use as a novel nutraceutical for the treatment of osteoarthritis. Biochem Pharmacol 76 (11):1426–1439

Shen L, Liu L, Ji H-F (2017) Regulative effects of curcumin spice administration on gut microbiota and its pharmacological implications. Food Nutr Res 61:1–4

Shome S, Talukdar AD, Choudhury MD et al (2016) Curcumin as potential therapeutic natural product: a nanobiotechnological perspective. J Pharm Pharmacol 68:1481–1500

Si HB, Zeng Y, Liu SY et al (2017) Intra-articular injection of microRNA-140 (miRNA-140) alleviates osteoarthritis (OA) progression by modulating extracellular matrix (ECM) homeostasis in rats. Osteoarthr Cart 25(10):1698–1707

Silvestri Y, D'amado S, Cetrullo D et al (2018) Chondroprotective and antioxidant activity of spermidine in human chondrocytes. Osteoarthr Cartil 26(1):S343. (Abstract)

Singh S, Kumar D, Kumar S et al (2015) Cartilage oligomeric matrix protein (COMP) and hyaluronic acid (HA): diagnostic biomarkers of knee osteoarthritis. MOJ Orthop Rheumatol 2(2):00044

Sluzalska KD, Liebisch G, Lochnit G et al (2017) Interleukin-1 beta affects the phospholipid biosynthesis of fibroblast-like synoviocytes from human osteoarthritic knee joints. Osteoarthr Cart 25 (11):1890–1899

Stabler TV, Huang Z, Montell E et al (2017) Chondroitin sulfate inhibits NF-kappa B activity induced by interaction of pathogenic and damage associated molecules. Osteoarthr Cart 25(1):166–174

Stoh SJ (2014) Safety and efficacy of shilajit (mumie, moomiyo). Phytother Res 28:475–479

Svala E, Jin C, Rüetschi U et al (2017) Characterization of lubricin in synovial fluid from horses with osteoarthritis. Equine Vet J 49:116–123

Trentham DE, Dynesius-Trentham RA et al (1993) Effects of oral administration of type-II collagen on rheumatoid arthritis. Science 262:1727–1730

Trumble TN (2005) The use of nutraceuticals for osteoarthritis in horses. Vet Clin North Am Equine Pract 21:575–597

Trumble TN, Billinghurst RC, McIlwraith CW (2004) Correlation of prostaglandin E_2 concentrations in synovial fluid with ground reaction forces and clinical variables for pain or inflammation in dogs with osteoarthritis induced by transection of the cranial cruciate ligament. Am J Vet Res 65:1269–1275

Upaganlawar A, Ghule B (2009) Pharmacological activities of *Boswellia serrata* RoxB.-mini review. Ethnobot Leaflets 13:766–774

Usha P, Naidu M (2004) Randomized, double-blind, parallel, placebo-controlled study of oral glucosamine, methylsulfonylmethane and their combination in osteoarthritis. Clin Drug Invest 24:353–363

Valvason C, Musacchio E, Pozzuoli A et al (2008) Influence of glucosamine sulfate on oxidative stress in human osteoarthritic chondrocytes: effects of HO-I, p^{22}Phox and iNOS expression. Rheumatology 47:31–35

Van Loon J, De Grauw J, Van Dierendonck M et al (2010) Intra-articular opioid analgesia is effective in reducing pain and inflammation in an equine LPS induced synovitis model. Equine Vet J 42:412–419

Van Meurs JBJ (2017) Osteoarthritis year in review 2016: genetics, genomics and epigenetics. Osteoarthr Cart 25:181–189

Vangness CT, Spiker W, Erickson J (2009) A review of evidence-based medicine for glucosamine and chondroitin sulfate use in knee osteoarthritis. Arthroscopy 25:86–94

Velmurugan C, Vivek B, Wilson E et al (2012) Evaluation of safety profile of black shilajit after 91 days repeated administration in rats. Asia Pac J Trop Biomed 2:210–214

Venable RO, Stoker AM, Cook CR et al (2008) Examination of synovial fluid hyaluronan quantity and quality in stifle joints of dogs with osteoarthritis. Am J Vet Res 69(12):1569–1573

Villalvilla A, Gómez R, Largo R et al (2013) Lipid transport and metabolism in healthy and osteoarthritic cartilage. Int J Med Sci 14:20793–20808

Wan Z-H, Zhao Q (2017) Gypenoside inhibits interleukin-1β-induced inflammatory response in human osteoarthritis chondrocytes. J Biochem Mol Toxicol 2017:e21926

Wang GL, Wu YB, Liu JT et al (2016) Upregulation of miR-98 inhibits apoptosis in cartilage cells in osteoarthritis. Gen Test Mol Biomark 20(11):645–653

Wang Q, Tan QY, Xu W et al (2017) Cartilage-specific deletion of Alk5 gene results in a progressive osteoarthritis-like phenotype in mice. Osteoarthr Cart 25(11):1868–1879

Wegener T, Lupke NP (2003) Treatment of patients with arthritis of hip or knee with an aqueous extract of devil's claw amazon. Phytother Res 17(10):1165–1172

Xu I, Peng H, Glasson S et al (2007) Increased expression of the collagen receptor discoidin domain receptor 2 in articular cartilage as a key event in the pathogenesis of osteoarthritis. Arthr Rheumat 56:2663–2673

Yang G, Chang C-C, Yang Y et al (2018) Resveratrol alleviates rheumatoid arthritis via reducing ROS and inflammation, inhibiting MAPK signaling pathways, and suppressing angiogenesis. J Agri Food Chem 66:12953–12960

Zainal Z, Longman AJ, Hurst S et al (2009) Relative efficacies of omega-3 polyunsaturated fatty acids in reducing expression of key proteins in a model system for studying osteoarthritis. Osteoarthr Cart 17(7):896–905

Zhang Z, Leong DJ, Xu L et al (2016) Curcumin slows osteoarthritis progression and relieves osteoarthritis-associated pain symptoms in a post-traumatic osteoarthritis mouse model. Arthr Res Ther 18:128

Nutraceuticals for Antiaging

Bhanushree Gupta, Bhupesh Kumar, Anshuman Sharma, Deeksha Sori, Rahul Sharma, and Saumya Mehta

Abstract

Aging is a complex biological phenomenon in which structural and functional changes take place over time in a living organism. The relationship between diet and aging is quite interesting. Antioxidants and decrease in caloric intake help in slowing the aging phenomenon. Oxygen plays many significant roles in the body. First and foremost it is required to sustain life. However, oxygen also produces reactive oxygen species (ROS) which is injurious to health and one of the major factors responsible for aging. Healthy food and lifestyle, in addition to dietary antioxidants, are required to increase quality of life and slow the aging process. Dietary antioxidants (adaptogens, coenzyme Q10, isoflavones, anthocyanins, probiotics, vitamin A, vitamin C, vitamin D, and vitamin E) and microelements such as manganese, zinc, selenium, and iron help in slowing the aging process because of their ability to reduce the amount of ROS in cells, which ultimately results in an increase in life span of organisms. The objective of this chapter is to highlight the importance of antiaging nutraceuticals and to discuss modern theories of aging. Further, the role of antiaging nutraceuticals as antioxidants is described along with mechanisms to increase lifespan.

Keywords

Aging · Antioxidants · Quality of life · Lifespan · Nutraceuticals

1 Introduction

Aging is a complex biological and unavoidable phenomenon which is characterized by physiological changes in cells and tissues that results in increased risk of disease and death. It also means to grow and become mature along with these physiological changes (Lee et al. 2006). Aging depends on both internal and external factors. Internal factors are normal naturally occurring processes which take place within the cell. External factors include nutritional deficiencies, hormonal imbalances, ultraviolet irradiation, chronic sun exposure, and other factors such as smoking and pollution. Skin aging can be diminished by proper preventive measures including skin care, a balanced diet, and antioxidant-enriched supplements. With these methods, harmful effects of free radicals can be reduced (Schagen et al. 2012).

The effect of nutrition on the aging process has long been a matter of interest not only in animal research but in humans as well. An element of nutrition can be provided through nutraceuticals. As the name suggests, "nutra" means food and "ceutical" refers to the medicinal product properties. According to the 1996 definition by the Foundation for Innovation in Medicine (FIM), nutraceuticals are "food or food ingredients providing medical and health benefits, including prevention and/or treatment of diseases (Vranešić-Bender 2010)". Such products include isolated nutrients, dietary supplements, genetically engineered "designer" foods, functional foods, and herbal extracts (Kwak and Jukes 2001). According to the above statement, nutraceuticals are distinct in that they add up to long-term health benefits with additional consumption in the form of supplements.

Author contributed equally with all other contributors. Bhupesh Kumar, Anshuman Sharma and Deeksha Sori

B. Gupta (✉) · B. Kumar · A. Sharma · D. Sori
Center for Basic Sciences, Pt. Ravishankar Shukla University, Raipur, Chhattisgarh, India

R. Sharma
Department of Plant Physiology, Agril. Biochemistry, Medicinal and Aromatic Plants, Indira Gandhi Agricultural University, Raipur, Chhattisgarh, India

S. Mehta
School of Pharmaceutical Sciences, Jamia Hamdard University, New Delhi, India

R. C. Gupta et al. (eds.), *Nutraceuticals in Veterinary Medicine*, https://doi.org/10.1007/978-3-030-04624-8_25

Scientific data suggest that antioxidants show favorable effects in many chronic and age-related diseases like cancer and neurodegenerative diseases. A large number of substances, such as vitamins, carotenoids, and flavonoids, show antioxidant properties in that they show significant benefit for prevention or treatment of chronic diseases, ultimately resulting in a healthy and increased life span. The antioxidant substances derived from foods are currently a large area of research, especially coenzyme Q10, phytoestrogens, probiotics, and omega-3 fatty acids. These substances antagonize inflammatory and degenerative processes in the body and provide favorable effects in the digestive and immune systems, thereby increasing quality of life (Patel and Singh 2014).

2 Signs of Aging

Wrinkles around the eyes, brows, and regions of the neck are clear signs of aging. The hair becomes gray, maximal heart rate declines due to a decrease in oxygen use capacity, approximately 10,000 neurons of the brain become dysfunctional, vision becomes less clear, and the maximal capacity of the kidney and liver to filter waste material decreases (Patel and Singh 2014).

2.1 Theories of Aging

Based on common assumptions, the following theories have been postulated to describe the phenomenon of aging:

2.1.1 Free Radical Theory

Free radicals are highly reactive, short-lived uncharged species having an unpaired valence electron. These free radicals are needed in various metabolic processes, including protection of the body from infection, in the synthesis of hormones, and for production of energy released from food. In all these conditions, free radicals are required in specific amounts. Excessive production of free radicals is responsible for specific damage to the body including DNA, elastin, and collagen (Agarwal 2013). Damage occurs when a free radical takes or pulls an electron from any molecule, most likely from a neighboring molecule, to pair its unpaired electron and convert the molecule to a free radical.

Oxidative damage to macromolecules like DNA and proteins is accumulated but is not single handedly significant enough to cause aging. Oxygen has many important roles in the body. Not only is it required for life, but it is also involved in the production of many potentially injurious compounds. Some reactive oxygen species (ROS) are given in Table 1.

When attacked by free radicals results, the DNA molecule undergoes genetic mutations which cause synthesis of dysfunctional protein. This mutation may be corrected by DNA-repairing enzymes. Free radicals are generated during energy production, i.e., ATP. During the aging process, mitochondria lose their efficiency for ATP production. There are several scientific studies (Agarwal 2013) which prove that mitochondrial DNA is more prone to mutation than core DNA.

Table 1 Reactive oxygen species

Superoxide anion radical	$(O_2)^-$
Hydroperoxyl radical	HO_2
Hydrogen peroxide	H_2O_2
Hydroxyl radical	OH^-
Peroxide radical (R=lipid)	ROO^-
Singlet oxygen	$^1O_2$

A decreased intake of calories aids in slowing the aging phenomenon and promotes life span in rats, spiders, flies, fish, etc. (Vranešić-Bender 2010). Older rodents are generally involved in these kinds of studies in which they are given a special diet full of vitamins and minerals (Wickens 2001; Cui et al. 2011).

Mechanism of Action

The imbalance between free radicals and prooxidants is defined as oxidative stress. Severe oxidative stress causes cell dysfunction. Oxidative stress occurs because of a disturbance in the amount of prooxidants and antioxidants. Humans have built-in antioxidative systems that help present free radical damage. Prevention is only possible with the intake of enough antioxidants included in the food. In stress conditions, there are increased amounts of free radicals. Free radicals take an electron from the neighboring stable molecule along with concomitant generation of free radicals. Increased electron transfer may cause damage to the membrane and repress the cell's defense system. It is the structure of the antioxidant which enables it to donate electrons for a free radical, resulting in stabilization of the molecule.

There is a shortening of telomeric sequences in DNA with cell replication and aging. Biological aging can be indicated by telomeric length. Recently, it has been shown that an increased uptake of multivitamins has a great impact on telomeric length because this modulates oxidative stress and the proteins involved in inflammation. Studies have shown that women with a higher intake of multivitamins have 5.1% longer telomeres as compared to women with a lower intake of multivitamins (George and Ritter 1996; Xu et al. 2009).

There are many compounds with antioxidative properties. Dietary antioxidants help in increased regulation of ROS, which aids in improved physiological functions in the human body (Lueckenotte 2000; Rath and Shinde 2012).

Figure 1 shows the potential of antioxidants in a hierarchical manner. Catalases and peroxidases are the most active

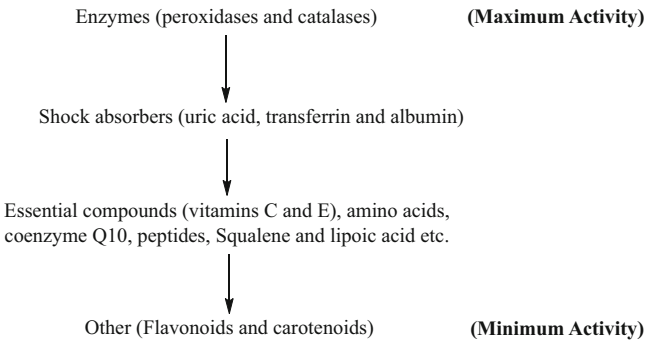


Fig. 1 Antioxidant hierarchy

antioxidants. The second class consists of shock absorbers, and the third is represented by essential antioxidants like vitamins, amino acids, and some complex macromolecules like coenzyme Q10. The last class is the largest consisting of natural compounds like flavonoids and carotenoids (Lueckenotte 2000).

Catalases and peroxidases are the most active antioxidants which play a vital role in antioxidant defense. The enzymes reduce the excess amount of free radicals and are commonly found in the subcellular organelles (Tabassum and Rasool 2012). These enzymes can perform their function properly only with the adequate supply of elements including zinc, selenium, manganese, copper, and iron.

2.1.2 Stochastic Theory

The stochastic theory is also called the wear and tear theory and was proposed by August Weismann (Peng et al. 2014). According to Weisman an excess of fat, certain injuries and infections, and excessive irradiation with UV light are the main causes of aging. This theory was later modified by Hart and Setlow (1974) who proposed that aging is due to DNA damage and mutation in proteins. According to their research, there is a positive correlation between DNA and life span.

2.1.3 Mitochondrial Decline Theory

According to this theory, cytochrome c oxidase, an enzyme which plays a vital role in the mitochondrial electron transport chain (ETC), continuously declines over time in both vertebrates and invertebrates (Benzi et al. 1992; Schwarze et al. 1998). Due to a deficiency in the activity of cytochrome c oxidase, activity of the electron transport chain is reduced. Therefore, there are close connections between MDTA and FRTA. Improving the antioxidant defense system also enhances the functional decline of mitochondria along with the decreasing amount of free radicals.

2.1.4 Theory of Ubiquitin Proteasomal System Decline

According to this theory, misfolding and aggregation of proteins are the reasons behind aging. 26S proteasome is responsible for degradation of the misfolded protein. It is a complex consisting of 20S core chamber and two 19S caps attached to it from each end (Thrower et al. 2000; Verma et al. 2001). It is reported that the activity of 26S proteasome declines over time, hence its capacity to degrade protein decreases with time. This causes an accumulation of misfolded proteins leading to the development of several neurodegenerative diseases such as Alzheimer's and Parkinson's.

2.1.5 Immunologic Theory

The immunologic theory states that a decline in activity of the immune system is the main force behind aging. A wide range of foreign molecules are detected and destroyed by the immune system to preserve the healthy cells. It is reported that a characteristic of the immune system to resist infectious diseases reduces over time, therefore increasing the risk of death in the elderly (Lueckenotte 2000).

In Vivo Model of Aging

Scientific research shows that sanitation and food intake increase the human life span, but an increasing life span does not mean that an individual remains in good health, i.e., living longer does not mean increased quality of life. A healthy diet and lifestyle improve the health condition, and in such cases nutraceuticals play a vital role in reducing aging. Before the divergence of species, the various orthologs of ancestral genes provide information regarding the similarities and differences in the species. Through this information, one can understand how a species can be useful as a model. Two often used biological models, rats and mice, possess 85% of human genes and are therefore widely used for various studies.

Caenorhabditis elegans as in vivo model:

Caenorhabditis elegans is a round worm which is distantly related to humans and provides many physiological similarities, such as the nervous and digestive systems (Barnett et al. 2016; Fitsanakis et al. 2019). *C. elegans* is an invertebrate, and its in vivo model is very closely related to previously mentioned vertebrate models. Between 40% and 80% of human genes have orthologs in *C. elegans* (Brenner 1974; Lai et al. 2000; Shaye and Greenwald 2011). This model was proposed in 1970 by scientist Sydney Brenner (1974) and is widely used in research. With the help of genetic tools like green fluorescent protein (GFP), the effect of nutraceuticals on the life span of worm can be measured. This extension of life span indicates the beneficial effects of nutraceuticals. Due to

similarities in genes of worms and humans, it has been expected that these nutraceuticals have beneficial effects on humans by inhibiting one or more aging condition.

3 Antiaging Nutraceuticals and Functional Foods

3.1 Adaptogens

Adaptogens are used in the stabilization of physiological processes and for maintaining homeostasis. They are used in herbal medicine. These compounds increase the body's ability to resist the damage from risk factors and decreases cellular sensitivity to stress. Adaptogens lead to protection from stress increasing the resistance to stress and also promoting or restoring normal physiological function.

Some of the most popular adaptogens include:

1. Brahmi (*Bacopa monnieri*)
2. Ginkgo (*Ginkgo biloba*)
3. Amla (*Emblica officinalis*)
4. Curcumin (*Curcuma longa*)
5. Liquorice (*Glycyrrhiza glabra*)
6. Ginseng (*Panax ginseng*)

3.1.1 Brahmi (*Bacopa monnieri*)

Brahmi is a perennial herb. It has purple flowers and small oblong leaves. The bacosides are the most valuable nootropic phytochemicals found in *Bacopa monnieri*. Its main function is to protect the brain from free radicals and to stimulate learning and cognitive functioning. Brahmi contains two important phytochemicals, herpestine (Fig. 2) and brahmine. Regular use of Brahmi oil prevents several diseases, such as amnesia and Alzheimer's disease (Talokar et al. 2017).

3.1.2 Ginkgo (*Ginkgo biloba*)

Ginkgo biloba is the scientific name of Ginkgo. The main function of this plant is to enhance the amount of oxygen availability to the tissues. The leaves of ginkgo help in maintaining glucose level in the brain and also aid in blood flow to the brain. It also improves mental functioning in human beings. Generally, 24% flavone glycosides are present in ginkgo leaf extract along with flavone glycosides, isorhamnetin (Fig. 3) lactone derivatives (ginkgolides), ascorbic acid, catechin, and shikimic acid. The components of ginkgo are active scavengers of free radicals which cause the death of premature cells (Talokar et al. 2017). For further details on *G. biloba*, readers are referred to Dziwenka and Coppock (2016).

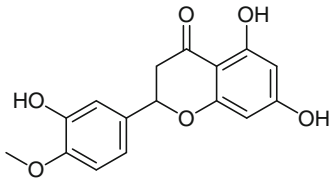


Fig. 2 Herpestine

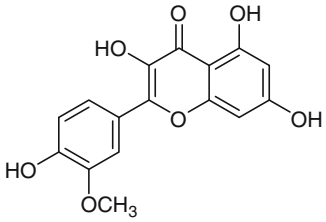


Fig. 3 Isorhamnetin

3.1.3 Amla (*Emblica officinalis*)

Emblica officinalis is commonly known as amla and belongs to the family Phyllanthaceae. The amla churn possesses memory enhancing activity due to its cholesterol reducing properties in the brain along with total cholesterol in the body, which has proven helpful in the treatment of Alzheimer's disease (Talokar et al. 2017).

3.1.4 Curcumin (*Curcuma longa*)

Curcumin is derived from turmeric which is a spice of the ginger family. It has yellow pigment, and it has many biological roles such as antioxidant, anticarcinogenic, and anti-inflammatory (Krausz et al. 2014; Pal et al. 2016). Due to these properties, curcumin is known as a potential drug for the treatment of cancer (Perrone et al. 2015). Curcumin has the capacity to suppress pro-inflammatory cytokines and prostaglandin E_2 (PGE_2), tumor necrosis factor-α (TNF-α), and cyclooxygenase-2 (COX-2). This suppressing activity shows anti-inflammatory properties of curcumin (Krausz et al. 2014; Perrone et al. 2015). Furthermore, curcumin fills in as an antioxidant agent by diminishing ROS production, hindering lipid peroxidation, and scavenging free oxygen radicals (Krausz et al. 2014; Perrone et al. 2015). Oral ingestion of curcumin in rodents showed amelioration of cystic fibrosis imperfections and hindrance of tumor expansion, yet human studies are lacking (Egan et al. 2004; Kunnumakkara et al. 2008; Draelos 2010).

3.1.5 Liquorice (*Glycyrrhiza glabra*)

The root of *Glycyrrhiza glabra* is liquorice, which belongs to the Fabaceae family. The roots and rhizomes of the plant act

as a brain tonic which helps to maintain blood sugar levels in the body. Glycyrrhizin is the main constituent of liquorice having antioxidant properties which help to increase memory, protect the brain from damage, and maintain neuronal function (Talokar et al. 2017).

3.1.6 Ginseng (*Panax ginseng*)

The roots of Ginseng plants are used for many medical purposes. The roots of this plant contain ginsenosides. Ginsenosides modulate the immune function which enhances the body's resistance against stress, trauma, anxiety, and fatigue. It helps in improving memory and learning performance and has anti-stress properties (Talokar et al. 2017).

3.2 Probiotics and Prebiotics

Probiotics are fermented products which change the diversity and activity of microorganism flora present in the gastrointestinal tract for the benefit of the individual. It improves the immune status of the person and makes up for malnutrition in aged people. Some of the probiotic microorganisms are *Lactobacillus rhamnosus* GG and *Lactobacillus reuteri* (Patel and Singh 2014).

Prebiotics are ingredients which cannot be digested but when taken in significant amounts can stimulate the activity of the friendly flora in the colon and improve health conditions.

3.3 Coenzyme Q10

Coenzyme Q10 (CoQ10) or ubiquinone is a fat-soluble benzoquinone compound with antioxidant properties. It aids in production of other antioxidants like α-tocopherol. It protects from UV radiation damages, serves as a cofactor in the production of ATP, and also works as membrane stabilizer. As the synthesis of CoQ10 reduces with aging, a regular dose of 60 mg/day helps to decrease wrinkles (Vranešić-Bender 2010; Talokar et al. 2017; Uekaji and Terao 2017).

3.4 Polyphenols

Polyphenols are present in plants as secondary metabolites and are mainly found in cereals, vegetables, fruits, and beverages. Polyphenols are attracting the attention of antiaging researchers due to their anti-inflammatory, antioxidant, and anticarcinogenic properties. These properties allow polyphenols to be able to prevent numerous health issues including asthma, cancer, diabetes, infection, and cardiovascular disease (Manach et al. 2004). Polyphenols are divided into various functional groups like flavonoids, lignans, phenolic acid, and stilbenes (Manach et al. 2004). Lab investigations of various polyphenols, such as grape seed proanthocyanidins, genistein, green tea polyphenols, silymarin, and resveratrol, led in small animal models of UV-prompted skin irritation, oxidative stress, and DNA damage. It is proposed that these polyphenols, joined with sunscreen protection, can shield skin from the adverse impacts of UV radiation, including the risk of skin cancers (Nichols and Katiyar 2010). Some flavonoids, polyphenols, and botanical antioxidants and their properties are described below.

3.4.1 Flavonoids (Phlorizin)

Phlorizin is a type of flavonoids that occurs naturally in a few plants. For more than 150 years, it has been widely utilized in the pharmaceutical industry and serves as a platform for physiological examination. An investigation to study the effect of phlorizin on life expectancy of the yeast *Saccharomyces cerevisiae* demonstrated a change in the reasonability of the yeast. Numerous other botanical extracts have been portrayed to have intense antioxidant properties. For example, apigenin (Sim et al. 2007), genistein, and silymarin have been shown to have beneficial effects on skin aging parameters (Katiyar 2002; Moore et al. 2006).

3.4.2 Resveratrol (Stilbenes)

Resveratrol is a natural polyphenol found in the skin of grapes and peanuts that exhibits antioxidant properties. It has been the subject of extraordinary studies over the last two decades because of its antiaging properties. Resveratrol works both as a chelating agent and as a radical scavenger, and moreover it exerts anti-inflammatory activity. It is also beneficial in cardiovascular, Alzheimer, and many other diseases (Di Franco et al. 2012; Risuleo 2016).

3.4.3 Apple Polyphenol

The apple contains many phytochemicals, mainly polyphenols which have antioxidant properties. Polyphenols, including chlorogenic acid, phloretin, proanthocyanidin B2, epicatechin, catechin, and rutin, are those most commonly found in the apple. Consumption of an apple is responsible for lowering the risk of cardiovascular disease and hypercholesterolemia. Several studies show that consumption of an apple reduces the risk of various cancers, especially lung cancer, and it is found more effective in women than in men (Feskanich et al. 2000; Marchand et al. 2000; Arts et al. 2001; Sesso et al. 2003).

3.4.4 Blueberry Extract

Blueberries are rich in polyphenols and also have higher antioxidant properties as compared to other fruits and vegetables. The natural compounds present in blueberries can reduce age-related losses (Joseph et al. 2005). It has been reported that daily consumption of blueberries can

improve memory function in adults. However, but this research is constrained due to a low number of samples and data (Krikorian et al. 2010).

3.4.5 Tea Catechins and Theaflavins

Tea is one of the most popular beverages, and it is consumed by a majority of the population. Tea leaves contain catechins and theaflavins, which are responsible for various health benefits. Several scientific studies showed that DNA oxidation reduces through consumption of black or green tea (Meng et al. 2001; Rietveld and Wiseman 2003). In vivo studies showed that catechins and theaflavins increased average life span of *Drosophila* (Bauer et al. 2004; Li et al. 2008).

3.4.6 Black Rice Anthocyanins

Black rice is rich in dietary antioxidants, and its supplementation has various beneficial effects, such as anticancer, anti-Alzheimer's, and anti-inflammatory. Black rice has an antioxidant property due to its abundant content of anthocyanins such as cyanidin-3-*o*-glucoside and peonidin-3-glucoside (Chiang et al. 2006).

3.5 Vitamins

3.5.1 Vitamin C

Vitamin C, a highly water-soluble vitamin (Fig. 4) also known as ascorbic acid, is a white colorless crystalline compound having strong reducing properties which make it a good antioxidant in a hydrophilic environment. It is photosensitive in nature (Schagen et al. 2012; Souyoul et al. 2018). Humans are unable to synthesize ascorbic acid naturally in their bodies and attempt to fulfill their requirements for vitamin C via ascorbic acid-enriched sources like green peppers, kiwifruit, oranges, grapefruit, brussel sprouts, strawberries, citrus fruit, and broccoli (Talokar et al. 2017). Vitamin C is oxidized into dehydroxy ascorbic acid, which enters the cells and is then converted back to ascorbic acid via the reduction process (Subramani et al. 2014). It is important to the human body for preventing disease related to connective tissue and for improvements in immune and cardiovascular cell function (Talokar et al. 2017). Deficiency of vitamin C causes a disease known as scurvy with clinical signs of fragility, gum bleeding, petechiae, and slow wound healing (Boyera et al. 1998). Vitamin C is a strong antioxidant and free radical scavenger that prevents damage to cell membranes, tissues, and DNA from free radicals (oxidation), and it is also important in collagen hydroxylation and beneficial in the maturation process of extracellular and intracellular collagen (Wu et al. 2017).

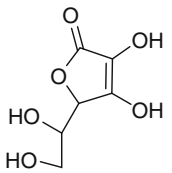


Fig. 4 Vitamin C

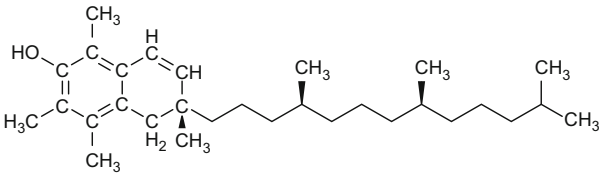


Fig. 5 Vitamin E

3.5.2 Vitamin E

Vitamin E (Fig. 5) is a group of fat-soluble vitamins collectively called "tocopherols." It is a membrane-bound antioxidant along with a free radical scavenger. Tocopherol is similar to vitamin C in that it is a nonenzymatic antioxidant (Lyons and O'Brien 2002; Ristow and Schmeisser 2011). It is present in vegetables, safflower oil, wheat germ oil, sunflower oil and seeds, almonds, peanuts, soy, corn, and meat (Stahl et al. 2006; Suganuma et al. 2010). Deficiency of vitamin E manifests in edema with seborrhiec changes or papular erythema, dryness, and depigmentation in prematurely born babies (Passi et al. 1991). Intake of vitamin E helps against collagen cross-connecting and lipid peroxidation, both of which are connected to maturation of the skin (Schagen et al. 2012).

Vitamin E and vitamin C are synergistic in nature. When UV-activated particles oxidize cell components, a chain reaction of lipid peroxidation in the membranes, which is rich in polyunsaturated fatty acids, is induced. In this reaction, the antioxidant agent D-α-tocopherol is oxidized and converted into the tocopheroxyl radical and then further recovered by ascorbic acid (Chan et al. 1991; Fryer 1993). Through this process, the cell membrane is stabilized by preventing the oxidation of polyunsaturated fatty acids. Vitamin E has been observed to inhibit erythema and endless UVB-initiated skin damage and reduce sunburned cells and photocarcinogenic effects (McVean and Liebler 1999; Makrantonaki and Zouboulis 2008).

3.5.3 Vitamin D

Vitamin D is a fat-soluble vitamin synthesized by the human body during exposure to sunlight. It also acts as a

prohormone. Small amounts of vitamins D2 and D3 (Fig. 6) originate from the dietary intake of animal-based foods, such as egg yolk or fish. Some products, like cereals, margarine, and milk, can be enriched with vitamin D (Schagen et al. 2012). The skin is a significant site for vitamin D3 UVB intervention. Vitamin D is stored in the lipid parts of the body, and an overabundance of this vitamin can have unwanted effects like weakness, poor appetite, weight loss, and vomiting. Vitamin D serves an important role in bone integrity and calcium homeostasis. It is also important for various physiological functions including release of inflammatory cytokines, regulation of growth, and immune response (Reichrath et al. 2007). It shields human skin cells from UV-incited cell passage and apoptosis and restrains the initiation of stress-actuated protein kinase (De Haes et al. 2003, 2005). Many studies show that oral vitamin D treatment prevents skin cancer, which is connected with antiaging effects (Lehmann 2009). Production of vitamin D3 decreases with advancing age. Several factors such as malnutrition, infrequent sun exposure, and behavioral effects can be responsible for vitamin D deficiency. Therefore, supplementation of vitamin D and calcium is essential for humans and animals (Makrantonaki and Zouboulis 2008).

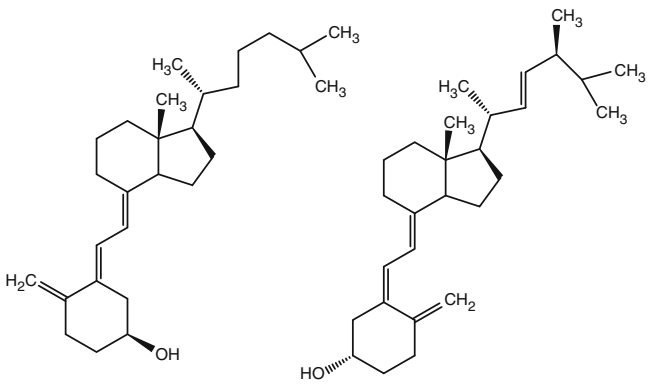


Fig. 6 Vitamin D2 (left) and D3 (right)

3.6 Carotenoids

Carotenoids are derivatives of vitamin A such as astaxanthin, β-carotene, retinol, and lycopene, which are generally exceedingly viable antioxidants and have been recorded to be photoprotective. Human skin is moderately enhanced by lycopene and β-carotene, contrasted with lutein and zeaxanthin, conceivably reflecting a particular function of hydrocarbon carotenoids in human skin photoprotection (Scarmo et al. 2010).

β-Carotene (Fig. 7) is the most important member of the carotenoids (Britton et al. 2008). It is found in the human diet in sweet potatoes, carrots, pumpkin, papaya, and mangos. An important role of β-carotene is to exert pro-vitamin-A activity. β-Carotene can be separated into two molecules of tansretinol by the BCMO1 enzyme. β-Carotene can also go about as a lipid radical scavenger and as a single oxygen quencher (Grune et al. 2010). On the basis of dissemination of BCMO1 in tissues, β-carotene digestion occurs in a number of organs, including the skin (Lindqvist and Andersson 2004). β-Carotene is an efficient photoprotector, and it prevents UV-prompted erythema (Sies and Stahl 2004).

Astaxanthin (Fig. 8) is found in algae, yeast, shrimp, salmon, crustacea, trout, crawfish, and krill. Astaxanthin has impressive potential and promising applications in human well-being and nutrition (Hussein et al. 2006). It has an exceptional potential for ensuring protector life form against a number of diseases (Higuera-Ciapara et al. 2006). The UV defensive impact of algal extract is due to 14% of astaxanthin compared to engineered astaxanthin.

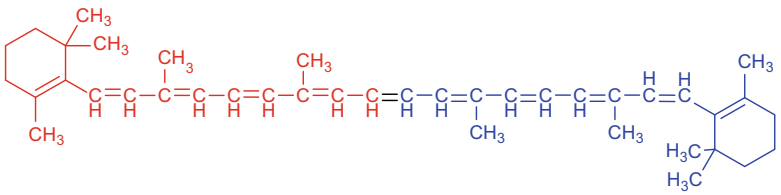


Fig. 7 β-Carotene

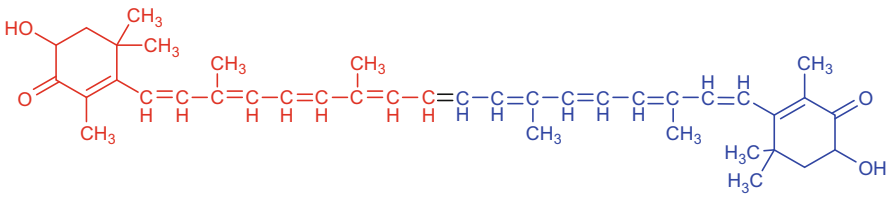


Fig. 8 Astaxanthin

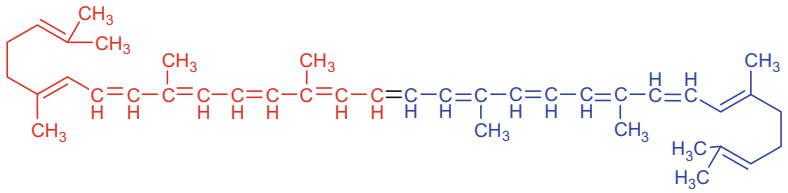


Fig. 9 Lycopene

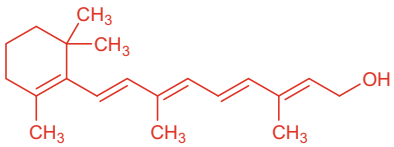


Fig. 10 Retinol

Preincubation with engineered astaxanthin or an algal extract could forestall UVA-induced changes in cell superoxide dismutase action and decline in cell glutathione content (Lyons and O'Brien 2002). Suganuma et al. (2010) showed that results of UVA radiation, such as skin wrinkling, can be prevented and minimized by topical or oral uses of astaxanthin.

Lycopene (Fig. 9) is a red carotene, carotenoid, and phytochemical present in red fruits and vegetables, tomatoes, and other red products such as papayas, red carrots, and watermelons (Schagen et al. 2012). In spite of the fact that lycopene is synthetically a carotene, lycopene has no vitamin A activity. It does however show strong tendencies as a single oxygen quencher (Evans and Johnson 2010). Some results indicate that lycopene and β-carotene protect the skin against sunburn by expanding the basal guard against UV light-intervened damage and their products are also known to be effective against cancer and in decreasing the activity of MMP-1, an enzyme that is responsible for the breakdown of collagen (Pohar et al. 2003; Etminan et al. 2004; Stahl et al. 2006; Stahl and Sies 2012).

Retinol (Fig. 10) is a fat-soluble vitamin consisting of isoprene units. Since it cannot be synthesized by the human body, it is necessary to fulfill the requirement of this vitamin by external sources (diet) such as with fatty fish, cheese, milk, egg yolks, etc. Its two derivatives, retinoic acid and retinaldehyde, are important for growth, helpful in reproduction, and responsible for epithelial tissues maintenance (Zouboulis et al. 2008). Retinaldehyde is also very important for vision and is produced in a reversible process by the oxidation of retinol.

Vitamin A applied topically has been shown to possess various counter actions against irregular pigmentation of skin aging and skin wrinkling. The effects of vitamin A and acid derivatives on bleaching agents and chemical peeling have been evaluated. The MMP-mediated and UV-induced collagen breakdown have been reduced by the treatment of topically applied retinoid (Fisher et al. 1997, 2000; Lee et al. 2012).

4 Concluding Remarks and Future Directions

Aging is a process that depends on many internal and external factors. Aging theories have linked aging with an excess of free radicals, an imbalance in diet and biological mutations. Nutraceuticals have been assumed to be a critical part of enhancing life span and helping the endogenous antioxidant framework, with antioxidant-containing items that are regularly present in the skin. A steady diet of antioxidant-rich products (vegetables, fruit, and wheat grains) has certain health properties, which can delay aging. The nutraceuticals lead to very few irrelevant symptoms in contrast with its significant impact on cell digestion. Nutraceuticals seem to hold a great promise to delay or combat aging process.

References

Agarwal V (2013) An ayurvedic insight to ageing with its preventive measures. Int J Res Ayurveda Pharm 4:31–33

Arts IC, Jacobs DR Jr, Harnack LJ et al (2001) Dietary catechins in relation to coronary heart disease death among postmenopausal women. Epidemiology 12(6):668–675

Barnett RE, Baily DC, Hatfield DE, Fitsanakis VA (2016) *Caenorhabditis elegans*: a model organism for nutraceutical safety and toxicity evaluation. In: Nutraceuticals: efficacy, safety and toxicity. Academic Press/Elsevier, Amsterdam, pp 341–354

Bauer JH, Goupil S, Garber JP et al (2004) An accelerated assay for the identification of lifespan-extending interventions in Drosophila melanogaster. Proc Natl Acad Sci U S A 101:12980–12985

Benzi G, Pastoris O, Marzatico F et al (1992) The mitochondrial electron transfer alteration as a factor involved in the brain aging. Neurobiol Aging 13:361–368

Boyera N, Galey I, Bernad BA (1998) Effect of vitamin C and its derivatives on collagen synthesis and crosslinking by normal human fibroblasts. Int J Cosmet Sci 20:151–158

Brenner S (1974) The genetics of Caenorhabditis elegans. Genetics 77:71–94

Britton G, Liaaen-Jensen S et al (2008) Carotenoids. Birkhäuser, Basel, pp 1–33

Chan AC, Tran K, Raynor T et al (1991) Regeneration of vitamin E in human platelets. J Biol Chem 266:17290–17295

Chiang AN, Wu HL, Yeh HI et al (2006) Antioxidant effects of black rice extract through the induction of superoxide dismutase and catalase activities. Lipids 41:797–803

Cui H, Kong Y, Zhang H (2011) Oxidative stress, mitochondrial dysfunction, and aging. J Signal Transduct 2012:1–13

De Haes P, Garmyn M, Degreef H et al (2003) 1,25-Dihydroxyvitamin D3 inhibits ultraviolet B-induced apoptosis, Jun kinase activation, and interleukin-6 production in primary human keratinocytes. J Cell Biochem 89:663–673

De Haes P, Garmyn M, Verstuyf A et al (2005) 1,25-Dihydroxyvitamin D3 and analogues protect primary human keratinocytes against UVB-induced DNA damage. J Photochem Photobiol B 78:141–148

Di Franco R, Calvanese M, Murino P et al (2012) Skin toxicity from external beam radiation therapy in breast cancer patients: protective effects of Resveratrol, Lycopene, Vitamin C and anthocyanin. Radiat Oncol 7:1–6

Draelos ZD (2010) Nutrition and enhancing youthful appearing skin. Clin Dermatol 28:400–408

Dziwenka M, Coppock RW (2016) *Ginkgo biloba*. In: Gupta RC (ed) Nutraceuticals: efficacy, safety and toxicity. Academic Press/Elsevier, Amsterdam, pp 681–691

Egan ME, Pearson M, Weiner SA et al (2004) Curcumin, a major constituent of turmeric, corrects cystic Fibrosis defects. Science 304:600–602

Etminan M, Takkouche B, Caamaño-Isorna F (2004) The role of tomato products and lycopene in the prevention of prostate cancer: a meta-analysis of observational studies. Cancer Epidemiol Biomark 13:340–345

Evans JA, Johnson EJ (2010) The role of phytonutrients in skin health. Nutrients 2:903–928

Feskanich D, Ziegler RG, Michaud DS et al (2000) Prospective study of fruit and vegetable consumption and risk of lung cancer among men and women. J Natl Cancer Inst 92(22):1812–1823

Fisher GJ, Wang ZQ, Datta SC et al (1997) Pathophysiology of premature skin aging induced by ultraviolet light. N Engl J Med 337:1419–1428

Fisher GJ, Datta S, Wang Z et al (2000) c-Jun-dependent inhibition of cutaneous procollagen Transcription following ultraviolet irradiation is reversed by all-trans retinoic acid. J Clin Invest 106:663–670

Fitsanakis VA, Negga R, Hatfield H (2019) Mechanistic toxicological biomarkers in *Caenorhabditis elegans*. In: Gupta RC (ed) Biomarkers in toxicology, 2nd edn. Academic Press/Elsevier, Amsterdam. (in press)

Fryer MJ (1993) Evidence for the photoprotective effects of vitamin E. Photochem Photobiol Sci 58:304–312

George AJ, Ritter MA (1996) Thymic involution with ageing: obsolescence or good housekeeping? Immunol Today 17(6):267–272

Grune T, Lietz G, Palou A et al (2010) β-Carotene is an important vitamin A source for humans. J Nutr 140(12):2268S–2285S

Hart RW, Setlow RB (1974) Correlation between deoxyribonucleic acid excision repair and life span in a number of mammalian species. Proc Natl Acad Sci U S A 71:2169–2173

Higuera-Ciapara I, Félix-Valenzuela L, Goycoolea FM (2006) Astaxanthin: a review of its chemistry and applications. Crit Rev Food Sci Nutr 46:185–196

Hussein G, Goto H, Oda S et al (2006) Antihypertensive potential and mechanism of action of astaxanthin: III. Antioxidant and histopathological effects in spontaneously hypertensive rats. Biol Pharm Bull 29:684–688

Joseph JA, Shukitt-Hale B, Casadesus G (2005) Reversing the deleterious effects of aging on neuronal communication and behavior: beneficial properties of fruit polyphenolic compounds. Am J Clin Nutr 81:313S–316S

Katiyar SK (2002) Treatment of silymarin, a plant flavonoid, prevents ultraviolet light-induced immune suppression and oxidative stress in mouse skin. Int J Oncol 21:1213–1222

Krausz A, Gunn H, Friedman A (2014) The basic science of natural ingredients. J Drugs Dermatol 13:937–943

Krikorian R, Shidler MD, Nash TA et al (2010) Blueberry supplementation improves memory in older adults. J Agric Food Chem 58:3996–4000

Kunnumakkara AB, Anand P, Aggarwal BB (2008) Curcumin inhibits proliferation, invasion, angiogenesis and metastasis of different cancers through interaction with multiple cell signaling proteins. Cancer Lett 269:199–225

Kwak NS, Jukes DJ (2001) Functional foods. Part 2: The impact on current regulatory terminology. Food Control 12:109–117

Lai CH, Chou CY, Chang LY et al (2000) Identification of novel human genes evolutionarily conserved in *Caenorhabditis elegans* by comparative proteomics. Genome Res 10:703–713

Lee J, Koo N, Min DB (2006) Reactive oxygen species, aging and antioxidative nutraceuticals. Compr Rev Food Sci Food Saf 3:21–33

Lee SJ, Cho SA, An SS et al (2012) Significantly inhibits retinoid-induced skin irritation *in vitro* and *in vivo*. Evid Based Complement Alternat Med 2012:1–11

Lehmann B (2009) Role of the vitamin D3 pathway in healthy and diseased skin–facts, contradictions and hypotheses. Exp Dermatol 18:97–108

Li YM, Chan HY, Yao XQ et al (2008) Green tea catechins and broccoli reduce fat-induced mortality in *Drosophila melanogaster*. J Nutr Biochem 19:376–383

Lindqvist A, Andersson S (2004) Cell type-specific expression of beta-carotene 15,15′-monooxygenase in human tissues. J Histochem Cytochem 52:491–499

Lueckenotte AG (2000) Theories of ageing. In: Gerontologic nursing, 2nd edn. Holly Evans Madison Publisher, pp 21–24

Lyons NM, O'Brien N (2002) Modulatory effects of an algal extract containing astaxanthin on UVA irradiated cells in culture. J Dermatol Sci 30:73–84

Makrantonaki E, Zouboulis C (2008) Skin alterations and diseases in advanced age. Drug Discov Today Dis Mech 5:153–162

Manach C, Scalbert A, Morand C et al (2004) Food sources and bioavailability. Am J Clin Nutr 79:727–747

Marchand LL, Murphy SP, Hankin JH et al (2000) Intake of flavonoids and lung cancer. J Natl Cancer Inst 92(2):154–160

McVean M, Liebler DC (1999) Prevention of DNA photodamage by vitamin E compounds and sunscreens: roles of ultraviolet absorbance and cellular uptake. Mol Carcinog 24:169–176

Meng J, Ren B, Xu Y et al (2001) Reduction of oxidative DNA damage (comet assay) in white blood cells by black tea consumption in smokers and non-smokers. Toxicol Sci 60:411–412

Moore JO, Wang Y, Stebbins WG et al (2006) Photoprotective effect of isoflavone genistein on ultraviolet B-induced pyrimidine dimer formation and PCNA expression in human reconstituted skin and its implications in dermatology and prevention of cutaneous carcinogenesis. Carcinogenesis 27:1627–1635

Nichols JA, Katiyar SK (2010) Skin photoprotection by natural polyphenols: anti inflammatory, antioxidant and DNA repair mechanisms. Arch Dermatol Res 302:71–83

Pal HC, Hunt KM, Diamond A et al (2016) Phytochemicals for the management of melanoma. Mini Rev Med Chem 16:953–979

Passi S, Morrone A, De Luca C et al (1991) Blood levels of vitamin E, polyunsaturated fatty acids of phospholipids, lipoperoxides and glutathione peroxidase in patients affected with seborrheic dermatitis. J Dermatol Sci 2:171–178

Patel P, Singh SK (2014) The aging gut and the role of prebiotics, probiotics, and synbiotics: a review. J Clin Gerontol Geriatr 5(1):3–6

Peng C, Wang X, Chen J et al (2014) Biology of ageing and role of dietary antioxidant. Bio Med Res Int 2014:1–13

Perrone D, Ardito F, Giannatempo G et al (2015) Biological and therapeutic activities, and anticancer properties of curcumin. Exp Ther Med 10(5):1615–1623

Pohar KS, Gong MC, Bahnson R et al (2003) Tomatoes, lycopene and prostate cancer: a clinician's guide for counseling those at risk for prostate cancer. World J Urol 21(1):9–14

Rath SK, Shinde A (2012) Review of antioxidant activity of Rasayana herbs ayurveda. Int J Ayurvedic Herb Med 2(1):202–217

Reichrath J, Lehmann B, Carlberg C et al (2007) Vitamins as hormones. Horm Metab Res 39(2):71–84

Rietveld A, Wiseman S (2003) Antioxidant effects of tea: evidence from human clinical trials. J Nutr 133(10):3285S–3292S

Ristow M, Schmeisser S (2011) Extending life span by increasing oxidative stress. Free Radic Biol Med 51:327–336

Risuleo G (2016) Resveratrol: multiple activities on the biological functionality of the cell. In: Gupta RC (ed) Nutraceuticals: efficacy, safety and toxicity. Academic Press/Elsevier, Amsterdam, pp 453–464

Scarmo S, Cartmel B, Lin H et al (2010) Significant correlations of dermal total carotenoids and dermal lycopene with their respective plasma levels in healthy adults. Arch Biochem Biophys 504:34–39

Schagen SK, Zampeli VA, Makrantonaki E et al (2012) Discovering the link between nutrition and skin aging. Dermato-Endocrinology 4 (3):298–307

Schwarze SR, Weindruch R, Aiken JM (1998) Oxidative stress and aging reduce cox I RNA and cytochrome oxidase activity in Drosophila. Free Radic Biol Med 25:740–747

Sesso HD, Gaziano JM, Liu S et al (2003) Flavonoid intake and the risk of cardiovascular disease in women. Am J Clin Nutr 77 (6):1400–1408

Shaye DD, Greenwald I (2011) OrthoList: a compendium of *C. elegans* genes with human orthologs. PLoS One 6:1–12

Sies H, Stahl W (2004) Nutritional protection against skin damage from sunlight. Annu Rev Nutr 24:173–200

Sim GS, Lee BC, Cho HS et al (2007) Structure activity relationship of antioxidative property of flavonoids and inhibitory effect on matrix metalloproteinase activity in UVA-irradiated human dermal fibroblast. Arch Pharm Res 30(3):290–298

Souyoul SA, Saussy KP, Lupo MP (2018) Nutraceuticals: a review. Dermatol Ther (Heidelb) 8(1):5–16

Stahl W, Sies H (2012) b-Carotene and other carotenoids in protection from sunlight. Am J Clin Nutr 96(5):1179S–1184S

Stahl W, Heinrich U, Aust O et al (2006) Lycopene-rich products and dietary photoprotection. Photochem Photobiol Sci 5:238–242

Subramani T, Yeap SK, Ho WY et al (2014) Vitamin C suppresses cell death in MCF-7 human breast cancer cells induced by tamoxifen. J Cell Mol Med 18:305–313

Suganuma K, Nakajima H, Ohtsuki M et al (2010) Astaxanthin attenuates the UVA-induced up-regulation of matrix metalloproteinase-1 and skin fibroblast elastase in human dermal fibroblasts. J Dermatol Sci 58:136–142

Tabassum N, Rasool S (2012) Natural cognitive enhancers. J Pharm Res 5(1):155

Talokar SS, Rajnikant VR, Salunkhe V et al (2017) A review on nootropics and nutraceuticals: the missile for ageing. Int Res J Pharmaceut Appl Sci 8:1–4

Thrower JS, Hoffman L, Rechsteiner M et al (2000) Recognition of the poly ubiquitin proteolytic signal. EMBO J 19:94–102

Uekaji Y, Terao K (2017) Coenzyme Q10 – gamma cyclodextrin complex is a Powerful nutraceutical for anti-aging and health improvements. Biomed Res Clin Pract 2:1–5

Verma R, McDonald H, Yates JR III et al (2001) Selective degradation of ubiquitinated Sic1 by purified 26S proteasome yields active S phase cyclin-Cdk. Mol Cell 8:439–448

Vranešić-Bender D (2010) The role of nutraceuticals in anti-aging medicine. Acta Clin Croat 49:537–544

Wickens AP (2001) Ageing and the free radical theory. Respir Physiol 128:380–381

Wu X, Cheng J, Wang X (2017) Dietary antioxidants: potential anticancer agents. Nutr Cancer 69:521–533

Xu Q, Parks CG et al (2009) Multivitamin use and telomere length in women. Am J Clin Nutr 89:1857–1863

Zouboulis CC, Schagen S, Alestas T (2008) The sebocyte culture: a model to study the pathophysiology of the sebaceous gland in sebostasis, seborrhea and acne. Arch Dermatol 300:397–413

Nutraceuticals for Cognitive Dysfunction

Ramesh C. Gupta, Robin B. Doss, Ajay Srivastava, Rajiv Lall, and Anita Sinha

Abstract

With increasing age, humans and animals suffer from partial or complete loss of cognition and memory. As a result, quality of life declines significantly. Among many underlying mechanisms, a significant decline in the neurotransmitter acetylcholine (ACh), an increase in *N*-methyl-*D*-aspartate (NMDA), and oxidative stress are the most recognized events involved in cognition impairment, especially memory and learning. Like chronic neurodegenerative Alzheimer's disease (AD) in humans, canines and felines suffer from memory loss as they become older. Currently, for AD treatment in humans, an NMDA receptor antagonist memantine in combination with the acetylcholinesterase (AChE) inhibitor donepezil, rivastigmine, or galantamine appears to be the best option. A number of therapeutic drugs (selegiline, gabapentin, buspirone, memantine, etc.) are also available for treatment of canine cognition dysfunction (CCD)/cognitive dysfunction syndrome (CDS). A large number of plant extracts, their ingredients, and bioactive compounds of animal origin have been investigated for anticholinesterase (anti-ChE), antioxidative, anti-inflammatory, and immunomodulatory activities, as well as anti-Aβ aggregation and deposition in the brain. Some of these substances have also been shown to normalize the blood-brain barrier permeability and integrity, while others have been demonstrated to restore mitochondrial function. A small number of plant extracts have also shown MAO-B inhibitory property. Currently, demented dogs and cats are given nutraceuticals and/or a therapeutic diet to improve their cognition and memory. This chapter describes various nutraceuticals and substances that have potential to improve cognition and memory in senior dogs and cats.

Keywords

Canine cognition dysfunction · Memory loss · Acetylcholine · Acetylcholinesterase · Butyrylcholinesterase · Anticholinesterase · Plant alkaloids · NMDA receptor antagonist · Oxidative stress

1 Introduction

Presently, like humans, dogs are also living longer because of better nutrition, environment, and veterinary care. As a result, a large population of dogs, often regarded as senior dogs, suffer from cognitive dysfunction and memory loss (Bain et al. 2001). Prevalence of cognitive dysfunction syndrome (CDS) is extremely high. According to a study of the University of California Davis, 28% of dogs aged 11–12 years and 68% of dogs aged 15–16 years showed one or more signs of cognitive impairment (Neilsen et al. 2001). The prevalence of CDS in cats was found to be 36% in a population of 11–21-year-old cats (Moffat and Landsberg 2003). CDS is underdiagnosed because caregivers may assume behavior changes are a result of normal aging, and veterinarians may not recognize the signs (Salvin et al. 2010). The brain is the most complex organ in the body, and as it ages, certain changes occur, such as (1) oxidative/nitrosative stress, (2) neuroinflammation, (3) decline in acetylcholine (ACh) level, (4) decrease in acetylcholinesterase (AChE) activity and increase in butyrylcholinesterase (BuChE) activity, (5) decrease in choline acetyltransferase (ChAT), (6) increase in *N*-methyl-*D*-aspartate (NMDA) receptors, and (7) microvascular changes (reviewed in Gupta and Dekundy 2005; Sadhukhan et al. 2018; Sen and Hongpaisan 2018; Seo et al. 2018). Although, neurodegenerative changes occur in many regions of the brain, in the context of memory, the

R. C. Gupta (✉) · R. B. Doss
Toxicology Department, Breathitt Veterinary Center, Murray State University, Hopkinsville, KY, USA
e-mail: rgupta@murraystate.edu

A. Srivastava · R. Lall · A. Sinha
Vets Plus Inc., Menomonie, WI, USA

R. C. Gupta et al. (eds.), *Nutraceuticals in Veterinary Medicine*, https://doi.org/10.1007/978-3-030-04624-8_26

cerebral cortex and hippocampus are of particular importance. Accumulation of a neurotoxic $A\beta_{1-42}$ peptide and hyperphosphorylation of tau, leading to the formation of senile plaques and neurofibrillary tangles in the cortex and hippocampus, are the two pathological hallmarks of AD in humans and CDS in canines and felines.

Plant extracts have been known for a long time to contain a cholinesterase (ChE) inhibiting property. Physostigmine from *Physostigma venenosum* (Calabar bean) and galantamine from *Galanthus nivalis* (snowdrop) are the two most common examples of such plants. Essential oil-rich plants, which are also known to possess an anti-ChE property, have traditionally been used for memory enhancement in humans, and they can also be used for CDS in canines and felines (Orhan et al. 2009). In addition to anti-AChE, antioxidative, anti-inflammatory, and neuroprotective properties (Table 1), some plants have monoamine oxidase (MAO) inhibitory property (Mazzio et al. 2013). Nutraceuticals, such as cinnamon, curcumin, and berberine, can also attenuate β-amyloid and tau levels in brain regions. Recently, Marcelli et al. (2018) proposed an interesting intracellular molecular mechanism implied in AD pathophysiology called SUMOylation. Since there are no pharmacological treatments known to normalize SUMOylation/deSUMOylation equilibrium, some natural compounds are suggested for future convincing therapies. Extracts of many plants or their ingredients offer hope for prevention and/or treatment of cognition decline (Cicero et al. 2018; Momtaz et al. 2018).

Cognition impairment has also been improved by nutraceuticals of non-plant sources. For example, anchovy (*Coilia mystus*) has been shown to contain a peptide, anchovy protein hydrolysate (AHP), which can regulate AChE activity and can also exert antioxidative and neuroprotective properties (Zhao et al. 2017b). Major royal jelly protein 1 is reported to prevent cognition impairment (Chen et al. 2017; Lin et al. 2018). Melatonin from pineal gland appears to offer positive effect on cognitive decline and related anxiety and sleep-cycle disorders in senior dogs.

This chapter describes various options, such as plant extracts, bioactive compounds from invertebrates and vertebrates, and many other nutraceuticals for prevention and treatment of CDS in elderly canines and felines.

2 Canine Cognitive Dysfunction

Senior dogs, aged more than 8 years, can spontaneously develop neurodegenerative cerebral changes and associated impairment of cognitive function (Schütt et al. 2015). Canine cognitive dysfunction (CCD), or cognitive dysfunction syndrome (CDS) in dogs and cats (Landsberg et al. 2012; Vite and Head 2014) appears to be canine/feline counterpart of senile dementia of the Alzheimer's type (Rofina et al. 2006). Schütt et al. (2015) stated that the prevalence of CCD ranges from 14 to 35% in companion dogs more than 8 years of age, and the risk of developing CDS increases exponentially with increasing age (Azkona et al. 2009; Osella et al. 2007; Neilsen et al. 2001: Salvin et al. 2010, 2011). Changes in behavior and daily routines are considered the most important clinical markers of cognitive dysfunction in aged dogs and cats.

3 Pathophysiology

The cellular and molecular biology of memory is very complex (Kandel 2012). The brains of older dogs and cats may show several anatomic and physiologic changes (Vite and Head 2014; Seibert 2017). These changes may include a reduction in overall brain mass (including atrophy of cerebral cortex and basal ganglia), a reduction in the number of neurons, generalized gliosis, degeneration of white matter, demyelination, neuroaxonal degeneration, increase in ventricular size, meningeal fibrosis and calcification, and the presence of β-amyloid (Aβ) plaques. Functional changes occur, such as depletion of neurotransmitters (norepinephrine, serotonin, and dopamine), a decline in the cholinergic system, an increase in monoamine oxidase B activity, and a reduction of endogenous antioxidants (Landsberg and Araujo 2005; D'Amilio et al. 2018). Sechi et al. (2015) also observed a decrease in brain-derived neurotrophic factor (BDNF) in serum correlates with cognitive decline and deficits in LTP and memory. Other etiopathologic factor in cognitive decline is reported to be neuroinflammation through overactivation of microglia (Balducci and Forloni 2018).

3.1 Cholinergic System and Cognition

The cholinergic neurotransmission in the central nervous system (CNS) plays an important role in modulating cognitive processes, such as learning, memory, arousal and deep sleep as well as in modulating locomotor activity (Terry and Buccafusco 2006; Wevers 2011). In neurodegenerative disease, such as CDS or AD, a deficit of ACh and an increase in NMDA receptors and free radicals (oxidative stress) are major underlying modulations (Zhao and Zhao 2013).

3.2 Oxidative Stress, Neuroinflammation, and Neurodegeneration

The brain is very vulnerable to oxidative stress because of (1) high oxygen consumption (>20% of body's total oxygen), (2) high percentage of PUFAs, and (3) low antioxidant

capacity. In the aged brain, excess generation of free radicals activates glia/astrocytes and leads to neuroinflammation (Seo et al. 2018). Oxidative stress has also been implicated in Aβ- or tau-induced neurotoxicity. In addition, evidence suggests that oxidative stress may augment the production and aggregation of Aβ and facilitate the phosphorylation and polymerization of tau, thus forming a vicious cycle that promotes the initiation and progression of neurodegeneration and AD (Zhao and Zhao 2013).

3.3 Pathological Changes

Recently, Schmidt et al. (2015) described that the aged brain develops an abnormal Aβ deposition in brain parenchyma and the walls of the cerebral blood vessels. The prefrontal cortex is the first area affected, followed by the temporal cortex, the hippocampus, and the occipital cortex. These investigators further emphasized that regardless of position, the amount and extent of Aβ deposits correlates with the severity of cognitive impairment.

4 Diagnosis of Cognition Dysfunction

Changes in behavior and daily routines are considered the most important clinical markers of cognitive dysfunction in aged dogs (Schütt et al. 2015). These investigators measured the plasma concentration of both $A\beta_{1–40}$ and $A\beta_{1–42}$ and noted highest levels of $A\beta_{1–42}$ in canine cognition decline (CCD). Concentrations of plasma $A\beta_{1–40}$ and $A\beta_{1–42}$ and the $A\beta_{1–42}/A\beta_{1–40}$ ratio can be used as biomarkers of CCD. Interestingly, Schütt et al. (2015) stated that dogs with CCD are not as cognitively impaired as people with Alzheimer's disease (AD) and thus may correspond to early AD or the mild cognitive impairment (MCI) phase. Dogs with CCD commonly display specific clinical signs, such as aimless wandering, staring blankly into space, avoiding being petted, and difficulty with finding dropped food.

Veterinarians often make a diagnosis of CCD/CDS based on the acronym DISHA (*D*isorientation, *I*nteraction changes, *S*leep/wake cycle, *H*ouse soiling, *A*ctivity level changes) (Landsberg et al. 2003; Landsberg 2005). In brief, DISHA can be described as:

- *D*isorientation—changes in spatial awareness, loss of ability to navigate around familiar obstacles, and wandering behavior
- *I*nteraction changes—decreased interest in social interactions, petting, greetings, dependent or "clingy" behaviors
- *S*leep/wake cycle changes—restlessness or frequent walking during the night, increased sleep during daytime hours
- *H*ouse soiling—no longer lets owner know when it needs to go outside, indoor elimination, incontinence
- *A*ctivity level changes—decreased exploration and response to things, people, and sounds around the house, decreased grooming, decreased appetite, increased anxiety, including restlessness, agitation, and/or separation distress

In addition to clinical signs, veterinarians should examine dogs using the Canine Dementia Scale (CADES). CADES is a statistically validated, highly sensitive rating scale for canine CDS. The scale contains 17 nonredundant items, distributed across 4 relevant domains: (1) spatial orientation, (2) social interactions, (3) sleep-wake cycles, and (4) house soiling. Recently, Madaria et al. (2015) reported that dogs with mild cognitive impairment frequently had impaired social interactions, while dogs with moderate cognitive dysfunction had abnormal social interactions and sleep/wake cycles. Dogs with severe cognitive dysfunction exhibited impairment in all four domains. It is noteworthy that no dog with CCD may show all of these signs. For further details, readers are referred to Landsberg et al. (2012).

Recently, Hampel et al. (2018) described that a comprehensive "omics"-based biomarker may guide the exploration of spatiotemporal systems-wide morpho-functional shifts along the continuum of AD or CDS pathophysiology, from adaptation to irreversible failure. Biomarkers of AD in humans and CCD in dogs have been described in detail elsewhere (Galasko 2006; Schütt et al. 2015; Pitt and Leung 2018).

5 Prevention and Treatment of Cognitive Dysfunction

5.1 Plants Extracts and Their Ingredients

A large number of plants and their ingredients have been found to possess AChE and/or BuChE inhibitory properties to treat AD in humans (Trevisan et al. 2003) and CDS in animals (unpublished). Many plants, in addition to ChE inhibitory property, have antioxidative, anti-inflammatory, immunomodulatory, anti-β amyloid deposition, and many other properties, thereby normalizing neurotransmitter levels and improving cognition and learning memory in canines and felines. Some important plants are discussed here in brief and along with these, the rest are listed in Table 1.

5.1.1 *Polygonum hydropiper L.*

Ayaz et al. (2015) analyzed the essential oils of flowers and leaves of *Polygonum hydropiper* L. and found caryophyllene as the major component in flowers and

Table 1 Plant extracts/nutraceuticals with potential for directly or indirectly improving cognition and memory

Plant extract/nutraceutical	Pharmacological/ biological constituents	Biological/pharmacological activities	References
Angelica archangelica	Imperatorin and xanthotoxin	AChE inhibitory	Sigurdsson and Gudbjarnason (2007)
Anisomeles indica L.	Polyphenols	AChE inhibitory, antioxidative, anti-inflammatory, anti-Alzheimer's disease (AD), antinociceptive, anxiolytic, and sedative	Uddin et al. (2016, 2018)
Aristotelia chilensis	F5	AChE, BuChE, and tyrosinase inhibitory	Cespedes et al. (2017)
Artemisia dracunculus (essential oil)	Estragole	AChE inhibitory	Dohi et al. (2009)
Ascorbic acid	Ascorbic acid	Antioxidative, anti-proteolytic, and anti-apoptotic	Olajide et al. (2017)
Asparagus cochinchinensis	Flavonoids, phenols, saponins, and protodioscin	Antioxidative, increased release of nerve growth factor, AChE inhibitory, and decreased $A\beta_{1\text{-}42}$ peptide production and deposition	Lee et al. (2018)
Bacopa Monnieri (Brahmi)	Bacopasides	AChE inhibitory, antioxidative, neuroprotective	Das et al. (2002) and Zhang et al. (2009)
Berberine (*Berberis vulgaris*, *B. aristata*, *B. aquifolium*, *Coptis chinensis*, *Hydrastis canadensis*) Protoberberine (*Stephania venosa*)	Berberine and berberine metabolites (thalifendine, berberrubine, and jatrorrhizine), protoberberines (stepharanine, cyclanoline, and *N*-methyl stepholidine)	AChE inhibitory, antioxidative, anti-inflammatory, immunomodulatory, neuroprotective, analgesic, Aβ42 inhibitor, and improved cognition and memory	Ingkaninan et al. (2006), Zhu and Qian (2006), Kulkarni and Dhir (2008, 2010), Asai et al. (2007), Kumar et al. (2015), Cai et al. (2016), and Hussein et al. (2018)
Cannabis sativa (hemp seed)	Lignanamides (cannabisin M, cannabisin N, cannabisin O, and 3,3-dimethyl-heliotropamide)	AChE inhibitory and antioxidative	Yan et al. (2015) and Hartsel et al. (2016)
Cashew nut shell liquid (*Anacardium occidentale*)	Anacardic acids	Anti-Alzheimer's disease	Filho et al. (2018)
Cinnamomum verum, C. tamale	Polyphenols, cinnamaldehyde	Anti-tau protein and Aβ aggregation, anti-Alzheimer's disease, and antidiabetes	Momtaz et al. (2018)
Curcumin/turmeric (*Curcuma longa*)	Curcumin, demethoxycurcumin, and bisdemethoxycurcumin	Anti-inflammatory, anti-neuroinflammatory, antioxidative, cytoprotective, prevents Aβ aggregation, and prevents memory impairment	Maheshwari et al. (2006), Chen (2006), Javeri and Chand (2016), Risuleo (2016), Sadhukhan et al. (2018), and Sorrenti et al. (2018)
Cyclotrichium niveum (essential oil)	Isomenthone and pulegone	AChE inhibitory and antioxidative	Orhan et al. (2009)
Elatostema papillosum	Phenolics and flavonoids	AChE and BuChE inhibitory, free radical scavenging, and antioxidative	Reza et al. (2018)
Eugenia dysenterica (cagaita)	Not yet identified	AChE inhibitory	Gasca et al. (2017)
Garcinia madruno	Morelloflavone, volkensiflavone, fukugiside, and amentoflavone	Reduced Aβ peptides deposition in the brain and improved learning	Sabogal-Guaqueta et al. (2018)
Ginkgo biloba	Ginkgolide A, B, and C, bilobalide, ginkgolic acid, and flavonoids (quercetin)	Inhibits Aβ-induced apoptosis in the hippocampus via upregulation of BDNF, neuroprotective, AChE inhibitory, enhances mitochondrial metabolism and ATP production, anti-platelet-activating factor, and cognition and memory enhancement	Das et al. (2002), Dugoua et al. (2006), Reichling et al. (2006), Nagahara et al. (2009), Xiao et al. (2010), Ihl et al. (2011), Islam et al. (2013), Mohanta et al. (2014) and Dziwenka, and Coppock (2016)
Ginseng	Ginsenosides	Decreasing oxidative stress, upregulating plasticity-related proteins in the hippocampus, anti-inflammatory, immunomodulatory, neuroprotective, and improve cognition and memory	Zhao et al. (2009), Li et al. (2015), and Peng et al. (2018)

(continued)

Table 1 (continued)

Plant extract/nutraceutical	Pharmacological/ biological constituents	Biological/pharmacological activities	References
Green tea (*Camellia sinensis*)	(−)-epigallocatechin-3-gallate	Prevents spatial learning and memory impairment by decreasing Aβ1-42 oligomers and upregulating synaptic plasticity proteins in the hippocampus	Li et al. (2009) and Coppock and Dziwenka (2016)
Highbush blueberry (*Vaccinium corymbosum* L.)	Flavonoids	Activation of BDNF/CREB/AKT signaling	Hong et al. (2017)
Inula graveolens (Essential oil)	Bornyl acetate and borneol	AChE inhibitory	Dohi et al. (2009)
Monsonia angustifolia	Justicidin A	Decreased Aβ formation, neuroprotective, and anti-autophagy	Gu et al. (2016) and Chun et al. (2017)
Mutellina purpurea	Pteryxin	BuChE inhibitory	Orhan et al. (2017)
Norea micrantha	Not yet identified	AChE and BuChE inhibitory and antioxidative	Imran et al. (2017)
Polygonum hydropiper L. (essential oil)	Caryophyllene oxide and decahydronaphthalene	AChE and BuChE inhibitory and antioxidative	Ayaz et al. (2015)
Piper hispidinervum (essential oil)	Asaricin	AChE inhibitory	Xiang et al. (2017)
N-3 PUFA	N-3 PUFA	Prevents Aβ-induced depression	Morgese et al. (2018)
Quercetin	Quercetin	Antioxidative, anti-inflammatory, AChE inhibitory, neuroprotective, mitoprotective, reduced Aβ peptide in the brain, and improves cognition and memory	Ay et al. (2016), Dziwenka and Coppock (2016), Islam et al. (2013), Sabogal-Guaqueta et al. (2015), and Teodoro et al. (2016)
Resveratrol	Resveratrol	Antioxidative, anti-inflammatory, increases mitochondrial function, neuroprotective, and prevents memory loss	Marambaud et al. (2005), Porquet et al. (2014), Risuleo (2016), Teodoro et al. (2016), and Sadhukhan et al. (2018)
Rosmarinic acid	Rosmarinic acid	Antioxidative, anti-β-amyloid, neuroprotective, and anti-Alzheimer's disease	Zhang et al. (2016) and Rong et al. (2018)
Safflower seed oil cake (*Carthamus tinctorius* L.)	(+/−)-carthatins A-F (1-6, respectively)	AChE inhibitory	Peng et al. (2017)
Saffron (*Crocus sativus*)	Picrocrocin, crocin, crocetin, dimethylcrocetin, and safranal	AChE inhibitory, antinociceptive, antidepressant, anxiolytic, anti-inflammatory, antioxidative, anti-amyloidogenic, and prevention of cognition and memory impairment	Ghadrdoost et al. (2011), Geromichalos et al. (2012), Ghaffari et al. (2015) and Adalier, and Parker (2016)
Salvia lavandulaefolia	1,8-cineol, and α-pinene	AChE inhibitory	Perry et al. (2000, 2003)
Senna multijuga	7′-Multijuguinone and 12′-hydroxy-7′-multijuguinone	AChE inhibitory	Serrano et al. (2010)
Soybean (*Glycine max* L.)	Di- and tripeptides, isoflavone, genistein	Increased neurotrophic factors (BDNF), normetanephrine, and noradrenaline and prevented cognitive impairment. Genistein alleviates Aβ-induced inflammation through toll-like receptor 4/NF-κB	Ding et al. (2013), Maebuchi et al. (2013), Katayama et al. (2014), Ma et al. (2015), and Imai et al. (2017)
Taurine	Taurine	Improves learning and retention	El Idrissi (2008)
Withania somnifera	Sitoindosides VII-X, withanolides, glycowithanolides, withanoside I, withanolide sulfoxide	Antioxidative, anti-inflammatory, immunomodulatory, antiaging, AChE inhibitory	Bhattacharya et al. (1995, 1997), Schliebs et al. (1997), Choudhary et al. (2004), Kuboyama et al. (2006), and Bharti et al. (2016)
Zephyranthes carinata	3-Epimacronine and lycoramine	AChE inhibitory	Cortes et al. (2015)

Abbreviations: *AChE* acetylcholinesterase, *BuChE* butyrylcholinesterase, *BDNF* brain-derived neurotrophic factor

decahydronaphthalene in leaves. In an in vitro study, these compounds exhibited dose-dependent ChE inhibitory and antioxidant activities, with these being higher in leaves than in flowers (Ayaz et al. 2015). IC_{50} values for AChE inhibition by essential oils from leaves and flowers were reported to be 120 and 220 μg/mL, respectively; and for BuChE inhibition were 130 and 225 μg/mL, respectively.

5.1.2 Piper Species

The essential oils derived from certain piper species (*Piper austrosinense*, *P. puberulum*, *P. flaviflorum*, *P. betle*, and *P. hispidinervum*) showed strong AChE inhibitory activity with IC_{50} values in the range of 1.51–13.9 mg/mL. The most active essential oil from *P. hispidinervum* contained a potent bioactive compound, asaricin, with an IC_{50} value of 0.44±0.02 mg/mL against AChE, comparable to galantamine with an IC_{50} 0.15±0.01 mg/mL (Xiang et al. 2017). The study suggested that the piper essential oils may be a good natural product source for compounds with therapeutic potential for AD or other CNS diseases. The possibility also exists that the oil can be used against CCD/CDS.

5.1.3 *Angelica archangelica* and *Geranium sylvaticum*

Sigurdsson and Gudbjarnason (2007) screened several Icelandic medicinal plants for AChE-inhibiting property. Ethanolic extracts of *Angelica archangelica* seeds and the aerial parts of *Geranium sylvaticum* proved to be effective with IC_{50} values of 2.2 mg/mL and 3.56 mg/mL, respectively. Analysis of *A. archangelica* extract revealed the presence of two major active principles (imperatorin and xanthotoxin), with xanthotoxin being more potent than imperatorin, with IC_{50} value of 155 μg/mL (0.72 mM) and 274 μg/mL (1.01 mM), respectively. Interestingly, Sigurdsson and Gudbjarnason (2007) found a synergistic interaction between the extract of *A. archangelica* and *G. sylvaticum* for anticholinesterase activity.

5.1.4 *Bacopa monnieri*

Bacopa monnieri, often called "Brahmi" meaning Creator, is a small creeping herb commonly found in marshy areas throughout India. The major active ingredients in Brahmi are steroidal saponins called bacopasides. In humans, they are used for many CNS disorders, such as cognitive dysfunction, epilepsy, seizures, anxiety, and depression. In humans, Brahmi has been found to improve the speed of visual information processing and spatial working memory accuracy, as well as reducing subjective anxiety levels (Stough et al. 2001; Morgan and Stevens 2010). It has been mentioned that bacopasides cross the BBB via lipid-mediated free diffusion due to their hydrophobic nature and effects on brain health and function; however there is no definitive evidence to support this fact (Pitt and Leung 2016). In in vitro and experimental studies, Brahmi has been reported to improve cognition by multiple mechanisms, such as its antioxidative, anti-lipid peroxidation, and neuroprotective properties, and by prevention of cell death (Limpeanchob et al. 2008; Hosmani 2009; Saini et al. 2012; Velaga et al. 2014; Le et al. 2015). In addition, Brahmi has been reported to provide neuroprotective and memory-restoring effects due to the ability of bacopasides to alter Ca^{2+} dynamics in smooth muscle cells inducing vasodilation (Kamkaew et al. 2011), thereby providing more oxygen and glucose (reviewed in Pitt and Leung 2016). Furthermore, the levels of neurotransmitters (serotonin, ACh, GABA, and glutamate) are reported to be elevated in the hippocampus of rats fed Brahmi, which appears to facilitate neuronal activity and synaptic signaling (Ranjan et al. 2011). In an in vitro study, Das et al. (2002) also demonstrated that *B. monnieri* extract exerted anti-ChE activity in mice.

In an experimental study, Piyabhan et al. (2016) demonstrated that treatment with Brahmi prior to phencyclidine (PCP, 2 mg/kg, ip, twice a day) prevented cognitive impairment by elevating vesicular glutamate transporter 3 (VGLUT3) in the prefrontal cortex (PFC), striatum, and hippocampus (CA1-3 sectors) of rats. Rats receiving Brahmi after PCP treatment restored cognitive deficit by increasing VGLUT3 in the PFC and striatum. Findings of this study and others suggest that Brahmi could be a new frontier of prevention and restoration of cognitive deficit associated with AD, PD, or schizophrenia.

The recommended dose of *B. monnieri* extract in dogs and cats is 250 mg and 100 mg/day, respectively.

5.1.5 *Curcuma longa* (Turmeric/Curcumin)

The root of *Curcuma longa* contains turmeric, which is composed of a number of compounds called curcuminoids. The main curcuminoids are curcumin, demethoxycurcumin, and bisdemethoxycurcumin. Turmeric has been used for thousands of years to heal humans and animals from a number of illnesses. Currently, curcumin is commonly used to prevent or treat diseases, such as pain, cancer, stroke, AD, epilepsy, depression, obesity, diabetes, and metabolic disorders (Javeri and Chand 2016). Many studies evidenced curcumin's anti-inflammatory action exerted via the activation of transcription factors such as NF-κB and AP-1, and its antioxidative as well as cytoprotective properties (Chen et al. 2006; Maheshwari et al. 2006; Risuleo 2016).

It is documented that low-dose (~50 mg/day) long-term use of curcumin may delay or halt the onset of cognitive impairment in the early stages of AD (Chandra et al. 2001; Hamaguchi et al. 2010). Lim et al. (2001) reported that low-dose curcumin feeding (160 ppm in feed) for 6 months in aged transgenic AD mice was found to reduce astrocytic marker glial fibrillary acidic protein (GAFP), insoluble Aβ, and soluble Aβ, with a 43–50% decrease in plaque level.

Curcumin has also been shown to bind to Aβ and increase brain clearance of Aβ in mouse models of AD and disaggregated fibrillary $A\beta_{40}$ in in vitro studies (Lim et al. 2001; Garcia-Alloza et al. 2007). In in vivo studies, aged transgenic 2576 mice with advanced amyloid accumulation fed curcumin (500 ppm in feed) for 5 months reduced the levels of amyloid and plaques. Findings of these studies and others demonstrated that curcumin crosses the BBB despite the low dose and poor bioavailability (reviewed in Javeri and Chand 2016; Sorrenti et al. 2018).

In experimental studies, high doses of systemically injected lipopolysaccharide (5–10 mg/kg) have been extensively used to study the interaction between peripheral inflammation and neurodegenerative disorders. Peripheral LPS induces synthesis of proinflammatory mediators in the brain, resulting in a variety of central effects, including synaptic dysfunction, neuronal cell degeneration, and cognitive impairment (Qin et al. 2007, 2013). Recently, Sorrenti et al. (2018) demonstrated that curcumin (50 mg/kg) administered orally for two consecutive days prior to a single ip injection of a high dose of LPS (5 mg/kg) in young adult mice prevented the immune response, neuroinflammation, and memory impairment. Additionally, short-term treatment with curcumin, administered at the time of LPS challenge, anticipated the recovery from memory impairments observed 1 month after the inflammatory stimulus, when mice had completely recovered from the acute neuroinflammation. Findings of this investigation suggested that the preventive effect of curcumin in inhibiting the acute effects of neuroinflammation could be of value in reducing the long-term consequences of brain inflammation, cognitive deficits, and memory dysfunction.

Curcumin appears to be a highly promising nutraceutical for prevention and treatment of cognition impairment in canines and felines.

5.1.6 Ginseng

Ginseng is the root of plants (genus *Panax*), including Chinese ginseng (*P. notoginseng*), Japanese ginseng (*P. japonica*), Korean ginseng (*P. ginseng*), Vietnamese ginseng (*P. vietnamensis*), and American ginseng (*P. quinquefolius*). Ginseng is cultivated or grown in mountainous regions (wild) and mainly produced in China, South Korea, the United States, and Canada. The root extract contains many active ingredients, called ginsenosides (ginseng saponins). The content of ginsenosides differs in the different species of ginseng as well as in the different parts of the plant. The total amount of dammarane-type saponins in *P. notoginseng* is reported to be greater than in *P. ginseng* or *P. quinquefolius* (Peng et al. 2018). Dammarane-type saponins can be divided into two groups: protopanaxadiol and protopanaxatriol. A large number of ginsenosides have been identified in *P. ginseng* (Choi 2008; Yang and Wu 2016) and in *P. notoginseng* (Peng et al. 2018). The efficacy of *P. ginseng* on the brain and nervous system has been investigated in several studies (reviewed in Yang and Wu 2016). Ginsenosides do not readily cross the BBB, and only trace amounts can be detected in the brain after oral administration. However, metabolites of ginsenosides cross the BBB and produce direct effects on neuronal cells (reviewed in Pitt and Leung 2016).

In general, *P. ginseng* is considered to be a stimulant to cognition. Xing et al. (2008) reviewed animal study data and reported that ginsenosides Rb1, Rc, Rg1, Rg2, and Rg3 had effects on the CNS. Both ginsenosides Rg1 and Rb1 enhanced activities of the CNS, whereas ginsenoside Rb1 had a much weaker potency. The improvement of cognition was attributed to the higher ratio of ginsenoside Rg1/Rb1 (Chen et al. 2008). Liu et al. (2011) found that ginsenoside Rb1 improved cognitive function and the survival of newborn hippocampal neurons in adult rats due to its anti-stress effects. Ginsenosides Rg1 and Rb1 also helped to facilitate acquisition and retrieval of memory (Wee et al. 2011). Furthermore, ginsenoside Rg1, Rg3, and Re were found to reduce β-amyloid peptide in animal brains following a single dose (Chen et al. 2006).

Ginsenoside-Rh1has been reported to activate estrogen receptor (ER)-dependent gene expression (Lee et al. 2003), and ER activity is correlated with memory function. At the cellular level, ER activity promotes many memory-associated changes, including increases in dendritic spine density, NMDA receptor expression, and long-term potentiation (LTP) magnitude (Smith and McMahon 2005). Many clinical trials have been conducted in healthy as well as in cognitively impaired patients. In some studies, ginseng was found to provide improvements in cognition and memory, while in other studies, very little or no effect (Attele et al. 1999; Kennedy et al. 2001; Scholey and Kennedy 2002; Xing et al. 2008; Kim et al. 2015; Asian ginseng 2016). Ginseng has been used in pets for a number of years to treat several diseases (Addison's disease, congestive heart failure, diabetes mellitus, chronic low-grade hepatitis) and improve cognition and memory, but no controlled clinical trials are reported in canines or felines.

5.1.7 *Ginkgo biloba*

The *Ginkgo biloba* is believed to be the world's oldest living tree, dating back to more than 250 million years ago. It grows in many countries, including Asia, North America, Europe, New Zealand, and Argentina. *G. biloba* is one of the most popular nutraceuticals used in the United States and Europe. Its extract or its active ingredient(s) has been used for thousands of years in a number of diseases. *G. biloba* leaf extract consists of terpene trilactones (ginkgolides A, B, and C and bilobalide), ginkgolic acid, and flavonoid glycosides (quercetin, kaempferol, and isorhamnetin) (Wang et al. 2014).

Gurley et al. (2012) described its therapeutic use for insufficient blood flow, memory deficits, cognitive disorders, AD, depression, vertigo, tinnitus, and intermittent claudication. In humans, *G. biloba* has been indicated for memory enhancement (Dugoua et al. 2006). The WHO has accepted the standardized extracts such as EGb 761 and Li 1370 as anti-dementia drugs based on the in vitro and in vivo pharmacological studies and the numerous clinical studies supporting the efficacy of EGb 761 in the CNS when taking a 240-mg daily dose.

Pharmacological and pharmacokinetic studies suggest that the target of EGb 761 is the CNS (Ude et al. 2013). It can improve cerebral blood flow by 50–100%, increase glucose uptake and utilization, reduce corticosteroid production, and improve mitochondrial metabolism and ATP production, as well as affect intracellular and extracellular ionic gradients (reviewed in Dziwenka and Coppock 2016). EGb 761 has also been shown to reduce cognitive dysfunction in a model of vascular dysfunction in gerbils. The study showed a significant recovery of spatial memory with regular postischemic treatment (Mohanta et al. 2014).

In a number of clinical trials, EGb proved to improve cognition and memory (Lebars et al. 1997; Ihl et al. 2011; Garvilova et al. 2014). In a double-blinded clinical study, mild to moderate AD patients received EGb 761 (240 mg) or placebo daily for 24 weeks. EGb 761-treated patients improved significantly when evaluated on the Short Cognitive Performance Test (Syndrome Kurz Test, SKT) and the Neuropsychiatric Inventory (NPI) test (Ihl et al. 2011). In a similar study, Garvilova et al. (2014) reported that EGb 761 improved neuropsychiatric symptoms and cognitive performance with mild cognitive impairment (MCI). However, in some clinical trials, no difference was found between *G. biloba* and placebo groups.

G. biloba can protect neuronal damage by its direct action on neurons or indirectly by increasing blood flow and antioxidant action (Chan et al. 2007). DeFeudis and Drieu (2000) demonstrated that the high concentrations of EG 761enhanced the uptake of radiolabeled norepinephrine, dopamine, and 5-HT into the synaptosomes of the rat brain. EGb 761 influences the neuromodulation of adrenergic neurotransmission, and this could explain the positive effect on cognition seen in mice and humans. In vivo studies in mice revealed that in EGb 761 extract, terpenoids act by decreasing the generation of free radicals, and flavonoids act by scavenging the free radicals (DeFeudis and Drieu 2000). In addition to anti-apoptotic effect, ginkgolides and bilobalide in EGb 761 protect mitochondria from age-related damage and to improve mitochondrial function and energy metabolism (reviewed in Dziwenka and Coppock 2016). The two proposed mechanisms for mitochondrial protection are (1) inhibition of platelet-activating factor (PAF) at the PAF receptor and (2) blockage of glycine-activated chloride channels in glycine receptors in hippocampal neurons (Ude et al. 2013). Ginkgolide B is proven to be the most potent mitochondrial protectant. In an in vitro study, *G. biloba* extract also exerted anticholinesterase activity in mice (Das et al. 2002).

In canines, *G. biloba* products are indicated in health conditions, such as cognition/memory impairment, depression, anxiety, and tinnitus. In an open clinical trial, Ginkgo leaf extract was administered to 42 elderly dogs at a daily dose of 40 mg/10 kg body wt for 8 weeks (Reichling et al. 2006). The findings revealed that the Ginkgo leaf extract appears to be an efficacious agent that provides a safe dietary supplement for the elderly dogs with age-related behavioral disturbances. *G. biloba* has been included in Senilife®, which is indicated for canines with cognitive dysfunction, but due to limited evidence, it offers weak anti-dementic effect. Senilife® also contains resveratrol, which lacks scientific evidence to support that it improves cognition or memory.

5.1.8 *Crocus sativus* L. (Saffron)

Saffron, commonly referred to as "Red Gold," frequently used spice, has been identified as a memory-enhancing agent (Adalier and Parker 2016). Saffron possesses bioactive compounds (crocin, crocetin, dimethylcrocetin, and safranal), which may exert antioxidative, anti-inflammatory, antinociceptive, anti-ChE, and anti-amyloidogenic activities. Geromichalos et al. (2012) reported the AChE inhibitory activities of crocetin, dimethylcrocetin, and safranal with IC_{50} values of 96.33, 107.1, and 21.09 μM, respectively. Kinetic analysis showed that safranal interacts only with the binding site of AChE, but crocetin and dimethylcrocetin bind simultaneously to the catalytic and peripheral anionic sites. Papandreou et al. (2006) unraveled the underlying mechanism of saffron in AD as antioxidative and the inhibitory activity on Aβ aggregation. *Trans*-crocin-4 (the digentibiosyl ester of crocetin) was found to be the most potent compared to other constituents in saffron. Due to these properties, saffron has been found to be very effective in cognitive impairment and AD (Ghadrdoost et al. 2011; Geromichalos et al. 2012; Ghaffari et al. 2015). In a clinical study, patients receiving 15 mg saffron extract twice daily for 22 weeks had a better outcome on cognitive functions than the placebo group (Akhondzadeh et al. 2010). In another clinical trial, saffron was found to be as effective as donepezil in treatment of mild to moderate AD patients (reviewed in Adalier and Parker 2016). No clinical trials have been reported on saffron in CCD/CDS.

5.1.9 *Monsonia angustifolia*

Monsonia angustifolia is an indigenous vegetable to Tanzania, and it has several bioactive compounds, such as justicidin A, 5-methoxyjusticidin A, chinensinaphthol, retrochinensinaphthol methyl ether, and suchilactone. Among these compounds, justicidin A has been found to possess a neuroprotective property. Gu et al. (2016) reported

that justicidin A protects SH-SY5Y cells from $A\beta_{25–35}$-induced neuronal cell death through inhibition of hyperphosphorylation of tau and induction of autophagy via regulation of the activity of GSK-3β and AMPK and also provided some insights into the relationship between tau protein hyperphosphorylation and autophagy. Chun et al. (2017) investigated the effect of ethanol extract of *M. angustifolia* on Aβ production and spatial learning ability as protection against AD. In an in vitro study, the formation of Aβ peptides was significantly reduced in HeLa cells. In an in vivo study, Tg2576 mice were treated with *M. angustifolia* extract for 6 months and then subjected to a Morris water maze and a novel object recognition test. Findings revealed that the treated mice showed ameliorated behavioral deficits of the AD transgenic mice and reduced levels of insoluble Aβ42 in the cerebral cortex and hippocampus. Justicidin A significantly decreased the formation of Aβ in APPsw-transfected cells. These studies suggest that *M. angustifolia* extract or justicidin A have the potential to be developed as a treatment of AD in humans and CDS in canines and felines.

5.1.10 Berberine-Containing Plants

Berberine (BBR), a nonbasic, quaternary benzylisoquinoline alkaloid, can be obtained from a number of plants (*Berberis vulgaris*, *B. aristata*, *B. aquifolium*, *Hydrastis canadensis*, *Coptis chinensis*, *Argemone mexicana*, and others). Currently, BBR is also available in the synthesized form (BBR chloride and BBR sulfate). BBR derived from plants is found to be of high potency and safer than the synthesized form. For thousands of years, BBR-containing plant extracts have been used in many health conditions, including diabetes, hyperlipidemia, hypertension, ischemic stroke, microbial diarrhea, cancer, etc. In a number of reports, the BBR alkaloid has been indicated in neurological diseases, such as AD, PD, anxiety, depression, and epilepsy (Peng et al. 2004; Yoo et al. 2006; Zhu and Qian 2006; Peng et al. 2007; Kulkarni and Dhir 2008, 2010; Ye et al. 2009; Kumar et al. 2015).

Tan et al. (2013) investigated the tissue distribution and pharmacokinetics of BBR and its metabolites in rats after oral administration of BBR (200 mg/kg). The results revealed that berberine was quickly distributed in the liver, kidneys, muscle, lungs, brain, heart, pancreas, and fat in a descending order of its amount. The concentrations of berberine were higher in tissues than in plasma. Metabolites of BBR, such as thalifendine, berberrubine, and jatrorrhizine were detected in the liver and kidney. Pharmacokinetic studies by Wang et al. (2004, 2005) further confirmed that berberine crosses the BBB and accumulates in the hippocampus, where it provides anti-apoptotic and neuroprotective effects.

Berberine exerts multiple pharmacological actions, such as antioxidant, anti-inflammatory, neuroprotective, analgesic, anxiolytic, and immunomodulatory actions, thereby providing preventive and therapeutic effects in neurological conditions (Kulkarni and Dhir 2008, 2010; Kumar et al. 2015; Hussein et al. 2018). In an investigation conducted on aluminum-overloaded rats, berberine improved learning and memory and protected hippocampal neurons from death by normalizing AChE, ChAT, SOD, and MAO-B activities and MDA content (Zhang et al. 2009). Kulkarni and Dhir (2008, 2010) and Kumar et al. (2015) reported that acute or chronic administration of BBR (5 mg/kg, ip) for 15 days increased the levels of neurotransmitters (norepinephrine, dopamine, and 5-HT) in discrete brain regions. Its neuroprotective role is also attributed to its ability to block potassium channels in hippocampal CA1 neurons leading to suppression of apoptosis (Wang et al. 2004). Cell death rate of neurons treated with BBR (5 μmol/L) was found to be significantly lower than that of non-treated neurons. Further, BBR was reported to protect the hippocampal CA1 region from ischemic injury by inhibiting NMDA receptor immunoreactivity in ischemic gerbil brains (reviewed in Ye et al. 2009). Hussein et al. (2018) demonstrated that rats preexposed to a mixture of heavy metals (Al, 50 mg/kg; Cd, 5 mg/kg; and Fl, 20 mg/kg) daily for 90 days, when treated with BBR (50 mg/kg/day, po) for a month, downregulated AChE expression and inhibited its activity in brain tissue and normalized the production of TNF-α, IL-1β, IL-6, and IL-12. Additionally, BBR inhibited the formation of Aβ42 and improved cognition and memory. In a few other studies, BBR has been reported to prevent or delay the process of AD (Abd El-Wahab et al. 2013; Cai et al. 2016). Thus, BBR appears to be a novel preventive and therapeutic modality for CDS in canine and felines.

Quaternary protoberberine alkaloids, such as stepharanine, cyclanoline, and *N*-methyl stepholidine were isolated from a Thai medicinal plant *Stephania venosa* Spreng (Ingkaninan et al. 2006). These alkaloids expressed inhibitory activity of AChE with IC_{50} values of 14.1±0.81, 9.23±3.47, and 31.30±3.67 μM, respectively, and have great potential for anti-CDS in canines and felines.

5.1.11 *Huperzia serrata* (Chinese Club Moss)

Huperzine A, derived from *Huperzia serrata*, is commonly used for the treatment of AD in China. It exerts anti-AChE, NMDA receptor antagonist, and neuroprotective properties (Landsberg et al. 2012). Patients with AD receiving huperzine A have shown improvement in general cognitive function, global clinical status, functional performance, and reduced behavioral disturbance compared to patients taking placebos. Thus, huperzine A has potential as nutraceutical for the treatment of CDS in elderly dogs.

5.1.12 Soybean Peptides and Isoflavones

Currently, soy proteins are being used as a source of nutrients around the world. These proteins are well-known for different functional properties and are widely recognized as a

potential source of bioactive peptides (Katayama et al. 2014; Imai et al. 2017). Soy peptides have been reported to exhibit a number of functional properties, such as hypocholesterolemic, antihypertensive, antiviral, anti-inflammatory, and immunostimulatory. Katayama et al. (2014) reported that administration of soy peptides via diet (7% w/w daily for 26 weeks) suppressed cognitive decline (Morris-Water Maze for Cognitive Testing) by induction of brain-derived neurotrophic factors (BDNF) in SAMP8 mice. These investigators demonstrated that soy peptides-fed mice improved special learning and memory when compared with control mice. Interestingly, soy peptides significantly upregulated neurotrophic factors (NGF, BDNF, and NT-3), which promote neurogenesis, neurodifferentiation, neuroprotection, and neuroplasticity. It has been suggested that the NT-3 enhancement in the brain is a potential therapeutic strategy for preventing age-related cognitive decline.

Ding et al. (2013) reported that 80 mg/kg/day of soybean isoflavone by gavage as pretreatment could alleviate the synaptic structural damage and antagonize the downregulation of expressions of proteins [(1) mRNA and protein of the synaptophysin and postsynaptic density protein 95 (PDS-95); (2) protein of calmodulin (CaM), Ca^{2+}/calmodulin-dependent protein kinase II (CaMK II), and cAMP response element-binding protein (CREB); and (3) phosphorylation levels of CaMK II and CREB (pCAM II, pCREB) induced by $A\beta_{1–42}$ in rats]. These findings suggested that soybean isoflavone pretreatment could ameliorate the impairment of learning and memory ability in rats induced by $A\beta_{1–42}$. Genistein, a major isoflavone in soybeans, provided a neuroprotective effect through anti-inflammatory activity in C6 glial cells caused by $A\beta_{25–35}$. The findings revealed that genistein could (1) alleviate $A\beta_{25–35}$-induced cell apoptosis, (2) prevent TNF-α and IL-1β release, (3) upregulate the gene and protein expression of TLR4, and (4) significantly upregulate the expression of 1κB-α in C6 cells. The study suggested that genistein may provide a neuroprotective effect via alleviating $A\beta_{25–35}$-induced inflammatory stress by regulating the TLR4/NF-κβ signaling pathway. Genistein can be an adjunctive therapy for treating cognition dysfunction syndrome in canines or felines.

5.2 Monoamine Oxidase B (MAO-B) Inhibitors

Age-related increase in monoamine oxidase B (MAO-B) is known to contribute to CNS neurodegenerative diseases (Mazzio et al. 2013). MAO-B inhibitors are indicated for patients with AD and PD as they could enhance catecholamine neuroactivity and increase dopamine levels in dogs. Currently, FDA approved Anipryl® (selegiline hydrochloride, L-deprenyl hydrochloride) is available on the market for the control of clinical signs associated with CDS in dogs. The recommended dose in dogs with CDS is 0.5–1.0 mg/kg body wt po, once daily, preferably in the morning. The extracts of several plants (such as *Phellodendron amurense*, *Cyamopsis psoralioides*, *Glycyrrhiza glabra*, *Glycyrrhiza uralensis*, and *Psoralea corylifolia*) have been reported for strong MAO-B inhibiting potency (Mazzio et al. 2013). Berberine from *Coptis chinensis* is known to inhibit MAO-B activity. The extracts of some plants (such as turmeric, comfrey, bhringraj, skullcap, kava kava, wild indigo, gentian, and green tea) are identified with lesser MAO-B inhibiting potency. Plant extracts with a strong MAO-B inhibiting activity can be used as nutraceuticals for managing the symptoms of dementia and related anxiety and sleep-cycle disorders in aging dogs.

5.3 Calcium-Buffering Proteins

Calcium-binding proteins are involved in cellular activities and functions. Cells possess numerous calcium-binding proteins that regulate calcium concentration in the cytosol by buffering free calcium ion (Moran et al. 2014). Intracellular calcium dysregulation has been associated with aging and may be linked to CDS in dogs (Milgram et al. 2015). These investigators evaluated the effects of a calcium-buffering photoprotein apoaequorin (placebo, 2.5 or 5 mg) on discrimination learning, attention, and visuospatial memory tasks in beagle dogs. Findings revealed that the apoaequorin-treated dogs showed improved performance on the discrimination learning and attention tasks, but not on the spatial memory tasks. In another study, these authors found that dogs receiving 10 mg daily dose of apoaequorin showed cognition improvement equal to those receiving 1 mg selegiline (Anipryl®). Currently, apoaequorin is one of the ingredients of Neutricks®. Moran et al. (2013) determined the No-Observed-Adverse-Effect level (NOAEL) for apoaequorin as 666.7 mg/kg body wt/day in rats, and there appears to be no concern of safety due to unusual stability of the protein by ingestion (Moran et al. 2013).

5.4 Nutraceuticals with Antioxidative, Anti-inflammatory, and Neuroprotective Properties

Literature abounds showing that oxidative stress due to excess generation of free radicals is one of the leading causes of CCD/CDS in dogs (Skoumalova et al. 2003). This is due to the fact that the brain (1) consumes a high rate of oxygen, (2) has a high percentage of PUFAs, and (3) has low levels of endogenous antioxidant activity. Studies also support that

foods and nutraceuticals enriched with antioxidants can help reduce the effects of aging on canine cognition (Cotman et al. 2002; Sechi et al. 2015). Examples of such diets include flaxseed, spinach, citrus pulp, tomato pomace, grape pomace, α-lipoic acid, vitamin C, vitamin E, choline, L-carnitine, etc. Further improvement in cognition and learning ability was observed when dogs were provided behavioral stimulus/enrichment in addition to an antioxidant-rich diet (Milgram et al. 2005; Opii et al. 2008).

Some of the common antioxidants that have been shown to improve cognition and memory are discussed below in brief.

5.4.1 Ascorbic Acid

Recently, in a rat model of AD, Olajide et al. (2017) investigated efficacy of ascorbic acid in reversing aluminum chloride (AlCl3)-induced behavioral deficits and neuropathological alterations in the prefrontal cortex and hippocampus. Administration of ascorbic acid (100 mg/kg daily for 15 days) significantly attenuated behavioral deficits in rats through inhibition of molecular and cellular stressor proteins activated by $AlCl_3$. Findings revealed that the primary mechanisms underlying ascorbic acid therapy relates to its abilities to scavenge free radicals, prevent membrane lipid peroxidation, modulate neuronal bioenergetics, and its anti-proteolytic properties. It appears that ascorbic acid supplementation may inhibit progression of neurodegenerative processes and behavioral alterations in dogs and cats.

5.4.2 Lycopene

Lycopene, a carotenoid pigment, possesses potent antioxidative, anti-inflammatory, and neuroprotective properties. In an experimental study, Zhao et al. (2017a) determined the effects of lycopene on oxidative stress-induced cognitive defects and the underlying mechanisms. The behavioral tests (Y-maze test, locomotor activity, and Morris water maze test) revealed that chronic lycopene supplementation (50 mg/kg body wt/day) alleviated D-galactose-induced cognitive impairment in CD-1 mice. Lycopene restored BDNF levels and ameliorated histopathological changes in the hippocampus of mice. Lycopene treatment activated the mRNA expressions and significantly elevated activities of antioxidant enzymes (HO-1 and NQO-1) and reduced levels of inflammatory cytokines (IL-1β and TNF-α) in the hippocampus. Furthermore, lycopene attenuated neuronal oxidative damage through activation of Nrf2 signaling and inactivation of Nf-κB translocation in a H_2O_2-induced SH-SY5Y cell model. Zhao et al. (2018) also demonstrated that lycopene supplementation significantly reduced age-associated neuroinflammation and lowered the accumulation of $A\beta_{1–42}$ in the brains of aged CD-1 mice. Hwang et al. (2017) revealed that lycopene inhibited apoptosis by reducing ROS and by inhibiting mitochondrial dysfunction and the NF-κB-target gene Nucling expression in neuronal cells. It appears that lycopene has a potential to improve cognitive impairment and memory by ameliorating oxidative stress, neuroinflammation, activation of Nrf2/NF-κB transcriptional pathway, and accumulation of $A\beta_{1–42}$.

5.4.3 Vitamin E

Vitamin E (vit E) is a potent antioxidant that may have beneficial health effects in AD by ameliorating oxidative stress and Aβ-associated free radicals (Yatin et al. 2000; Morris et al. 2005; Geraldo et al. 2014; Adalier and Parker 2016). Out of eight different forms of vit E, α-tocopherol and γ-tocopherol are the two forms that are most associated with the slowing down of cognitive decline (Morris et al. 2005). α-Tocopherol is the most bioavailable form of vit E and the most helpful in AD (Joshi and Pratico 2012), while γ-tocopherol has been found to be important for its neuroprotective effect (Morris et al. 2015). Vit E mainly acts as a chain breaking antioxidant and radical scavenger, protecting cell membranes against oxidative damage. In addition, vitamin E regulates ROS production, maintains oxidative phosphorylation in the mitochondria, and accelerates restitution of high-energy metabolites.

In a number of clinical studies, vitamin E (2000 IU/day) has been shown to delay the progression of mild to moderate AD because of its potent antioxidative and neuroprotective effects (reviewed in Adalier and Parker 2016). When vit E was given in combination with a ChE inhibitor (donepezil, rivastigmine, or galantamine), it provided greater improvement in cognition than the ChE inhibitor alone (Bittner 2009). However, when vit E was given in combination with memantine (an NMDA receptor antagonist), it did not offer any additional benefits (Dysken et al. 2014).

No clinical studies have been conducted in cognitively declined canines and felines. Of course, vita E is included in the therapeutic diet Senilife®.

5.4.4 Omega Fatty Acids

Polyunsaturated fatty acids (PUFA) are important for the formation of cellular membranes and physiological functions in the brain (Layé et al. 2018). There are two main families of PUFA, n-3 and n-6 (commonly referred as omega-3 and omega-6 fatty acids, respectively). α-Linolenic acid (ALA) is the precursor of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), and linolenic acid (LA) is the precursor of arachidonic acid (AA). AA, DHA, and EPA are consumed in the diet, since they are not produced in the brain. The major dietary sources of ALA are green plants, nuts, flaxseed, and rapeseed oil, whereas fish oil is the main source of EPA and DHA (Layé et al. 2018).

The brain is highly enriched in AA and DHA, and both omega-3 and omega-6 PUFAs are esterified into phospholipids, which are well known to play a critical role

in the structures and functions of brain cell membranes. Brain regional distribution and accumulation of LC-PUFAs vary. For example, in adult C57BL6/J mice, the highest level of AA is found in the hippocampus (10.2%), followed by the prefrontal cortex (9.7%), the hypothalamus (8.5%), the cortex (7.7%), the cerebellum (6.5%), and the brainstem (5.5%) The highest level of DHA is found in the prefrontal cortex (14.3%) and in the hippocampus (13.7%), followed by cerebellum (12.2%), cortex (11.9%), hypothalamus (10.1%), and brainstem (8.2%) (Joffre et al. 2016). It is noteworthy that the hippocampus and cortex are important brain regions in the context of memory, although other brain regions are also involved.

In humans, higher n-3 LC-PUFA consumption is associated with a lower risk of inflammation-associated neurologic disorders (Bazinet and Layé 2014). Supplementation of patients diagnosed with AD with a DHA-risk diet led to a reduced release of proinflammatory cytokines from blood mononuclear leukocytes (Vedin et al. 2008). High levels of brain DHA are linked to the reduced expression of proinflammatory cytokines in several rodent models of acute (LPS) or chronic (aging) neuroinflammation (Orr et al. 2013). Brain PUFAs contribute to microglial homeostasis and regulate their role in neuroinflammatory cascades (Layé et al. 2018). In a randomized, double-blind, placebo-controlled clinical trial, findings did not support supplementing older adults with DHA-rich fish oil to prevent cognitive decline (Danthiir et al. 2018). In rats, Morgese et al. (2018) demonstrated that an n-3 PUFA-enriched diet prevented the Aβ-induced AD-associated depressive-like behavior and also reverted serotonin and neurotrophin levels that were reduced in the prefrontal cortex. Brain PUFA metabolism is altered in neurological conditions, such as dementia, so nutraceuticals appear to intervene and restore brain lipid metabolism. These studies suggest that omega-3 fatty acids have a great potential and can be used as nutraceuticals in canines and felines with cognitive dysfunction.

5.4.5 Medium-Chain Triglycerides

Glucose is the main energy source of neurons, and its metabolism is reduced with aging (Seibert 2017). It has been suggested that dietary medium-chain triglycerides (MCTs) can increase the levels of ketones in the blood, which can be used as an alternate energy source for neurons and cerebral functioning. Pan (2011) reported that fatty acids derived from MCTs could provide up to 20% of the brain's energy requirements, and long-term supplementation with MCTs has been shown to improve cognitive function in aged dogs. MCTs have been reported to elevate mitochondrial function to boost the level of ATP production and reduce the levels of Aβ deposits (Taha et al. 2009; Pan et al. 2010).

Coconut oil has MCTs, which are converted into ketones that can provide energy to the brain. Scientific studies and clinical trials have yet to be done to support the facts and its use in CCD.

5.4.6 Phosphatidylserine

Phosphatidylserine is a phospholipid that occurs in cell membranes. It facilitates normal function of nerve cell membranes and influences levels of various neurotransmitters. Baumeister et al. (2008) investigated the influence of phosphatidylserine on cognitive performance and cortical activity before and after induced stress. Findings revealed that chronic supplementation of phosphatidylserine significantly decreased beta-1 power in right hemispheric frontal brain regions before and after induced stress. Pedata et al. (1985) found that acetylcholine release in 24-month-old rats receiving a single administration of phosphatidylserine (15 mg/kg, ip) or phosphatidylcholine (15 mg/kg, ip) for 30 days was as low as in the 24-month-old rats receiving the Tris buffer only. These investigators suggested that chronic phosphatidylserine treatment may reduce the age-related decrease in ACh release by acting on the stimulus-secretion coupling mechanism.

Crook et al. (1991) evaluated patients with age-associated memory impairment receiving phosphatidylserine (100 mg tid) for 12 weeks. Treated patients showed improvement on both computerized and standardized neuropsychological performance tests and also on clinical global ratings of improvement. Based on the clinical literature in humans, the FDA concluded that the evidence did not support assertions that phosphatidylserine has preventative or therapeutic benefit for cognitive dysfunction and dementia in people. However, the agency did approve claim for the product as "Consumption of phosphatidylserine may reduce the risk of cognitive dysfunction or dementia in the elderly." Phosphatidylserine is one of the ingredients in Senilife® and Aktivait®, but no clinical trials have been found on phosphatidylserine alone in senior dogs.

5.4.7 *S*-Adenosylmethionine

S-Adenosylmethionine (SAMe) is an endogenous molecule that has an antioxidant property. SAMe is involved in the synthesis of some neurotransmitters and is known to play a role in the regulation of nerve cell membrane structure and function. Based on limited scientific data, SAMe showed evidence for an improvement in daytime activity and possibly some improvement in sleep problems, but it did not offer any improvement for confusion or disorientation.

5.4.8 Melatonin

Melatonin (*N*-acetyl-5-methoxytryptamine) is a naturally occurring hormone produced by the pineal gland. It has been shown to exert antioxidative, anti-inflammatory, and immunomodulatory effects (Sharman and Bondy 2016). It has been suggested that the effective range of melatonin on

cognitive function and mood may exist within physiological levels. In a number of studies, physiological melatonin levels were found to be reduced in patients with AD or major depressive disorder (Mishima et al. 1999; Wu et al. 2003). Higher melatonin levels within the physiological range were associated with lower prevalence of cognitive impairment and depressed mood (Obayashi et al. 2015). The association between physiological melatonin levels and cognitive function was independent of depressive symptoms. In a meta-analysis of ten randomized controlled trials, Hansen et al. (2014) concluded that there was no significant effect of melatonin against depression. In most of these trials, the daily dose of melatonin was 2.5–10 mg, approximately 10- to 40-fold higher melatonin levels than physiological levels, when prolonged-release tablets were used. Fast-release tablets tend to shorten the effective period of melatonin.

Leuner et al. (2007) reported that diminished neurogenesis precedes old age, and this decline can be delayed by supplementation with melatonin (Ramirez-Rodriguez et al. 2012). In addition, the maintenance of dendritic complexity is enhanced by melatonin (Ramirez-Rodriguez et al. 2012).

Melatonin is used for anxiety or disturbed sleep/wake pattern in dogs with dementia. According to the American Kennel Club website, the recommended dose of melatonin for dogs is 1 mg for under 10 lbs, 1.5 mg for 10–25 lbs, 3 mg for 25–100 lbs, and 3–6 mg for over 100 lbs. Melatonin works within just few minutes after taking it, and the effect can last for about 8 h.

5.4.9 Rosmarinic Acid

Rosmarinic acid (RosA) is a water-soluble polyphenol derived from various medicinal herbs, such as *Salvia miltiorrhiza* Bunge and *Rosmarinus officinalis*, which has been used in folk medicine to treat various forms of dementias (Zhang et al. 2016). RosA has been demonstrated to exert antioxidant, anti-inflammatory, anti-proliferative, cytoprotective, neuroprotective, and anti-depressant properties in a variety of model systems, including AD (Taguchi et al. 2017). Nuclear factor E2-related factor 2 (Nrf2) appears to be the master regulator to protect neuronal cells from oxidative stress via activating expression of the antioxidant response element (ARE)-bearing genes. In an in vitro study, Rong et al. (2018) reported that RosA attenuated Aβ-induced cellular ROS generation and lipid peroxidation in PC12 cells. RosA also mediated neuroprotection in Aβ-challenged PC12 cells. The antioxidant effects of RosA are mediated predominantly by Akt/GSK-3β/Fyn pathway through increased activity of Nrf2. Zhang et al. (2017) presented compelling evidence that inhibition of GSK-3βactivity by dietary supplements prevent cognitive impairment in transgenic AD mice. It can be suggested that RosA appears to be a promising candidate for neuroprotective treatment of CDS in canines and felines.

5.4.10 Brown Rice

Brown rice (*Oryza sativus* L.) is commonly consumed around the world. It is generally processed to remove the germ layer and bran because they are very hard, difficult to cook, and poorly digested. However, these parts contain ingredients, such as ferulic acid, gamma-aminobutyric acid (GABA), vitamin B6, vitamin B12, and folic acid (Okuda et al. 2018). Ferulic acid is an antioxidant (Kanski et al. 2002), inhibitor of Aβ production and aggregation (Ono et al. 2005), and a β-secretase modulator (Mori et al. 2013). Additionally, it destabilizes preformed β-amyloid fibrils (Ono et al. 2005) and provides neuroprotection (Yan et al. 2001).

Highly water pressurized brown rice (HPBR) treatment increases the water absorbency of brown rice without losing nutrients and enhances its digestibility. Okuda et al. (2018) administered HPBR in 3-month-old male senescence-accelerated mouse-prone 8 (SAMP8) mice for 2 months. Findings revealed that HPBR significantly reduced the levels of $A\beta_{1-42}$ in the brain, improved motor function, and ameliorated cognitive dysfunction in an AD mouse model. Recently, whole grain brown rice is also reported for a slower gastric emptying rate, compared to white rice, which in part may explain for its low glycemic response Pletsch and Hamaker (2018).

HPBR can be used as a nutraceutical for preventing cognitive dysfunction syndrome in canines and felines. Being high in dietary fiber, it may block the absorption of sugar and fat from the GI tract and contributes to its beneficial effects in diabetes or obesity (Okuda et al. 2018).

5.5 Bioactive Substances from Invertebrates for Cognitive Dysfunction

5.5.1 Royal Jelly Proteins

Royal Jelly (RJ), the main food of the early larvae of worker bees (*Apis mellifera* L.) and the honeybee queen, is secreted by nurse honeybees from their hypopharyngeal and mandibular glands. The composition of RJ is complex, as it contains proteins, peptides, free amino acids, sugars, lipids, vitamins, and water (Lin et al. 2018; Mureşan et al. 2018). Major royal jelly proteins (MRJPs) are water-soluble proteins composed of nine members with a molecular weight of 49–87 kDa. Recently, Lin et al. (2018) developed an UPLC-MS method to quantify MRJP1, MRJP2, MRJP3, and other signature peptides that could have potential applications in many health conditions. The proposed method could also be used to evaluate the quality of MRJPs to avoid adulteration, which is quite common in the case of RJ proteins.

The MRJP1, designated as apalbumin 1, constitutes 45% of water-soluble proteins and is the most abundant protein in RJ. The RJ proteins have been used as functional foods and supplements for decades. It exerts antihypertensive,

Table 2 Nutraceuticals of non-plant origin for cognition and memory improvement

Nutraceutical	Bioactive ingredient	Biological/pharmacological activity	References
Royal jelly proteins from *Apis mellifera* L.	Major royal jelly protein 1	Prevention of cognition impairment	Chen et al. (2017), Lin et al. (2018), and Mureşan et al. (2018)
Anchovy (*Coilia mystus*	Anchovy protein hydrolysate	AChE inhibitory, antioxidative, neuroprotective, and memory and cognition improvement	Zhao et al. (2017b)
Brazilian red macroalgae extract (*Ochtodes secundiramea*)	Halogenated monoterpene	AChE inhibitory	Machado et al. (2015)
Melatonin	Melatonin	Antioxidative, anti-inflammatory, immunomodulatory, antidepressant, antiaging, anti-AD	Mishima et al. (1999), Wu et al. (2003), Obayashi et al. (2015) and Sharman, and Bondy (2016)

antidiabetic, anti-obesity, and increased cell proliferation activities in addition to regulation of mouse macrophage to release TNF-α (Yoshida et al. 2017; Lin et al. 2018). Reported studies also suggest that these proteins may improve cognition and memory (Drapeau et al. 2006; Zamani et al. 2012; Pyrzanowska et al. 2014; Chen et al. 2017). Chen et al. (2017) reported that aged male rats fed MRJP for 14 weeks showed improved spatial memory up to 48.5% when compared to controls. Metabolomic analysis using time-of-flight mass spectrometry revealed that the compounds altered were nicotinate and nicotinamide, cysteine taurine, and energy metabolism pathways (Table 2).

In a number of studies, bioactive peptides from RJ have been found to possess antihypertensive property (Matsui et al. 2002, Tokunaga et al. 2004; Sultana et al. 2008). Sultana et al. (2008) investigated the renin inhibitory effect of an RJ-derived peptide (dipeptide YY). The dipeptide YY was found to inhibit human renin activity. The inhibition constant (K_1) of YY was estimated to be 10 μM when the K_m was 0.16 μM using sheep angiotensinogen as the substrate. The peptide was observed to lower blood pressure in spontaneously hypertensive rats.

Yoshida et al. (2017) investigated whether RJ could prevent obesity and ameliorate hyperglycemia in type 2 diabetes. The findings indicated that administration of RJ (10 mg/kg, po) in KK-Ay mice for 4 weeks improved hyperglycemia, which may be due to suppression of G6Pase expression through the upregulation of *AdipoQ* and *AdipoR1* mRNA and pAMPK protein expressions. RJ did not influence insulin resistance or body weight.

The RJ dose as a supplement in human diets is typically 1 g per os (Khoshpey et al. 2016). This dose contains on average 120 mg of proteins, comprising 31% MRJP1 (~37 mg), 16% MRJP2 (~19 mg), and 26% MRJP3 (~31 mg). Approximately 87% of MRJP1 are in the oligomeric form (~32 mg), and 13% of MRJP1 are present as monomer (~5 mg). Royal jelly proteins have been shown to exert antioxidative, anti-inflammatory, and immunomodulatory effects (Šver et al. 1996; Kohno et al. 2004; Jamnik et al. 2007: It can be suggested that RJ proteins can be used to improve cognition and memory in canines and felines.

5.5.2 Anchovy Peptides

In addition to cholinergic system dysfunction and oxidative stress, apoptosis of neurons plays a pivotal role in the development of memory loss (Rosello et al. 2012). Recently, Zhao et al. (2017b) demonstrated that anchovy protein hydrolysate (APH) could attenuate scopolamine-induced memory deficits in mice by regulating AChE activity. These investigators identified two strong AChE-inhibiting peptides (Pro-Ala-Tyr-Cys-Ser, PAYCS, and Cys-Val-Gly-Ser-Tyr, CVGSY), which were also shown to increase cell viability and reduce LDH release, ROS production, malondialdehyde content, and the ratio of Bax/Bcl-2 of glutamate-induced apoptosis in PC12 cells, as well as increase of SOD and GSP-px activities. Both peptides protected the PC12 cells against glutamate-induced apoptosis via inhibiting ROS production and Ca^{2+} influx. Zhao et al. (2017b) suggested that PAYCS and CVGSY might be considered as nutraceuticals for alleviating memory deficits.

5.6 Bioactive Substances from Vertebrates for Cognitive Dysfunction

During the past several decades, venoms from several species are under investigation for the treatment of a variety of pathologies, including cardiovascular disorders, pain, cancer, and several neurodegenerative diseases (reviewed in DeSouza et al. 2018). Several toxins/substances have been shown to improve cognition and memory in humans and experimental models.

Fasciculins from *Dendroaspis angusticeps* (green mamba snake), RVV-V from *Daboia russelii russelii* (Indian viper), K-49-P1-20 peptide from *Bothrops asper* (viper), SVHRP from *Buthus martensii* Karsch (scorpion), PhTx3-1 and PhTx4-5-5 from *Phoneutria nigriventer* (spider), and $BVPLA_2$ from *Apis mellifera* (honey bee) are just a few

examples. These toxins improve cognition and memory via different mechanisms of action. For example, fasciculins inhibit AChE activity, RVV-V reduces Aβ plaque deposition, SVHRP increases BDNF levels and neurogenesis and reduces Aβ plaques, and $BVPLA_2$ reduces Aβ plaque deposition and exerts an anti-inflammatory activity. These toxins have a great potential and need to be tested in canine and feline cognition dysfunction (DeSouza et al. 2018).

5.7 Nutraceuticals for Blood-Brain Barrier Protection

In neurodegenerative diseases, such as AD, disruption of the blood-brain barrier (BBB) has been reported to be one of the pathophysiologies (reviewed in Gupta and Gupta 2018). Delivery across the BBB for a nutraceutical or a pharmaceutical compound is necessary in preventing or ameliorating the neurodegenerative diseases. Various in vitro and in vivo models are available to screen these compounds for crossing the BBB and negatively or positively influencing its structure and function (Gupta et al. 2015). Recently, Li et al. (2017) has suggested a zebrafish model for assessing the delivery of natural products and drugs across the BBB. Excessive ROS generation has been suggested to be one of the main mechanisms accounting for BBB dysfunction (reviewed in Li et al. 2018). Li et al. (2018) demonstrated that astragaloside IV (obtained from *Astragalus membranaceus*) pretreatment protected the BBB endothelial cells integrity against LPS-induced BBB disruption, by activating Nrf2 signaling pathway. Findings of this study suggested that astragaloside IV might be a potential antioxidant and neuroprotective nutraceutical/drug targeting BBB. In another study, Zhang et al. (2013) demonstrated that in an ischemia model, berberine upregulated pAkt, pGSK, pCREB, and claudin-5 and downregulated NF-κB expression, thereby ameliorating BBB permeability and providing neuroprotection.

5.8 Nutraceuticals for Mitochondrial Protection and Activation

The role of mitochondria in health, disease, and toxicity has been recognized for a long time. In addition to its primary function of ATP production (through oxidative phosphorylation), mitochondria plays a vital role in the modulation of apoptosis, autophagy, aging, and signaling pathways controlling neurogenesis and neuroplasticity (Morán et al. 2012; Go et al. 2018, Meyer et al. 2018; Wu et al. 2018; Yu et al. 2018). Mitochondrial dysfunction has been implicated in a number of neurodegenerative diseases, such as Alzheimer's disease (AD), Parkinson's disease (PD), and Huntington's disease (HD). Respiratory chain dysfunction (Maurer et al. 2000; Lin and Beal 2006; Morán et al. 2012), increase in ROS production and oxidative stress (Nunomura et al. 2006; Federico et al. 2012; Go et al. 2018), mitochondrial oxidative stress-induced hyperphosphorylation of tau (Melov et al. 2007), increased endoplasmic reticulum membranes (Schon and Area-Gomez 2013), overexpression of voltage-dependent anion channel 1 protein (Shoshan-Barmatz et al. 2018), and abnormal mitochondrial dynamics (Wang et al. 2009) have been reported in the pathogenesis of AD. Reddy and Beal (2008) also reported that the accumulation of mitochondrial DNA changes might increase ROS production and reduce mitochondrial ATP in an age-dependent manner, and this may contribute to AD development.

A number of natural and synthetic compounds have been shown to protect mitochondria through multiple mechanisms, thereby reducing cognition and memory impairment (Porquet et al. 2014; Risuleo 2016; Pitt and Leung 2016; Teodoro et al. 2016).

5.8.1 Resveratrol

Resveratrol, commonly referred to as "French Paradox," is a polyphenol naturally found in grapes and red wine. It exists in both *cis* and *trans* conformations. Resveratrol is reported to increase metabolic rate by mitochondrial biogenesis, and it has been postulated as an option to prevent AD (Jayasena et al. 2013; Porquet et al. 2014). Porquet et al. (2014) elucidated the interplay between the amyloidogenic pathway, sirtuin 1 (SIRT1) and 5-adenosine monophosphate-activated protein kinase (AMPK) signaling. Findings of this investigation supported the claim of resveratrol-induced neuroprotection by maintaining the equilibrium among SIRT1 and AMPK signaling, mitochondrial status, and the inflammatory changes. The onset of AD may be delayed or mitigated employing dietary resveratrol, which is able to prevent Aβ plaque formation and cognitive loss. Marambaud et al. (2005) demonstrated a proteasome-dependent anti-amyloidogenic activity of resveratrol but stated that resveratrol does not inhibit Aβ production because it has no effect on the Aβ-producing enzymes β- and γ-secretases. Instead, it promotes intracellular degradation of Aβ via a mechanism that involves the proteasome. In the context of AD, Teodoro et al. (2016) and Jardim (2018) summarized the neuroprotective effects of resveratrol as (1) to reduce intracellular and secreted levels of Aβ; (2) to reduce Aβ fibril formation by directly binding to Aβ; (3) to activate protein kinase C, which protects against Aβ-induced apoptosis; (4) to increase glutathione production, thereby contributing to antioxidant defense; and (5) to reduce NF-κB pathway activity, causing a decrease in inflammation and neuronal cell loss. It can be suggested that resveratrol has a strong therapeutic potential in AD in humans and CDS in pets. Currently, resveratrol is included in Senilife® for canines.

5.8.2 Quercetin

Quercetin is one of the most widely distributed flavonoids, which has strong antioxidant, free radical scavenging, and anti-inflammatory properties. In a number of in vitro, in silico, and in vivo studies, quercetin has been shown to exert multiple mechanisms of actions and improve learning and memory (Phachonpai et al. 2010; Islam et al. 2013; Sabogal-Guaqueta et al. 2015; Ay et al. 2016). Ghobeh et al. (2014) used quercetin and resveratrol to protect mitochondria from amyloid fragment fibrillogenesis. Quercetin destabilizes amyloid aggregates, thereby inhibiting the fibrillation process and rescuing the mitochondrial function, providing some optimistic insights into the prevention of AD.

Curcumin, vitamin E, *G. biloba*, *B. monnieri*, and medium-chain triglycerides (MCTs) may also protect mitochondria or elevate its function to prevent/improve cognition and memory impairment by different mechanisms (Ahmed 2012; Javeri and Chand 2016; Pitt and Leung 2016; Teodoro et al. 2016).

5.8.3 Thiamine and Its Derivatives

Thiamine-dependent processes are critical in glucose metabolism and are diminished in brains of AD. In a mouse model, it has also been demonstrated that thiamine deficiency causes the impaired hippocampal neurogenesis, which is greatly involved in cognitive dysfunction at early pre-pathological legion stage (Zhao et al. 2008). In animal models, thiamine deficiency is known to exacerbate plaque formation, promotes phosphorylation of tau, and impairs memory (Gibson et al. 2013). In contrast, treatment of mouse models of AD with the thiamine derivative benfotiamine diminishes plaques, decreases phosphorylation of tau, and reverses memory deficits. Another thiamine derivative is sulbutiamine (isobutyryl thiamine disulfide), which is often used to improve mental fatigue and memory. Sulbutiamine exerts its effects by acting on dopamine and no other catecholamines. Nootropic benefits of sulbutiamine are due to its effects on dopamine, glutamatergic, and cholinergic systems. Due to its higher absorption rate than thiamine, it acts to increase the efficiency of the BBB. It can be proposed that the use of benfotiamine or sulbutiamine could provide a safe intervention to reverse biological and clinical processes of CDS progression in canines and felines.

5.9 Therapeutic Diets

Currently, therapeutic diets/prescription diets, such as Canine b/d® (brain diet: fatty acids, antioxidants, and dl-α-lipoic acid, and L-carnitine), Purina Pro Plan Bright Mind® (medium-chain triglycerides), Senilife® (*G. biloba*, resveratrol, phosphatidylserine, vitamin E, and vitamin B6), Aktivait® (DHA, EPA, α-lipoic acid, glutathione, *N*-acetyl cysteine, vitamin C, vitamin E, L-carnitine, CoQ10, phosphatidylserine, and selenium), Novifit® (*S*-adenosylmethionine), and Neutricks® (apoaequorin), Nutramind®(omega-3 fatty acids, *G. biloba* extract, vitamin B1, vitamin B3, vitamin B6, vitamin B8, vitamin B12, vitamin E, and phosphatidylserine) are available for senior dogs with CCD/CDS. Nutramind® is also recommended for cats. Such diets are often fortified with a combination of fatty acids, vitamins, antioxidants, anti-inflammatory substances, mitochondrial cofactors, etc. In a number of studies, fortified and therapeutic diets have been shown to improve cognition and memory in senior dogs and cats (Cotman et al. 2002; Head 2007; Dodd et al. 2003; Ikeda-Douglas et al. 2004; Orlando 2018).

6 Concluding Remarks and Future Directions

A high percentage of senior dogs and cats suffer from cognitive dysfunction syndrome (CDS), and often they are underdiagnosed. The diagnosis of CDS is based on clinical signs (acronym DISHA) and cognitive dementia scale. The pathophysiology of CDS is very complex due to multiple underlying mechanisms, such as cholinergic neurotransmitter (ACh) deficit, oxidative stress, neuroinflammation, and Aβ peptides aggregation and deposition. Currently, several nutraceuticals are on the market for prevention and therapeutic intervention to improve or restore cognition and memory. This chapter describes the pathophysiology of CDS in canines and felines and offers various preventive and treatment modalities, including nutraceuticals that have anti-AChE, antioxidative, anti-inflammatory, anti-amyloidogenic, immunomodulatory, antidepressant, and anxiolytic properties. The chapter also provides a brief coverage of therapeutic diets available for prevention and treatment of CDS in canines and felines. Clinical trials are needed to validate claims for the most nutraceuticals available for canines and felines.

References

Abd El-Wahab AE, Ghareeb DA, Sarhan EEM et al (2013) *In vitro* biological assessment of *Berberis vulgaris* and its active constituent, berberine: antioxidant, anti-acetylcholinesterase, anti-diabetic and anticancer effects. BMC Compliment Altern Med 13:218–244

Adalier N, Parker H (2016) Vitamin E, turmeric and saffron in treatment of Alzheimer's disease. Antioxidants 5:40

Ahmed H (2012) Modulatory effects of vitamin E, acetyl-L-carnitine and α-lipoic acid on new potential biomarkers for Alzheimer's disease in rat model. Exp Toxicol Pathol 64(6):549–556

Akhondzadeh S, Shafiee SM, Harirchian MH et al (2010) A 22-week, multicenter, randomized, double-blind controlled trial of *Crocus sativus* in the treatment of mild-to-moderate Alzheimer's disease. Psychopharmacology 207:637–643

Asai M, Iwata N, Yoshikawa A et al (2007) Berberine alters the processing of Alzheimer's amyloid precursor protein to decrease Abeta secretion. Biochem Biophys Res Commun 352(2):498–502

Asian Ginseng (2016) National center for complementary and integrative health. US National Institutes of Health, Bethesda

Attele AS, Wu JA, Yuan CS (1999) Ginseng pharmacology: multiple constituents and multiple actions. Biochem Pharmacol 58 (11):1685–1693

Ay M, Charli A, Jin H et al (2016) Quercetin. In: Gupta RC (ed) Nutraceuticals: efficacy, safety and toxicity. Academic Press/ Elsevier, Amsterdam, pp 447–452

Ayaz M, Junaid M, Ullah F et al (2015) Comparative chemical profiling, cholinesterase inhibitions and anti-radicals properties of essential oils from *Polygonum hydropiper* L: a preliminary anti-Alzheimer's study. Lipids Health Dis 14:141

Azkona G, Garcia-Belenguer S, Chacon G et al (2009) Prevalence and risk factors of behavioral changes associated with age-related cognitive impairment in geriatric dogs. J Small Anim Pract 50:87–91

Bain MJ, Cliff KD, Ruehl WW (2001) Predicting behavioral changes associated with age-related cognitive impairment in dogs. J Am Vet Med Assoc 218:1792–1795

Balducci C, Forloni G (2018) Novel targets in Alzheimer's disease: a special focus on microglia. Pharmacol Res 130:402–413

Baumeister J, Barthel T, Geis KR et al (2008) Influence of phosphatidylserine on cognitive performance and cortical activity after induced stress. Nutr Neurosci 11:103–110

Bazinet RP, Layé S (2014) Polyunsaturated fatty acids and their metabolites in brain function and disease. Nat Rev Neurosci 15:771–785

Bharti VK, Malik JK, Gupta RC (2016) Ashwagandha: multiple health effects. In: Gupta RC (ed) Nutraceuticals: efficacy, safety and toxicity. Academic Press/Elsevier, Amsterdam, pp 717–733

Bhattacharya SK, Kumar A, Ghosal S (1995) Effects of glycowithanolides from *Withania somnifera* on an animal model of Alzheimer's disease and perturbed central cholinergic markers of cognition in rats. Phytother Res 9:110–113

Bhattacharya SK, Satyan KS, Ghosal S (1997) Antioxidant activity of glycowithanolides from *Withania somnifera*. Indian J Exp Biol 35 (3):236–239

Bittner DM (2009) Combination therapy of acetylcholinesterase inhibitor and vitamin E in Alzheimer disease. J Clin Psychopharmacol 29:511–513

Cai Z, Wang C, Yang W (2016) Role of berberine in Alzheimer's. Neuropsychiatr Dis Treat 12:2509–2520

Cespedes CL, Balbontin C, Avila JG et al (2017) Inhibition on cholinesterase and tyrosinase by alkaloids and phenolics from *Aristotelia chilensis leaves*. Food Chem Toxicol 109:984–995

Chan P, Xia Q, Fu P (2007) *Ginkgo biloba* leaves extract: biological, medicinal and toxicological effects. J Environ Sci Health C Environ Carcinog Ecotoxicol Rev 25:211–244

Chandra V, Pandav R, Dodge HH et al (2001) Incidence of Alzheimer's disease in a rural community in India. The Indo-US study. Neurology 57:985–989

Chen WF (2006) Curcumin and its analogues as potent inhibitors of low density lipoprotein oxidation. Free Radic Biol Med 40 (3):526–535

Chen F, Eckman EA, Eckman CB (2006) Reduction in levels of the Alzheimer's amyloid beta peptide after oral administration of ginsenosides. FASEB J 20:1269–1271

Chen CF, Chiou WF, Zhang JT (2008) Comparison of the pharmacological effects of *Panax ginseng* and *Panax quinquefolium*. Acta Pharmacol Sin 29:1103–1108

Chen D, Liu F, Wan J-B et al (2017) Effect of Royal jelly proteins on spatial memory in aged rats: metabolomics analysis in urine. J Agric Food Chem 65(15):3151–3159

Choi KT (2008) Botanical characteristics, pharmacological effects and medicinal components of Korean *Panax ginseng* C.A. Meyer. Acta Pharmacol Sin 29:1109–1118

Choudhary MI, Yousuf S, Nawaz SA et al (2004) Cholinesterase inhibiting withanolides from *Withania somnifera*. Chem Pharm Bull 52(11):1358–1361

Chun YS, Kim J, Chung S et al (2017) Protective roles of *Monsonia angustifolia* and its active compounds in experimental models of Alzheimer's disease. J Agric Food Chem 65:3133–3140

Cicero AFG, Fogacci F, Banach M (2018) Botanicals and phytochemicals active on cognitive decline: the clinical evidence. Pharmacol Res 130:204–212

Coppock RW, Dziwenka M (2016) Green tea extract. In: Gupta RC (ed) Nutraceuticals: efficacy, safety and toxicity. Academic Press/ Elsevier, Amsterdam, pp 633–652

Cortes N, Alvarez R, Osorio EH (2015) Alkaloid metabolite profiles by GC/MS and acetylcholinesterase inhibitory activities with binding-mode predictions of five *Amaryllidaceae* plants. J Pharm Biomed Anal 102:222–228

Cotman CW, Head E, Muggenburg BA et al (2002) Brain aging in the canine: a diet enriched in antioxidants reduces cognitive dysfunction. Neurobiol Aging 23(5):809–818

Crook TH, Tinklenberg J, Yesavage J et al (1991) Effects of phosphatidylserine in age-associated memory impairment. Neurology 41:644–649

D'Amilio M, Puglisi-Allegra S, Mercuri N (2018) The role of dopaminergic midbrain in Alzheimer's disease: translating basic science into clinical practice. Pharmacol Res 130:414–419

Danthiir V, Hosking DE, Nettelbeck T et al (2018) An 18-month randomized, double-blind, placebo-controlled trial of DHA-rich fish oil to prevent age-related cognitive decline in cognitively normal older adults. Am J Clin Nutr 107:754–762

Das A, Shanker G, Nath C et al (2002) A comparative study in rodents of standardized extracts of *Bacopa monnieri* and *Ginkgo biloba*: anticholinesterase and cognitive enhancing activities. Pharmacol Biochem Behav 73:893–900

DeFeudis F, Drieu K (2000) *Ginkgo biloba* extract (EGb 761) and CNS functions: basic studies and clinical applications. Curr Drug Targets 1:25–58

DeSouza JM, Goncalves BDC, Gomez MV et al (2018) Animal toxins as therapeutic tools to treat neurodegenerative diseases. Front Pharmacol 9:145

Ding J, Xi YD, Zhang DD et al (2013) Soybean isoflavones ameliorates β-amyloid 1-42-induced learning and memory deficit in rats by protecting synaptic structure and function. Synapse 67(12):856–864

Dodd CE, Zicker SC, Jewell DE et al (2003) Can a fortified food affect the behavioral manifestations of age-related cognitive decline in dogs. Vet Med 98:396–408

Dohi S, Terasaki M, Makino M (2009) Acetylcholinesterase inhibitory activity and chemical composition of commercial essential oil. J Agric Food Chem 57:4313–4318

Drapeau MD, Albert S, Kucharski R et al (2006) Evaluation of the yellow/major royal jelly protein family and the emergence of social behavior in honey bees. Genome Res 16:1385–1394

Dugoua JJ, Mills E, Perri D et al (2006) Safety and efficacy of ginkgo (*Ginkgo biloba*) during pregnancy and lactation. Can J Clin Pharmacol 13:e277–e284

Dysken MW, Sano M, Asthana S et al (2014) Effect of vitamin E and memantine on functional decline in Alzheimer disease. The TEAM-AD VA Cooperative Randomized Trial. JAMA 311:33–44

Dziwenka M, Coppock RW (2016) Ginkgo biloba. In: Gupta RC (ed) Nutraceuticals: efficacy, safety and toxicity. Academic Press/ Elsevier, Amsterdam, pp 681–691

El Idrissi A (2008) Taurine improves learning and retention in aged mice. Neurosci Lett 436:19–22

Federico A, Cardaioloi E, Da Pozzo P et al (2012) Mitochondria, oxidative stress and neurodegeneration. J Neurol Sci 322:254–262

Filho FO, Alcântra DB, Rodrigues THS et al (2018) Development and validation of a reversed phase HPLC method for determination of anacardic acids in cashew (Anacardium occidentale) nut shell liquid. J Chromatogr Sci 56(4):300–306

Galasko D (2006) Biological markers. In: Gauthier S (ed) Clinical diagnosis and management of Alzheimer's disease, 3rd edn. Informa Healthcare, Boca Raton, pp 125–133

Garcia-Alloza M, Borrelli LA, Rozkalne A et al (2007) Curcumin labels amyloid pathology *in vivo*, disrupts existing plaques, and partially restores distorted neurites in an Alzheimer mouse model. J Neurochem 102:1095–1104

Garvilova SI, Preuss UW, Wong JWM et al (2014) Efficacy and safety of *Ginkgo biloba* extract EGb 761 in mild cognitive impairment with neuropsychiatric symptoms: a randomized, placebo-controlled, double-blind, multi-center trial. In J Geriatr Psychiatry 29: 1087–1095

Gasca CA, Castillo WO, Takahashi CS et al (2017) Assessment of anti-cholinesterase activity and cytotoxicity of cagaita (Eugenia dysenterica) leaves. Food Chem Toxicol 109:996–1002

Geraldo E, Lloret A, Fuchsberger T et al (2014) Aβ and tau toxicities in Alzheimer's are linked via oxidative stress-induced P38 activation: protective role of vitamin E. Redox Biol 2:873–877

Geromichalos GD, Lamari FN, Papandreou MA et al (2012) Saffron as a source of novel acetylcholinesterase inhibitors: molecular docking and *in vitro* enzymatic studies. J Agric Food Chem 60:6131–6138

Ghadrdoost B, Vafaei AA, Rashidy-Pour A et al (2011) Protective effects of saffron extract and its active constituent crocin against oxidative stress and spatial learning and memory deficits induced by chronic stress in rats. Eur J Pharmacol 667:222–229

Ghaffari SH, Hatami H, Dehghan G (2015) Saffron ethanolic extract attenuates oxidative stress, spatial learning, and memory impairments induced by local injection of ethidium bromide. Res Pharm Sci 10:222–232

Ghobeh M, Ahmadian S, Meratan AA et al (2014) Interaction of $A\beta_{25-35}$ fibrillation products with mitochondria: effects of small molecule natural products. Biopolymers 102(6):473–486

Gibson GE, Hirsch JA, Cirio RT et al (2013) Abnormal thiamine-dependent processes in Alzheimer's disease: lessons from diabetes. Mol Cell Neurosci 55:17–25

Go Y-M, Fernandes J, Hu X et al (2018) Mitochondrial network responses in oxidative physiology and disease. Free Radic Biol Med 116:31–40

Gu MY, Kim J, Yang YO (2016) The neuroprotective effects Justicidin A on amyloid $beta_{25-35}$-induced neuronal cell death through inhibition of tau hyperphosphorylation and induction of autophagy in SH-SY5Y cells. Neurochem Res 41:1458–1467

Gupta RC, Dekundy A (2005) memantine does not influence AChE inhibition in rat brain by donepezil or rivastigmine but does with DFP. Drug Develop Res 64:71–81

Gupta RK, Gupta RC (2018) Biomarkers of blood-brain barrier dysfunction. In: Gupta RC (ed) Biomarkers in toxicology, 2nd edn. Academic Press/Elsevier, Amsterdam

Gupta RC, Pitt J, Zaja-Milatovic S (2015) Blood-brain barrier damage and dysfunction by chemical toxicity. In: Gupta RC (ed) Handbook of toxicology of chemical warfare agents. Academic Press/Elsevier, Amsterdam, pp 725–739

Gurley BJ, Fifer EK, Gardner Z (2012) Pharmacokinetic herb-drug interactions (Part 2): drug interactions involving popular botanical supplements and their clinical relevance. Planta Med 78:1490–1514

Hamaguchi T, Ono K, Yamada M (2010) Curcumin and Alzheimer's disease. CNS Neurosci Ther 16:285–297

Hampel H, Vergallo A, Aguilar LF et al (2018) Precision pharmacology for Alzheimer's disease. Pharmacol Res 130:331–365

Hansen MV, Danielsen AK, Hageman I et al (2014) The therapeutic or prophylactic effect of exogenous melatonin against depression and depressive symptoms: a systematic review and meta-analysis. Eur Neuropsychopharmacol 24:1719–1728

Hartsel JA, Eades J, Hickory B et al (2016) *Cannabis sativa* and Hemp. In: Gupta RC (ed) Nutraceuticals: efficacy, safety and toxicity. Academic Press/Elsevier, Amsterdam, pp 735–754

Head E (2007) Combining an antioxidant-fortified diet with behavioral enrichment leads to cognitive improvement and reduced brain pathology in aging canines: strategies for healthy aging. Ann N Y Acad Sci 1114:398–406

Hong SM, Soe KH, Lee TH et al (2017) Cognitive improving effects by highbush blueberry (Vaccinium corymbosum L.) vinegar on Scopolamine-induced amnesia mice model. J Agric Food Chem 66 (1):99–107

Hosmani R (2009) Neuroprotective efficacy of *Bacopa Monnieri* against rotenone induced oxidative stress and neurotoxicity in *Drosophila melanogaster*. Neurotoxicology 30:977–985

Hussein HM, Abd-Elmegied A, Ghareeb DA et al (2018) Neuroprotective effect of berberine against environmental heavy metals-induced neurotoxicity and Alzheimer's-like disease in rats. Food Chem Toxicol 111:432–444

Hwang S, Lim JW, Kim H (2017) Inhibitory effect of lycopene on amyloid-β-induced apoptosis in neuronal cells. Nutrients 9:883

Ihl R, Bachinskaya N, Korczyn AD et al (2011) Efficacy and safety of a once-daily formulation of *Ginkgo biloba* extract EGb 761 in dementia with neuropsychiatric features: a randomized controlled trial. Int J Geriatr Psychiatry 26:1186–1194

Ikeda-Douglas CJ, Zicker SC, Estrada J et al (2004) Prior experience, antioxidants, and mitochondrial cofactors improve cognitive dysfunction in aged beagles. Vet Ther 5:5–16

Imai H, Moriyasu K, Nakahata A et al (2017) Soy peptide ingestion augments the synthesis and metabolism of noradrenaline in the mouse brain. Biosci Biotechnol Biochem 81(5):1007–1013

Imran M, Ullah F, Ayaz M et al (2017) Anticholinesterase and antioxidant potentials of *Nonea micrantha* Bioss. And Reut along with GC-MS analysis. BMC Compliment Altern Med 17:499

Ingkaninan K, Phengpa P, Yuenyongsawad S et al (2006) Acetylcholinesterase inhibitors from *Stephania venosa* tuber. J Pharm Pharmacol 58(5):695–700

Islam MR, Zaman A, Jahan I et al (2013) *In silico* QSAR analysis of quercetin reveals its potential as therapeutic drug for Alzheimer's disease. J Young Pharm 5:173–179

Jamnik P, Goranovič D, Raspor P (2007) Antioxidative action of royal jelly in the yeast cell. Exp Gerontol 42:494–600

Jardim FR (2018) Resveratrol and brain mitochondria: a review. Mol Neurobiol 55(3):2085–2101

Javeri I, Chand N (2016) Curcumin. In: Gupta RC (ed) Nutraceuticals: efficacy, safety and toxicity. Academic Press/Elsevier, Amsterdam, pp 435–445

Jayasena T, Poljak A, Smythe G et al (2013) The role of polyphenols in the modulation of sirtuins and other pathways involved in Alzheimer's disease. Ageing Res Rev 12:867–883

Joffre C, Grégoire S, De Smelt V et al (2016) Modulation of brain PUFA content in different experimental models of mice. Prostaglandins Leukot Essent Fatty Acids 114:1–10

Joshi YB, Pratico D (2012) Vitamin E in aging, dementia, and Alzheimer's disease. Biofactors 38:90–97

Kamkaew N, Scholfield CN, Ingkaninan K et al (2011) *Bacopa monnieri* and its constituents is hypotensive in anesthetized rats and vasodilator in various artery types. J Ethnopharmacol 137:790–795

Kandel ER (2012) The molecular biology of memory: cAMP, PKA. CRE, CREB-1, CREB-2, and CPEB. Mol Brain 5:14

Kanski J, Aksenova M, Stoyanova A et al (2002) Ferulic acid antioxidant protection against hydroxyl and peroxyl radical oxidation in

synaptosomal and neuronal cell culture systems *in vitro*: structure-activity studies. J Nutr Biochem 13(5):273–281
Katayama S, Imai R, Sugiyama H et al (2014) Oral administration of soy peptides suppresses cognitive decline by induction of neurotrophic factors in SAMP8 mice. J Agric Food Chem 62:3563–3569
Kennedy DO, Scholey AB, Wesnes KA (2001) Dose dependent changes in cognitive performance and mood following acute administration of ginseng to healthy young volunteers. Nutr Neurosci 4:295–310
Khoshpey B, Djazayeri S, Amiri F et al (2016) Effect of royal jelly intake on serum glucose, apolipoprotein A-1 (ApoA-1), apolipoprotein B (ApoB) and ApoB/ApoA-1 ratios in patients with type 2 diabetes: a randomized, double-blind clinical trial study. Can J Diabetes 40:324–328
Kim Y-S, Woo Y-Y, Han C-K et al (2015) Safety analysis of *Panax Ginseng* in randomized clinical trials: a systematic review. Medicines 2(2):106–126
Kohno K, Okamoto I, Sano O et al (2004) Royal jelly inhibits the production of proinflammatory cytokines by activated macrophages. Biosci Biotechnol Biochem 68:138–145
Kuboyama T, Tohda C, Komatsu K (2006) Withanoside IV and its active metabolite, sominone, attenuate $A\beta_{25-35}$-induced neurodegeneration. Eur J Neurosci 23(6):1417–1426
Kulkarni SK, Dhir A (2008) On the mechanism of antidepressant-like action of berberine chloride. Eur J Pharmacol 589(1–3):163–172
Kulkarni SK, Dhir A (2010) Berberine: a plant alkaloid with therapeutic potential for central nervous system disorders. Phytother Res 24 (3):317–324
Kumar A, Chopra EK, Mukherjee M et al (2015) Current knowledge and pharmacological profile of berberine: an update. Eur J Pharmacol 761:288–297
Landsberg G (2005) Therapeutic agents for the treatment of cognitive dysfunction syndrome in senior dogs. Progr Neropsychopharmacol Biol Psychiatry 29:471–479
Landsberg GM, Araujo JA (2005) Behavior problems in geriatric pets. Vet Clin North Am Small Anim Pract 35(3):675–698
Landsberg GM, Hunthausen W, Ackerman L (2003) The effect of aging on the behavior of senior pets. In: Landsberg GM, Hunthausen W, Ackerman W (eds) Handbook of behavior problems of the dog and cat. Saunders, Edinburgh, pp 269–304
Landsberg GM, Nichol J, Araujo JA (2012) Cognitive dysfunction syndrome: a disease of canine and feline brain aging. Vet Clin North Am Small Anim Pract 42:749–768
Layé S, Nadjar A, Joffre C et al (2018) Anti-inflammatory effects of omega-3 fatty acids in the brain: physiological mechanisms and relevance to pharmacology. Pharmacol Rev 70:12–38
Le XT, Pham HTN, Van Nguyen T et al (2015) Protective effects of *Bacopa monnieri* on ischemia-induced cognitive deficits in mice: the possible contribution of bacopaside I and underlying mechanism. J Ethnopharmacol 164:37–45
Lebars PL, Katz MM, Berman N et al (1997) A placebo-controlled, double blinded, randomized trial of an extract of *Ginkgo biloba* for dementia. North American EGb Study Group. JAMA 278:1327–1332
Lee Y, Jin Y, Lim W et al (2003) A ginsenoside-Rh1, a component of ginseng saponin, activates estrogen receptor in human breast carcinoma MCF-7 cells. J Steroid Biochem Mol Biol 84:463–468
Lee HA, Kim JE, Sung JE et al (2018) *Asparagus cochinchinensis* stimulates release of nerve growth factor and abrogates oxidative stress in the Tg2576 model for Alzheimer's disease. BMC Compliment Altern Med 18:125
Leuner B, Kozorovitskiy Y, Gross CG et al (2007) Diminished adult neurogenesis in the marmoset brain precedes old age. Proc Natl Acad Sci USA 104:17169–17173
Li Q, Zhao HF, Zhang ZF et al (2009) Long-term green tea catechin administration prevents spatial learning and memory impairment in senescence-accelerated mouse prone-8 mice by decreasing a beta (1-42) oligomers and up-regulating synaptic plasticity-related proteins in the hippocampus. Neuroscience 163:741–749
Li NJ, Zhou L, Li W et al (2015) Protective effects of ginsenosides Rg1 and Rb1 on an Alzheimer's disease mouse model: a metabolomic study. J Chromatogr B Anal Technol Biomed Life Sci 985:54–61
Li Y, Chen T, Miao X et al (2017) Zebrafish: a promising *in vivo* model for assessing the delivery of natural products, fluorescence dyes and drugs across the blood-brain barrier. Pharmacol Res 125:246–257
Li H, Wang P, Huang F et al (2018) Astragaloside IV protects blood-brain barrier integrity from LPS-induced disruption via activating Nrf2 antioxidant signaling pathway in mice. Toxicol Appl Pharmacol 340:58–66
Lim GP, Chu T, Yang F et al (2001) The curry spice curcumin reduces oxidative damage and amyloid pathology in an Alzheimer transgenic mouse. J Neurosci 21:8370–8477
Limpeanchob N, Jaipan S, Rattanakaruna S et al (2008) Neuroprotective effect of *Bacopa monnieri* on beta-amyloid-induced cell death in primary cortical culture. J Ethnopharmacol 120:112–117
Lin MT, Beal MF (2006) Mitochondrial dysfunction and oxidative stress in neurodegenerative diseases. Nature 443:787–795
Lin N, Chen S, Zhang H et al (2018) Quantification of major royal jelly protein 1 in fresh royal jelly by ultraperformance liquid chromatography-Tandem mass spectrometry. J Agric Food Chem 66:1270–1278
Liu L, Hoang-Gia T, Wu H et al (2011) Ginsenoside Rb1 improves spatial learning and memory by regulation of cell genesis in the hippocampal subregions of rats. Brain Res 1382:147–154
Ma W, Ding B, Yu H et al (2015) Genistein alleviates β-amyloid-induced inflammatory damage through regulating toll-like receptor 4/nuclear factor κB. J Med Food 18(3):273–279
Machado LP, Caralho LR, Young MCM et al (2015) Evaluation of acetylcholinesterase inhibitory activity of Brazilian red macroalgae organic extract. Rev Bras Farmacogn 25:657–662
Madaria A, Farbakova J, Katina S et al (2015) Assessment of severity and progression of canine cognitive dysfunction using the Canine Dementia Scale (CADES). Appl Anim Behav Sci 171:138–145
Maebuchi M, Kishi Y, Koikeda T et al (2013) Soy peptide dietary supplementation increases serum dopamine level and improves cognitive dysfunction in subjects with mild cognitive impairment. Jpn Pharmacol Ther 41:67–73
Maheshwari RK, Singh AK, Gaddipati J et al (2006) Multiple biological activities of curcumin: a short review. Life Sci 78:2081–2087
Marambaud P, Zhao H, Davies P (2005) Resveratrol promotes clearance of Alzheimer's disease amyloid-beta peptides. J Biol Chem 280:37377–37382
Marcelli S, Ficulle E, Oiccolo L et al (2018) An overview of the possible therapeutic role of SUMOylation in the treatment of Alzheimer's disease. Pharmacol Res 130:420–437
Matsui T, Yukiyoshi A, Doi S et al (2002) Gastrointestinal enzyme production of bioactive peptides from royal jelly protein and their antihypertensive ability in SHR. J Nutr Biochem 13:80–86
Maurer I, Zierz S, Möller HJ (2000) A selective defect of cytochrome c oxidase is present in brain of Alzheimer disease patients. Neurobiol Aging 21:455–462
Mazzio E, Deiab S, Park K et al (2013) High throughput screening to identify natural human monoamine oxidase B inhibitors. Phytother Res 27(6):818–828
Melov S, Adlard PA, Morten K et al (2007) Mitochondrial oxidative stress causes hyperphosphorylation of tau. PLoS One 2:536
Meyer JN, Hartman JH, Mello DF (2018) Mitochondrial toxicity. Toxicol Sci 162(1):15–23
Milgram NW, Head E, Zicker SC et al (2005) Learning ability in aged beagle dogs is preserved by behavioral enrichment and dietary fortification: a two-year longitudinal study. Neurobiol Aging 26 (1):77–90

Milgram NW, Landsberg G, Merrick D et al (2015) A novel mechanism for cognitive enhancement in aged dogs with the use of a calcium-buffering protein. J Vet Behav Clin Appl Res 10(3):217–222

Mishima K, Tozawa T, Satoh K et al (1999) Melatonin secretion rhythm disorders in patients with senile dementia of Alzheimer's type with disturbed sleep-waking. Biol Psychiatry 45(4):417–421

Moffat KS, Landsberg GM (2003) An investigation of the prevalence of clinical signs of cognitive dysfunction syndrome (CDS) in cats. JAAHA 39(5):512

Mohanta T, Tamboli Y, Zubaidha P (2014) Phytochemical and medicinal importance of *Ginkgo biloba* L. Nat Prod Res 28:746–752

Momtaz S, Hassani S, Khan F et al (2018) Cinnamon, a promising prospect towards Alzheimer's disease. Pharmacol Res 130:241–258

Morán M, Moreno-Lastres D, Marín-Buera L et al (2012) Mitochondrial respiratory chain dysfunction: implications in neurodegeneration. Free Radic Biol Med 53:595–609

Moran DL, Marone PA, Bauter MR et al (2013) Safety assessment of apoaequorin, a protein preparation: subchronic toxicity study in rats. Food Chem Toxicol 57:1–10

Moran DL, Tetteh AO, Goodman RE et al (2014) Safety assessment of the calcium-binding protein, apoaequorin, expressed by *Escherichia* coli. Regul Toxicol Pharmacol 69(2):243–249

Morgan A, Stevens J (2010) *Bacopa monnieri* improve memory performance in older persons? Results of a randomized, placebo-controlled, double-blind trial. J Altern Complement Med 16:753–759

Morgese MG, Schiavone S, Mhillaj E et al (2018) N-3 PUFA diet enrichment prevents amyloid beta-induced depressive-like phenotype. Pharmacol Res 129:526–534

Mori T, Koyama N, Guillot-Sestier MV et al (2013) Ferulic acid is a nutraceutical β-secretase modulator that improves behavioral impairment and Alzheimer-like pathology in transgenic mice. PLoS One 8(2):e55774

Morris MC, Evans DA, Tangney CC et al (2005) Relation of the tocopherol forms to incident Alzheimer disease and to cognitive change. Am J Clin Nutr 81:508–514

Morris MC, Schneider JA, Li H et al (2015) Tocopherols relation to Alzheimer disease neuropathology in humans. Alzheimers Dement 11:32–39

Mureşan CI, Schierhorn A, Buttstedt A (2018) The fate of major royal jelly proteins during proteolytic digestion in the human gastrointestinal tract. J Food Agric Chem 66:4164–4170

Nagahara AH, Merrill DA, Coppola G et al (2009) Neuroprotective effects of brain-derived neurotrophic factor in rodent and primate models for Alzheimer's disease. Nat Med 15:331–337

Neilsen JC, Hart BL, Cliff KD et al (2001) Prevalence of behavioral changes associated with age-related cognitive impairment in dogs. J Am Vet Med Assoc 218:1787–1791

Nunomura A, Castellani RJ, Zhu X et al (2006) Involvement of oxidative stress in Alzheimer disease. J Neuropathol Exp Neurol 65:631–641

Obayashi K, Saeki K, Iwamoto J et al (2015) Physiological levels of melatonin relate to cognitive function and depressive symptoms: the HEIJO-KYO cohort. J Clin Endocrinol Metab 100(8):3090–3096

Okuda M, Fijita Y, Katsube T et al (2018) Highly water pressurized brown rice improves cognitive dysfunction in senescence-accelerated mouse prone 8 and reduces amyloid beta in the brain. BMC Compliment Altern Med 18:110

Olajide OJ, Yawson EO, Gbadamosi IT et al (2017) Ascorbic acid ameliorates behavioral deficits and neuropathological alterations in rat model of Alzheimer's disease. Environ Toxicol Pharmacol 50:200–211

Ono K, Hirohata M, Yamada M et al (2005) Ferulic acid destabilizes preformed β-amyloid fibrils *in vitro*. Biochem Biophys Res Commun 336:444–449

Opii WO, Joshi G, Head E et al (2008) Proteomic identification of brain proteins in the canine model of human aging following a long-term treatment with antioxidants and a program of behavioral enrichment: relevance to Alzheimer's disease. Neurobiol Aging 29(1):51–70

Orhan I, Şenol FS, Gülpinar AR et al (2009) Acetylcholinesterase inhibitory and antioxidant properties of *Cyclotrichium niveum*, *Thymus praecox* subsp, *caucasicus* var. *caucasicus*, *Echinacea purpurea* and *E. pallida*. Food Chem Toxicol 47:1304–1310

Orhan IE, Senol FS, Shekfeh S et al (2017) Pteryxin-A promising butyrylcholinesterase-inhibiting coumarin derivative from *Muttelina purpurea*. Food Chem Toxicol 109:970–974

Orlando JM (2018) Behavioral nutraceuticals and diets. Vet Clin North Am Small Anim Pract 48(3):473

Orr SK, Trépanier MO, Bazinet RP (2013) n-3 Polyunsaturated fatty acids in animal models with neuroinflammation. Prostaglandins Leukot Essent Fatty Acids 88:97–103

Osella MC, Re G, Odore R et al (2007) Canine cognitive dysfunction syndrome: prevalence, clinical signs and treatment with a neuroprotective nutraceutical. Appl Anim Behav Sci 105:297–310

Pan Y (2011) Enhancing brain function in senior dogs: a new nutritional approach. Top Companion Anim Med 26(1):10–16

Pan Y, Larson B, Araujo JA et al (2010) Dietary supplementation with medium-chain TAG has long-lasting cognition-enhancing effects in aged dogs. Br J Nutr 103:1746–1754

Papandreou MA, Kanakis CD, Polissiou MG et al (2006) Inhibitory activity on amyloid-β-aggregation and antioxidant properties of *Crocus sativus* stigmas extract and its crocin constituents. J Agric Food Chem 54:8762–8768

Pedata F, Giovannelli L, Spignoli G et al (1985) Phosphatidylserine increases acetylcholine release from cortical slices in aged rats. Neurobiol Aging 6:337–339

Peng WH, Wu CR, Chen CS et al (2004) Anxiolytic effect of berberine on exploratory activity of the mouse in two experimental anxiety models, interaction with drugs acting at 5-HT receptors. Life Sci 75:2451–2462

Peng WH, Lo KL, Lee YH et al (2007) Berberine produces antidepressant-like effects in the forced swim test and in the tail suspension test in mice. Life Sci 81(11):933–938

Peng XR, Wang X, Dong JR et al (2017) Rare hybrid dimers with anti-acetylcholinesterase activities from a Safflower (*Carthamus tinctorius* L.) seed oil cake. J Agric Food Chem 65(43):9453–9459

Peng M, Yi YX, Zhang T et al (2018) Stereoisomers of saponins in *Panax notoginseng* (Sanqi): a review. Front Pharmacol 9:188

Perry NSL, Houghton P, Theobald A et al (2000) *In vitro* inhibition of human erythrocyte acetylcholinesterase by *Sativa lavandulaefolia* essential oil and constituent terpenes. J Pharm Pharmacol 52:895–902

Perry NSL, Bollen C, Perry EK et al (2003) *Salvia* for dementia therapy: review of pharmacological activity and pilot tolerability clinical trial. Pharmacol Biochem Behav 75:651–659

Phachonpai W, Wattanathorn J, Muchimapura S et al (2010) Neuroprotective effect of quercetin encapsulated liposomes: a novel therapeutic strategy against Alzheimer's disease. Am J Appl Sci 7(4):480–485

Pitt J, Leung Y (2016) Cognitive effects of nutraceuticals. In: Gupta RC (ed) Nutraceuticals: efficacy, safety and toxicity. Academic Press/Elsevier, Amsterdam, pp 29–48

Pitt J, Leung Y (2018) Biomarkers of Alzheimer's disease. In: Gupta RC (ed) Biomarkers in toxicology. Academic Press/Elsevier, Amsterdam. In press

Piyabhan P, Wannasiri S, Naowaboot J (2016) *Bacopa Monnieri* (Brahmi) improved novel object recognition task and increased cerebral vesicular glutamate transporter type 3 in sub-chronic phencyclidine rat model of schizophrenia. Clin Exp Pharmacol Physiol 43 (12):1234–1242

Pletsch EA, Hamaker BR (2018) Brown rice compared to white rice slows gastric emptying in humans. Eur J Clin Nutr 72(3):367–373
Porquet D, Griñán-FerréC FI et al (2014) Neuroprotcive role of trans-resveratrol in murine model of familial Alzheimer's disease. J Alzheimers Dis 42:1209–1220
Pyrzanowska J, Piechal A, Blecharz-Klin K et al (2014) Long-term administration of Greek royal jelly improves spatial memory and influences the concentration of brain neurotransmitters in naturally aged Wistar male rats. J Ethnopharmacol 155:343–351
Qin L, Wu X, Block ML et al (2007) Systemic LPS causes chronic neuroinflammation and progressive neurodegeneration. Glia 55:453–462
Qin L, Liu Y, Hong JS et al (2013) NADPH oxidase and aging drive microglial activation, oxidative stress, and dopaminergic neurodegeneration following systemic LPS administration. Glia 61:855–868
Ramirez-Rodriguez G, Ortiz-Lopez L, Dominguez-Alonso A et al (2012) Chronic treatment with melatonin stimulates dendritic maturation and complexity in adult hippocampal neurogenesis of mice. J Pineal Res 50:29–37
Ranjan KE, Singh HK, Parkavi A et al (2011) Attenuation of 1-(m-chlorophenyl)-biguanide induced hippocampus-dependent memory impairment by a standardized extract of *Bacopa monnieri* (BESEB CDRI-08). Neurochem Res 36:2136–2144
Reddy PH, Beal MF (2008) Amyloid beta, mitochondrial dysfunction and synaptic damage: implications for cognitive decline in aging and Alzheimer's disease. Trends Mol Med 14:45–53
Reichling J, Frater-Schröder M, Herzog K et al (2006) Reduction of behavioral disturbances in elderly dogs supplemented with a standardized Ginkgo leaf extract. Schweiz Arch Tierheilkd 148 (5):257–263
Reza ASM, Hossain MS, Akhter S et al (2018) *In vitro* antioxidant and cholinesterase inhibitory activities of *Elatostemma papillosum* leaves and correlation with their phytochemical profiles: a study relevant to the treatment of Alzheimer's disease. BMC Complement Altern Med 18:123
Risuleo G (2016) Resveratrol: multiple activities on the biological functionality of the cell. In: Gupta RC (ed) Nutraceuticals: efficacy, safety and toxicity. Academic Press/Elsevier, Amsterdam, pp 453–464
Rofina JE, Van Ederen AM, Toussaint MJM et al (2006) Cognitive disturbances in old dogs suffering from the canine counterpart of Alzheimer's disease. Brain Res 1069:216–226
Rong H, Liang Y, Niu Y (2018) Rosmarinic acid attenuates β-amyloid-induced oxidative stress via Akt/GSK-3β/Fyn-mediated Nrf2 activation in PC2 cells. Free Radic Biol Med 120:114–123
Rosello A, Warnes G, Meier UC (2012) Cell death pathways and autophagy in the central nervous system and its involvement in neurodegeneration, immunity and central nervous system infection: to die or not to die-that is the question. Clin Exp Immunol 168:52–57
Sabogal-Guaqueta AM, Carrillo-Hormaza L, Osorio E et al (2015) Effects of bioflavonoids from *Garcinia madruno* on a triple transgenic mouse model of Alzheimer's disease. Pharmacol Res 129:128–138
Sabogal-Guaqueta AM, Munoz-Manco JI, Ramirez-Pineda JR et al (2018) The flavonoid quercetin ameliorates Alzheimer's disease pathology and protects cognitive and emotional function in aged triple transgenic Alzheimer's disease model mice. Neuropharmacology 93C:134–145
Sadhukhan P, Saha S, Dutta S et al (2018) Nutraceuticals: an emerging therapeutic approach against the pathogenesis of Alzheimer's disease. Pharmacol Res 129:100–114
Saini N, Singh D, Sandhir R (2012) Neuroprotective effects of *Bacopa monnieri* in experimental model of dementia. Neurochem Res 37:1928–1937
Salvin HE, McGreevy PD, Sachdev PS et al (2010) Under diagnosis of canine cognitive dysfunction: a cross-sectional survey of older companion dogs. Vet J 184(3):277–281
Salvin HE, McGreevy PD, Sachdev PS et al (2011) The canine cognitive dysfunction rating scale (CCDR): a data-driven and ecologically relevant assessment tool. Vet J 188:331–336
Schliebs R, Liebmann A, Bhattacharya SK et al (1997) Systemic administration of defined extracts from *Withania somnifera* (Indian ginseng) and Shilajit differentially affects cholinergic but not glutamatergic and GABAergic markers in rat brain. Neurochem Int 30(2):181–190
Schmidt F, Boltze J, Jager C et al (2015) Detection and quantification of β-amyloid, pyroglutamil Aβ, and tau in aged canines. J Neuropathol Exp Neurol 74(9):912–923
Scholey AB, Kennedy DO (2002) Acute, dose-dependent cognitive effects of *Ginkgo biloba*, *Panax ginseng* and their combination in healthy young volunteers: differential interactions with cognitive demand. Hum Psychopharmacol 17:35–44
Schon EA, Area-Gomez E (2013) Mitochondria-associated ER membranes in Alzheimer's disease. Mol Cell Neurosci 55:26–36
Schütt T, Toft N, Berendt M (2015) Cognitive function, progression of age-related behavioral changes, biomarkers, and survival in dogs more than 8 years old. J Vet Intern Med 29(6):1569–1577
Sechi S, Chiavolelli F, Spissu N et al (2015) An antioxidant dietary supplement improves brain-derived neurotrophic factor levels in serum of aged dogs: preliminary results. J Vet Med 2015:412501. https://doi.org/10.1155/2015/412501
Seibert L (2017) Management of dogs and cats with cognitive dysfunction. Today's. Vet Pract 7(5):1–8
Sen A, Hongpaisan J (2018) Hippocampal microvasculature changes in association with oxidative stress in Alzheimer's disease. Free Radic Biol Med 120:192–203
Seo EJ, Fischer N, Efferth T (2018) Phytochemicals as inhibitors of NF-kappa B for treatment of Alzheimer's disease. Pharmacol Res 129:262–273
Serrano MAR, Pivatto M, Francisco W et al (2010) Acetylcholinesterase inhibitory pyridine alkaloids of the leaves of *Senna multijuga*. J Nat Prod 73:482–484
Sharman EH, Bondy SC (2016) Melatonin: a safe nutraceutical and clinical agent. In: Gupta RC (ed) Nutraceuticals: efficacy, safety and toxicity. Academic Press/Elsevier, Amsterdam, pp 501–509
Shoshan-Barmatz V, Nahon-Crystal E, Shteinfer-Kuzmine A, Gupta R (2018) VDAC1, mitochondrial dysfunction, and Alzheimer's disease. Pharmacol Res 131:87–101
Sigurdsson S, Gudbjarnason S (2007) Inhibition of acetylcholinesterase by extracts and constituents from *Angelica archangelica* and *Geranium sylvaticum*. Z Naturforsch C 62(9–10):689–693
Skoumalova A, Rofina J, Schwippelova Z et al (2003) The role of free radicals in canine counterpart of senile dementia of the Alzheimer type. Exp Gerontol 38(6):711–719
Smith CC, McMahon LL (2005) Estrogen-induced increase in the magnitude of long-term potentiation occurs only when the ratio of NMDA transmission to AMPA transmission is increased. J Neurosci 25:7780–7791
Sorrenti V, Contarini G, Sut S et al (2018) Curcumin prevents acute neuroinflammation and long-term memory impairment induced by systemic lipopolysaccharide in mice. Front Pharmacol 9:183
Stough C, Lloyd J, Clarke J et al (2001) The chronic effects of an extract of *Bacopa monnieri* (Brahmi) on cognitive function in healthy human subjects. Psychopharmacology (Berlin) 156:481–484
Sultana A, Nurun Nabi AHM, Nasir UM et al (2008) A dipeptide YY derived from royal jelly proteins inhibits renin activity. Int J Mol Med 21:677–681
Šver L, Oršolić N, Tadić Z et al (1996) A royal jelly as a new potential immunomodulator in rats and mice. Comp Immunol Microbiol Infect Dis 19:31–38

Taguchi R, Hatayama K, Takahashi T et al (2017) Structure-activity relations of rosmarinic acid derivatives for the amyloid β aggregation inhibition and antioxidant properties. Eur J Med Chem 138:1066–1075

Taha AY, Henderson ST, Burnham MW (2009) Dietary enrichment with medium chain triglycerides (AC-1203) elevates polyunsaturated fatty acids in the parietal cortex of aged dogs: implications for treating age-related cognitive decline. Neurochem Res 34 (9):1619–1625

Tan X-S, Ma J-Y, Feng R et al (2013) Tissue distribution of berberine and its metabolites after oral administration in rats. PLoS One 8(10): e77969

Teodoro JS, Duarte FV, Rolo AP, Palmeira CM (2016) Mitochondria as a target for safety and toxicity evaluation of nutraceuticals. In: Gupta RC (ed) Nutraceuticals: efficacy, safety and toxicity. Academic Press/Elsevier, Amsterdam, pp 387–400

Terry AV, Buccafusco JJ (2006) The cholinergic hypothesis of age and Alzheimer's disease related cognitive deficits: recent challenges and their implications for novel drug development. J Pharmacol Exp Ther 306:821–827

Tokunaga K, Yoshida C, Suzuki K et al (2004) Antihypertensive effect of peptide from royal jelly in spontaneously hypertensive rats. Biol Pharm Bull 27:189–192

Trevisan MTS, Macedo FW, Meent M et al (2003) Screening for acetylcholinesterase inhibitors from plants to treat Alzheimer's disease. Quím Nova 26(3):301–304

Uddin MJ, Abdullah-Al-Mamun M, Biswas K et al (2016) Assessment of anticholinesterase activities and antioxidant potentials of *Anisomeles indica* relevant to the treatment of Alzheimer's disease. Orient Pharm Exp Med 16:113–121

Uddin MJ, Ali Reza ASM, Abdullah-Al-Mamun M et al (2018) Antinociceptive and anxiolytic and sedative effects of methanol extract of *Anisomeles indica*: an experimental assessment in mice and computer aided models. Front Pharmacol 9:246

Ude C, Schubert-Zsilavecz M, Wurglics M (2013) *Ginkgo biloba* extracts: a review of the pharmacokinetics of the active ingredients. Clin Pharmacokinet 52:727–749

Vedin I, Cederholm T, Freund Levi Y et al (2008) Effects of docosahexaenoic acid-rich n-3 fatty acid supplementation on cytokine release from blood mononuclear leukocytes: the Omega AD study. Am J Clin Nutr 87:1616–1622

Velaga MK, Basuri CKR, Taylor KS et al (2014) Ameliorative effects of *Bacopa monnieri* on lead-induced oxidative stress in different regions of rat brain. Drug Chem Toxicol 37:357–364

Vite CH, Head E (2014) Aging in the canine and feline brain. Vet Clin North Am Small Anim Pract 44:1113–1129

Wang F, Zhao G, Cheng L et al (2004) Effects of berberine on potassium currents in acutely isolated CA1 pyramidal neurons of rat hippocampus. Brain Res 999:91–97

Wang X, Wang R, Xing D et al (2005) Kinetic difference of berberine between hippocampus and plasma in rat after intravenous administration of *Coptidis rhizoma* extract. Life Sci 77(24):3058–3067

Wang X, Su B, Zheng L et al (2009) The role of abnormal mitochondrial dynamics in the pathogenesis of Alzheimer's disease. J Neurochem 109:153–159

Wang M, Zhao J, Avula B et al (2014) High-resolution gas chromatography/mass spectrometry method for characterization and quantitative analysis of ginkgolic acids in *Ginkgo biloba* plants, extracts, and dietary supplements. J Agric Food Chem 62: 12103–12111

Wee JJ, Mee PK, Chung AS (2011) Biological activities of ginseng and its application to human health. In: Benzie IFF, Wachtel-Galor S (eds) Herbal medicine: biomolecular and clinical aspects. CRC Press, Boca Raton

Wevers A (2011) Localization of pre- and postsynaptic cholinergic markers in the human brain. Behav Brain Res 221:341–355

Wu YH, Feenstra MG, Zhou JN et al (2003) Molecular changes underlying reduced pineal melatonin levels in Alzheimer's disease: alterations in preclinical and clinical stages. J Clin Endocrinol Metab 88(12):5898–5906

Wu D, Wang X, Sun H (2018) The role of mitochondria in cellular toxicity as a potential drug target. Cell Biol Toxicol 34:87–91

Xiang C-P, Han J-X, Li X-C et al (2017) Chemical composition and acetylcholinesterase inhibitory activity of essential oils from Piper species. J Agric Food Chem 65:3702–3710

Xiao Q, Wang C, Li J et al (2010) Ginkgolide B protects hippocampal neurons from apoptosis induced by beta-amyloid 25-35 partly via up-regulation of brain derived neurotrophic factor. Eur J Pharmacol 647:48–54

Xing YZ, Shang HC, Gao XM et al (2008) A comparison of the ancient use of ginseng in traditional Chinese medicine with modern pharmacological experiments and clinical trials. Phytother Res 22:851–858

Yan JJ, Cho JY, Kim HS et al (2001) Protection against beta-amyloid peptide toxicity *in vivo* with long-term administration of ferulic acid. Br J Pharmacol 133(1):89–96

Yan X, Tang J, dos Santos Passos C et al (2015) Characterization of lignanamides from hemp (*Cannabis sativa* L.) seed and their antioxidant and acetylcholinesterase inhibitory activities. J Agric Food Chem 63:10611–10619

Yang MS, Wu MY (2016) Chinese ginseng. In: Gupta RC (ed) Nutraceuticals: efficacy, safety and toxicity. Academic Press/ Elsevier, Amsterdam, pp 693–705

Yatin SM, Varadarajan S, Butterfield DA (2000) Vitamin E prevents Alzheimer's amyloid β-peptide (1-42)-induced neuronal protein oxidation and reactive oxygen species production. J Alzheimers Dis 2:123–131

Ye M, Fu S, Pi R et al (2009) Neuropharmacological and pharmacokinetic properties of berberine: a review of recent research. J Pharm Pharmacol 61:831–837

Yoo KY, Hwang IK, Lim BO et al (2006) Berberry extract reduces neuronal damage and *N*-methyl-*D*-aspartate receptor 1 immunoreactivity in the gerbil hippocampus after transient forebrain ischemia. Biol Pharm Bull 29:623–628

Yoshida M, Hayashi K, Watadani R et al (2017) Royal jelly improves hyperglycemia in obese/diabetic KK-Ay mice. J Vet Med Sci 79 (2):299–307

Yu H, Wang D, Zou L et al (2018) Proteomic alterations of brain subcellular organelles caused by low-dose copper exposure: implication for Alzheimer's disease. Arch Toxicol 92:1363–1382

Zamani Z, Reisi P, Alaei H et al (2012) Effect of royal jelly on special learning and memory in rat model of streptozotocin-induced sporadic Alzheimer's disease. Adv Biomed Res 1:26

Zhang J, Yang JQ, He BC et al (2009) Berberine and total base from rhizoma *Coptis chinensis* attenuate brain injury in an aluminum-induced rat model of neurodegenerative disease. Saudi Med J 30 (6):760–766

Zhang X, Zhang X, Wang C et al (2013) Neuroprotection of early and short-time applying berberine in the acute phase of cerebral ischemia: up-regulated pAkt, pGSK and pCREB, down-regulated NF-κB expression, ameliorated BBB permeability. Brain Res 1459 (6):61–70

Zhang XZ, Qian SS, Zhang YJ et al (2016) *Salvia miltiorrhiza*: a source for anti-Alzheimer's disease drugs. Pharm Biol 54:18–24

Zhang ZH, Wen L, Yu QY et al (2017) Long-term dietary supplementation with selenium-enriched yeast improves cognitive impairment, reserves synaptic deficits, and mitigates tau pathology in a triple

transgenic mouse model of Alzheimer's disease. J Agric Food Chem 65:4970–4979

Zhao Y, Zhao B (2013) Oxidative stress and the pathogenesis of Alzheimer's disease. Oxid Med Cell Longev 2013:316523

Zhao N, Zhong C, Wang Y et al (2008) Impaired hippocampal neurogenesis is involved in cognitive dysfunction induced by thiamine deficiency at early pre-pathological legion stage. Neurobiol Dis 29(2):176–185

Zhao HF, Li Q, Zhang ZF et al (2009) Long-term ginsenoside consumption prevents memory loss in aged SAMP8 mice by decreasing oxidative stress and up-regulating the plasticity-related proteins in hippocampus. Brain Res 1256:111–122

Zhao B, Ren B, Guo R et al (2017a) Supplementation of lycopene attenuates oxidative stress induced neuroinflammation and cognitive impairment via Nrf2/NF-κB transcriptional pathway. Food Chem Toxicol 109:505–516

Zhao T, Su G, Wang S et al (2017b) Neuroprotective effects of acetylcholinesterase inhibitory peptides from anchovy (*Coilia mystus*) against glutamate-induced toxicity in PC12 cells. J Agric Food Chem 65:11192–11201

Zhao B, Liu H, Wang J et al (2018) Lycopene supplementation attenuates oxidative stress, neuroinflammation, and cognitive impairment in aged CD-1 mice. J Agric Food Chem 66:3127–3136

Zhu F, Qian C (2006) Berberine chloride can ameliorate the spatial memory impairment and increase the expression of interleukin-1beta and inducible nitric oxide synthase in the rat model of Alzheimer's disease. BMC Neurosci 7:78

Nutraceuticals for Calming and Stress

Anitha Alex and Ajay Srivastava

Abstract

Stress in animals is evident through the disruptive behaviors exhibited, including excessive barking, restlessness, repetitive behavior, extreme vigilance, etc. Sociability is a key factor in determining the successful adaptation of pets to their environment. Sociable dogs are more comfortable with strangers and unfamiliar situations. Thus, reducing stress and anxiety in pets is essential in providing positive social interactions and to improve the quality of their life and that of the owners. γ-Aminobutyric acid (GABA) is the main inhibitory neurotransmitter in the mammalian brain, and many anxiolytic drugs exert their action through interactions with the GABA receptors. In addition to the GABAergic system, serotonergic, dopaminergic, and noradrenergic systems are also implicated in the development of anxiety and stress in various animal models and in humans. Furthermore, the involvement of the hypothalamic-pituitary-adrenal (HPA) axis and dysregulation of the immune system may also mediate social stress in animals that produces aggression and/or depression. While a number of anxiolytic drugs are available on the market, dietary supplements and herbal extracts are shown to exert equivalent calming effects with no or minimal addictive or aversive side effects. This chapter describes the underlying mechanisms involved in the development of stress and anxiety and various nutraceuticals and substances that have potential to reduce the stress behavior and improve social interactions in canines.

Keywords

Canine stress · Anxiety · Calming · GABA · Behavior

A. Alex
Salt Lake City, UT, USA

A. Srivastava (✉)
Vets Plus Inc., Menomonie, WI, USA
e-mail: drajay@vets-plus.com

1 Introduction

Stress and anxiety disorders in dogs like in humans are due to an imbalance in neurotransmitters in the brain. The key players in the pathogenesis of anxiety disorders are γ-aminobutyric acid (GABA), serotonin, dopamine, and norepinephrine. In addition, neuropeptides like cholecystokinin (CCK), oxytocin, vasopressin, atrial natriuretic peptide (ANP), and substance P are also implicated in the modulation of stress-related behaviors and anxiety (Bandelow et al. 2017; MacLean et al. 2017). The role of the hypothalamic-pituitary-adrenal (HPA) axis in the pathophysiology of anxiety disorders has also been widely studied. Studies indicate that overactivation of the HPA axis leads to the stimulation of the adrenal gland to release cortisol, dehydroepiandrosterone (DHEA), adrenaline, and noradrenaline. Cortisol, the stress hormone in humans and dogs (corticosteroids in rodents), mediates a variety of effects throughout the body and brain and exerts a negative feedback on the HPA axis, thereby inhibiting its own production (Herman and Cullinan 1997).

Stress in dogs can be recognized by their behavioral response, like active vigilance and repetitive movements such as jumping, pacing, and excessive barking. Domestic dogs are highly social animals and sociability is an essential trait for successful interaction with humans (Grigg et al. 2017). Dogs are faced with a variety of stressors in their lives, including novelty, spatial restrictions, increased noise levels, etc. Social isolation also acts as a stressor which results in alterations in reactivity to stress, social behavior, function of neurochemical and neuroendocrine systems, and physiological, anatomical, and behavioral changes in both animals and humans (Mumtaz et al. 2018). Another stress factor is veterinary hospital visits. Reducing stress levels in pets must be a priority during their hospital visits and this is often overlooked. There is mounting evidence to suggest that routine veterinary care may be contributing to lifelong patient anxiety, starting with the first puppy or kitten physical examination (Overall 2013). Reports suggest that while a "no

R. C. Gupta et al. (eds.), *Nutraceuticals in Veterinary Medicine*, https://doi.org/10.1007/978-3-030-04624-8_27

stress" environment is not possible, understanding how to create a "low stress" environment and how to handle animals in a less stressful manner greatly benefits patients, staff, and the hospital (Lloyd 2017).

Growing evidence suggests that herbal extracts and dietary nutrients can effectively reduce anxiety by altering both neurotransmitter levels and the HPA axis. For example, vitamins C, D, and E, omega-3 fatty acids, and the green tea amino acid L-theanine are dietary supplements known to increase the production of dopamine. Further, supplementation with the amino acid L-tryptophan and its precursor, 5-HTP, the B vitamins, vitamin D, selenium, and omega-3 fatty acids increases serotonin production. These amino acid supplements are neurotransmitter building blocks, and the vitamins act as cofactors in neurotransmitter biosynthesis pathways. This dietary approach can correct the underlying neurochemistry without the side effects associated with anxiolytic drugs that may simply mask the problem (Alramadhan et al. 2012).

This chapter describes the key neurotransmitters involved in anxiety and stress disorders, biomarkers of stress, various dietary supplements, and herbal products that can help in calming dogs and reducing their stress levels.

2 Neurotransmitters, Neuropeptides, and HPA Axis in Stress and Anxiety

2.1 γ-Aminobutyric Acid (GABA)

GABA is the major inhibitory neurotransmitter in the mammalian brain, and it plays a central role in the maintenance of inhibitory and excitatory balance. The fast inhibitory action of GABA is mediated through $GABA_A$ receptors, a chloride-gated ion channel. Preclinical and clinical studies strongly suggest that impairments in GABAergic neurotransmission underpin human stress and anxiety disorders (Goddard 2016). Hyperexcitability of the amygdala, in particular, is strongly associated with anxiety, hypervigilance, and an inability to regulate emotions. Several rodent models have highlighted the role of the GABA synthetic isoenzymes glutamic acid decarboxylase 65 (GAD65) and GAD67 in the expression of normal mammalian fear. Knockdown of GAD67 protein in the mouse amygdala impaired normal fear extinction and decreased sensitivity to diazepam (Heldt et al. 2012). Low cortical levels of GABA have been observed in panic disorders and may serve as a potential biomarker for panic and other related stress disorders (Goddard et al. 2004). Experiments in various animal models have implicated the role of GABA in separation anxiety and trait anxiety (Tasan et al. 2011; Feng et al. 2014). Additionally, chronic restraint stress can also lead to decreased expression of $GABA_A$ receptor subunits in the cortex and hippocampus, suggesting a role in HPA axis dysfunction and stress symptomatology (Wisłowska-Stanek et al. 2013). Several anxiolytic agents available on the market today mediate their calming effects by targeting the GABA receptors. Benzodiazepine (BZD) is a full agonist at the BZD site of $GABA_A$ receptors and allosterically enhances the physiological GABA responses. Preclinical work has further defined the role of discrete $GABA_A$ receptor subunits on the separate clinical effects of the BZDs such as anxiolysis, sedation, muscle relaxation, and anticonvulsant effects (Rudolph and Knoflach 2011).

Recent interest in dietary supplements has spiked the use of GABA as a food supplement. In Europe and the United States, GABA is considered a "food constituent" and a "dietary supplement," respectively (Boonstra et al. 2015). The calming effects of GABA by oral administration were investigated in adult Shih Tzu dogs and were found to decrease time spent standing and walking compared to non-treated dogs. Similarly, a significant depression in the urinary cortisol level was also observed at 7 h after administration. These results indicate that orally administrated GABA exerts calming effects on dogs just as in humans (Uetake et al. 2012). However, it should be noted that the ability of GABA to cross the blood-brain barrier (BBB) in humans is unclear as of now and available reports are at the very least contradictory (Boonstra et al. 2015).

There may be, however, other mechanisms through which GABA exerts its effects, such as through the enteric nervous system (ENS). In fact, gut microbiota was shown to improve mood and reduce anxiety in patients with chronic fatigue (Logan and Katzman 2005; Rao et al. 2009; Pisanu and Squassina 2017). Similarly, studies indicate that oral intake of probiotics resulted in reduced urinary cortisol and perceived psychological stress (Messaoudi et al. 2011). Certain bacterial strains such as *Lactobacillus* and *Bifidobacterium* can produce GABA in vivo and were found to be effective in increasing GABA concentrations in the ENS (Barrett et al. 2012). This might account for the reported calming effect of dietary GABA; however further research is required to support the beneficial effects of GABA through the ENS.

2.2 Serotonin (5-HT)

Serotonin or 5-HT is a monoamine found in the CNS, in blood platelets, and in the gastrointestinal tract. The principal source of serotonin release in the brain are the raphe nuclei in the brainstem. Serotonin can inhibit periaqueductal gray matter-medicated fight/flight responses from threats, while it can also facilitate amygdala-mediated anxiety responses (Deakin 2013). Animal data and genetic and neuroimaging studies in humans point to a role of the 5HT1A receptor in the neural processing of anxiety (Akimova et al. 2009). Recently, a review of the 5HT2C receptor suggested that this receptor

may play a crucial role in anxiety (Chagraoui et al. 2016). The selective serotonin reuptake inhibitor class of antidepressants (SSRIs) act by inhibiting the serotonin reuptake as the name suggests and includes citalopram, escitalopram (active enantiomer of citalopram), fluoxetine, fluvoxamine, paroxetine, and sertraline. Fluoxetine is approved for veterinary use in the treatment of canine separation anxiety. Careful attention must be paid when treating canines with SSRIs as an overdose might result in serotonin syndrome, which may clinically manifest as nausea, vomiting, mydriasis, hypersalivation, hyperthermia, ataxia, tremors, muscle rigidity, diarrhea, and seizures. The goals of treatment in this intoxication are to support the animal, prevent further absorption of the drug, support the central nervous system, control hyperthermia, and halt any seizure activity. The relative safety of SSRIs in an overdose, despite the occurrence of serotonin syndrome, makes them more desirable than other antidepressants (Fitzgerald and Bronstein 2013).

2.3 Dopamine

Dopamine is involved in reward-motivated behavior and motor control. Nervous pointer dogs have been suggested as an animal model for pathological anxiety. There was a trend for lower [HVA] and [DOPAC] levels and a significantly lower [DOPAC]/[DA] ratio in nervous dogs, suggesting decreased dopaminergic function (Gurguis et al. 1990). Social isolation stress may alter the levels of neurotransmitters such as dopamine, serotonin, GABA, glutamate, the nitrergic system, and adrenaline as well as lead to an alteration in receptor sensitivity of *N*-methyl-D-aspartate (NMDA) and the opioid system (Mumtaz et al. 2018).

2.4 Norepinephrine

NE is a catecholaminergic neurotransmitter that plays a key role in the regulation of the autonomic nervous system. The metabolism and functions of norepinephrine have been studied extensively. While hypofunction is postulated for depression, hyperfunction is suggested as the reason for anxiety disorders. Stimulation of the locus coeruleus, an area containing most of the noradrenergic cell bodies of the brain, has been shown to induce anxiety and to raise the concentration of the main central NE metabolite, 3-methoxy-4-hydroxyphenyl glycol (MHPG) in patients with panic attacks. It also produces somatic symptoms, increases in blood pressure, and elevation of cortisol levels (Bandelow et al. 2017).

2.5 Cholecystokinin (CCK)

CCK is a neuropeptide that is widely distributed in the brain and has been shown to induce excitation of central neurons as well as inhibitory postsynaptic effects (Bourin and Dailly 2004). Reports indicate that CCK might be an important modulator of the neuronal networks that are involved in anxiety (Bandelow et al. 2017). CCK interacts with the serotonergic, GABAergic, and noradrenergic systems, as well as with endocannabinoids and neuropeptides Y and S (Zwanzger et al. 2012). CCK-B receptor expression and binding are increased in animal models of anxiety. In humans, CCK-induced anxiety may be mediated via CCK-B receptors, whereas in mice it is mediated by both CCK-B and CCK-A receptors (Li et al. 2013).

2.6 Oxytocin (OT)

In humans, OT induces a reduced response to aversive stimuli in the amygdala. It is also reported to promote trust and reduces the level of anxiety (Kirsch et al. 2005; Kosfeld et al. 2005; Heinrichs et al. 2009). Recent studies in dogs suggest that OT facilitates and responds to affiliative forms of human-animal interaction (HAI). It has been shown that in a group of Labrador retrievers and Labrador retriever/Golden retriever crosses, dogs participating in HAI exhibited a significant increase in both salivary OT (+39%) and plasma OT (+5.7%), whereas dogs in the control group with no human interaction did not. These results suggest that in dogs exposed to HAI, changes in salivary OT may reflect the extent of affiliative behavior between the dog and the human, indicating the potential of salivary OT to be a useful biomarker in studies of HAI (MacLean et al. 2017). Interestingly, the effects of oxytocin on the dogs' social behavior may not be universal, but it may be constrained by situations and other individual factors. Further research in this field is warranted, and the understanding of how OT mediates social behavior might be useful in improving its application in pharmacotherapy (Turcsán et al. 2017).

2.7 Vasopressin (AVP)

Vasopressin, along with OT, has been linked to both affiliative and aggressive behavior in domestic dogs (MacLean et al. 2017). Reports indicate that while OT may play a larger role in affiliative social behavior, anxiolysis, and inhibition of aggression, AVP may play a larger role in anxiogenesis and aggression (Coccaro et al. 1998; Thompson et al. 2006). Aggressive behavior in dogs is a serious concern,

and in the United States alone, the estimated number of dog bites is ~4.5 million annually, with approximately half of these bites directed toward children (Centers for Disease Control and Prevention 2003; Gilchrist et al. 2008). In an experiment in pet dogs with chronic aggression, dogs with a history of aggression exhibited more aggressive behavior during simulated encounters with conspecifics and had lower free but higher total plasma AVP than matched controls. In addition, in assistance dog population, dogs who behaved more aggressively toward a threatening stranger had higher total AVP than dogs who did not, suggesting the role of AVP in shaping dog social behavior (MacLean et al. 2017).

2.8 HPA Axis

The HPA axis is the main physiological system that mediates the body's stress response. It plays a pivotal role in regulating the synthesis and release of endocrine hormones associated with the central nervous system, including cortisol, a major stress hormone. Cortisol has widespread effects throughout the body and brain and passes through the BBB to downregulate the HPA axis activity by triggering negative feedback mechanisms, thereby inhibiting its own production (Herman and Cullinan 1997). Cortisol exerts its effects through interactions with glucocorticoid and mineralocorticoid receptors and regulates the expression of several genes, including cytokines (Lee and Rhee 2017). Several reports indicate that psychological stress can trigger cytokine release (Black 2003; Zalcman and Siegel 2006), and growing evidence has shown an important role of the immune system in regulating negative emotional states as well as personality (Maes et al. 2009; Réus et al. 2015; Takahashi et al. 2018). Activation of the HPA axis is implicated in many behavioral disorders including social and spatial restriction (Beerda et al. 1999). Studies indicate that when placed into a kennel environment, dogs experience a spike in cortisol levels followed by a decrease to original at-home levels (Protopopova 2016).

3 Dietary Supplements in Calming

The well-being of dogs can be affected by changes in human lifestyle, eating habits, and increased environmental and social stressors, leading to behavioral changes and anxiety, followed by negative affective moods and poor welfare (Sechi et al. 2016). A chronic anxious status and nutrition have been demonstrated to significantly affect behavior (Bosch et al. 2007). A diet supplemented with amino acids, n-3 and n-6 polyunsaturated fatty acids (PUFAs), and a well-balanced amount of proteins and fiber was considered beneficial in dogs with evident behavioral disorders (Bosch et al. 2007). Similarly, dietary supplementation with minerals like calcium, magnesium, and selenium and vitamins like C, D, and E are also reported to reduce anxiety and improve mood in both humans and animals (Carroll et al. 2000; Benton and Cook 1990; Hughes et al. 2011).

Reports indicate that omega-3 deficiency may also be associated with mood and behavioral disorders (Owen et al. 2008) and dietary omega-3 fatty acids have been shown to be effective in improving mood and reducing the risk of anxiety (Appleton et al. 2008; Kiecolt-Glaser et al. 2011). In addition, studies show that a diet high in tryptophan can lower territorial aggression score, while a high-protein diet without tryptophan supplementation can induce a high dominance aggression score in dogs (DeNapoli et al. 2000). In canines, all these pathologies have been consistently reported to be associated with oxidative stress. Vitamin C, a cofactor for many enzymes, reduces anxiety by limiting the oxidative stress from metabolites and also by limiting cortisol (Hughes et al. 2011).

Oxidative stress results from excess generation of free radicals, such as peroxide ion, nitrogen monoxide, and hydroxyl radical (Pasquini et al. 2010). An improvement of overall tissue stability and protection against oxidative stress is observed when the diet is enriched with antioxidants (Sechi et al. 2015). These reports indicate that changes in oxidative stress-related factors, such as derivatives of reactive oxygen metabolites (dROMs) and biological antioxidant potential (BAP), must be evaluated to monitor the welfare and health of dogs under stressful conditions (Passantino et al. 2014; Sechi et al. 2015). In a randomized, controlled clinical evaluation, 69 dogs of different breeds with anxiety and chronic stress were fed a control diet or a nutraceutical diet for 45 days. The results from this study showed a significant increase in the plasma concentration of serotonin, dopamine, and β-endorphins in the nutraceutical diet group, whereas a significant decrease in the levels of noradrenaline, cortisol, and dROMs was observed, suggesting benefits from the use of diet and nutraceuticals in the treatment of behavioral disorders (Sechi et al. 2016).

In another study from the same group, 24 dogs of different ages and breeds with generalized anxiety and behavioral disorders received counterconditioning and desensitization behavioral therapy combined with a nutraceutical diet for 10 days. The diet consisted of a mixed formula of fish proteins, rice carbohydrates, *Punica granatum*, *Valeriana officinalis*, *Rosmarinus officinalis*, *Tilia* spp., tea extract, and L-tryptophan, with an omega-3/omega-6 ratio of 1:0.8. The results indicated that dogs treated with specific diets showed significant improvement in times of activity and times of rest after 10 days and significant improvement in clinical and behavioral symptoms (Di Cerbo et al. 2017). Overall, these studies demonstrate the positive effects of nutraceutical diets on neuroendocrine, behavioral, and social

parameters associated with stress, anxiety, aggression, and behavioral disturbances.

Protein Hydrolysates

Studies have shown that fish hydrolysate, a natural supplement derived from fish protein, helps in reducing fear and anxiety in Beagles. In a thunderstorm model, fish hydrolysate demonstrated reduction in a hyperactivity response to thunder (Landsberg et al. 2015). Other hydrolysates derived from milk protein have also shown efficacy in improving the anxiety symptoms in rats and dogs. A comparative study conducted in 38 dogs demonstrated that alpha-casozepine (a milk protein hydrolysate) and selegiline (Anipryl) were equally effective in alleviating anxiety symptoms (Beata et al. 2007a, b). Additionally, in a placebo-controlled trial, alpha-casozepine displayed improvement in fearful behavior and anxiety in cats due to socially stressful situations. Although not elucidated clearly, some studies have shown that the antianxiety effect of alpha-casozepine may be due to its structural similarity to GABA and its binding to GABA receptors in the brain.

Pheromones

Pheromones are chemical substances which affect social behavior in mammals. In a triple-blinded, randomized, and placebo-controlled study in puppies newly adopted from a pet store, dog-appeasing pheromone (DAP) showed reductions in behaviors associated with fear of unfamiliar people and new surroundings (Mills et al. 2006). In another open clinical study, DAP was able to reduce the symptoms of anxiety in fearful dogs that showed signs of fear in response to fireworks (Sheppard and Mills 2003). These pheromones have also shown some efficacy in reducing anxiety and fear in puppies during puppy classes and resulted in improved socialization (Denenberg et al. 2005). However, 11 of the 14 reports reviewed and published in the *Journal of the American Veterinary Medical Association* suggested that there is not enough evidence to support the effectiveness of pheromones for the treatment of undesirable behavior in cats and dogs.

4 Herbal Extracts in Calming

In addition to nutrients such as amino acids, minerals, and vitamins, herbs and plant products have also been shown to be effective in treating anxiety (Head and Kelly 2009; Saeed et al. 2007). Plant extracts provide an alternative and better tool in the pharmacotherapy of psychological disorders without the adverse effects associated with anxiolytic drugs. Drugs used to treat anxiety have many negative side effects including addiction, depression, suicide, seizures, sexual dysfunction, headaches, and more (Alramadhan et al. 2012). Anxiolytic medications do not restore normal levels of neurotransmitters but, instead, manipulate the brain chemistry.

4.1 *Ginkgo biloba*

Ginkgo biloba, the oldest existing tree in the world for over 200 million years, has long been implicated in the treatment of anxiety as a part of traditional medicine (Liu et al. 2015). Nutritional supplements of *Ginkgo biloba* have been shown to be effective in reducing anxiety in various animal models. *Ginkgo biloba* extract (EGB 761) prevented stress- and corticosterone-induced impairments of spatial memory in a chronic restrain stress model in rats (Walesiuk and Braszko 2009). Similarly, daily administration of ginkgolide-A (1 or 2 mg/kg, po) resulted in an anxiolytic-like effect in mice (Kuribara et al. 2003). Further, *Ginkgo biloba* extracts were shown to activate GABA pathways and act like a benzodiazepine to reduce anxiety in patients with generalized anxiety disorder. In elderly dogs (mean age 11.4 years), administered *Ginkgo* leaf extract at a daily dose of 40 mg/10 kg body weight for 8 weeks improved the "severity of the geriatric condition." The occurrence of typical age-related behavioral disturbances is well reported in elderly dogs. A significant decrease in five of the six clinical sign scores (disorientation, sleep/activity changes, behavioral changes, general behavior, and general physical condition/vitality) was observed during the treatment period, suggesting the utility of *Gingko biloba* in elderly dogs with age-related behavioral disturbances (Reichling et al. 2006). Overall, these results support the use of *Gingko biloba* extract as a key nutritional supplement to improve mood and behavior in dogs, thus increasing the quality of life for dogs and their owners.

4.2 St. John's Wort (*Hypericum perforatum*)

St. John's wort is an aromatic perennial plant native to Europe and parts of Asia, North America, and South America and has been used traditionally for the treatment of anxiety and depression and as a nerve tonic. A large amount of clinical and animal experimental data demonstrate that it exerts its action through mechanisms similar to the tricyclic antidepressants or serotonin reuptake inhibitors (Bukhari and Dar 2013). In fact, the majority of clinical studies that compare it with antidepressant drugs found it superior to the placebo (Sarris and Kavanagh 2009; Linde et al. 2008). St. John's wort increases brain levels of serotonin and also normalizes the HPA axis by reducing inflammatory and oxidative stress (Tadros et al. 2009; Head and Kelly 2009). Reports indicate that St. John's wort alleviates stress-induced

deterioration of memory in rats (Trofimiuk et al. 2006). In a force swim test experiment, *H. perforatum* extract (30–90 mg/kg i.p.) caused a dose-dependent reduction in immobility time in rats with maximal effect seen at 90 mg/kg, similar to that seen with fluoxetine and imipramine (30–70 mg/kg i.p.) (Bukhari and Dar 2013). These studies suggest that St. John's wort demonstrates antidepressant properties similar to standard antidepressants and that the antidepressant profile of *H. perforatum* is more closely related to the selective serotonin reuptake inhibitor class of antidepressants.

4.3 Ginseng

Ginseng is the root of plants within the genus *Panax* and it is traditionally used as a medicinal herb in Korea, Japan, and China. It shows potent antioxidant activity attributed to ginsenosides, which are extracted from the roots, leaves, stems, and fruit, and it has multiple pharmacological effects (Lee and Rhee 2017). Ginseng has been shown to regulate the HPA axis and is implicated in the treatment of depression, asthma, hypertension, and post-traumatic stress disorders (Park et al. 2005; Choi et al. 2011). Ginseng was also demonstrated to attenuate the rise in the concentration of corticosterone in plasma induced by restrictive stress through suppressing the activity of ACTH in the adrenal gland, suggesting its use as potential therapy for mental disorders associated with stress (Kim et al. 2003). In addition to suppressing the occurrence of psychological diseases, ginseng also prevents stress-associated physiological diseases and regulates the immune response and the hormonal changes due to stress, thus maintaining homeostasis (Lee and Rhee 2017).

4.4 Ashwagandha (*Withania somnifera*)

Withania somnifera belonging to the family Solanaceae has been used for centuries in Indian, Chinese, and Arabic traditional medicines. *Withania somnifera* extracts (root, leaf, or fruit) have been reported to possess anti-inflammatory, antitumor, anti-stress, antioxidant, immunomodulatory, hematopoietic, and rejuvenating properties (Mishra et al. 2000).

Reports indicate that rodents treated with ashwagandha showed reduced anxiety compared to control treatment. This reduction matched the reduction in anxiety in these rodents when treated with several benzodiazepine drugs (Ramanathan et al. 2011; Mohan et al. 2011). Ashwagandha has also been shown in clinical studies to reduce anxiety in patients and was superior in action compared to the control group receiving psychotherapy (Cooley et al. 2009).

4.5 Valerian (*Valeriana officinalis*)

Valerian is a temperate root that is commonly used for the treatment of insomnia and anxiety. Valerian extracts have been shown to both increase GABA synthesis and decrease synaptic GABA reuptake (Ortiz et al. 1999), through the activation of glutamic acid decarboxylase, an enzyme involved in the synthesis of GABA (Awad et al. 2007). Valerinic acid, an active compound from Valerian root, exerts its action through allosteric modulation of $GABA_A$ receptors (Benke et al. 2009). The anxiolytic properties of Valerian root extracts are well reported in animal models and in humans, suggesting its use as an alternative to anxiolytic drugs (Benke et al. 2009; Hattesohl et al. 2008).

In addition to the abovementioned herbal extracts, there are reports on the beneficial use of rosenroot, lemon balm, chamomile, and lavender oil in reducing anxiety and stress in animals and humans (Weeks 2009; Bystritsky et al. 2008; Kennedy et al. 2006; Komiya et al. 2009). The phytochemicals contained in herbal extracts belong to different classes such as alkaloids, flavonoids, phenolic acids, lignans, cinnamates, terpenes, and saponins. The results presented here showed that all of these possess anxiolytic effects in a wide range of animal models of anxiety. The mechanisms involved may include interactions with $GABA_A$ receptors, serotonergic, noradrenergic, and dopaminergic systems. Phytochemicals also modulate the HPA axis, thereby regulating the levels of cortisol and the release of pro-inflammatory cytokines (Farzaei et al. 2016). Taken together, these studies demonstrate the potential use of phytochemicals as supplements to conventional anxiolytic therapies in order to improve efficacy and reduce adverse effects.

5 Concluding Remarks and Future Directions

Anxiety and stress can be stimulated by environmental, social, or age-related factors in dogs. The underlying pathophysiology of anxiety is diverse and may involve multiple neurotransmitters and neuroendocrine systems, including the HPA axis, neuropeptides, and deficiencies in certain dietary nutrients. While newer anxiolytics with fewer side effects and addictive potentials are available on the market, growing evidence shows that nutraceuticals are equally potent and in some cases more effective than psychotherapy. Additionally, while anxiolytics exert its action by manipulating the neurotransmitter systems in the brain, herbal supplements exert their action by bringing the levels of neurotransmitters to physiological levels to maintain homeostasis. This chapter describes the key neurotransmitter systems involved in the development of anxiety and stress as well as the various

herbal and dietary supplements that are beneficial in reducing stress and calming the animals, thus providing more meaningful and enjoyable interaction with their owners. It should be noted that standardized studies in dogs in this field are still in their infancy, and further studies with more standardized protocols are warranted to investigate and validate the results presented in this chapter.

References

Akimova E, Lanzenberger R, Kasper S (2009) The serotonin-1A receptor in anxiety disorders. Biol Psychiatry 66:627–635

Alramadhan E, Hanna MS, Hanna MS et al (2012) Dietary and botanical anxiolytics. Med Sci Monit 18(4):RA40–RA48

Appleton KM, Rogers PJ, Ness AR (2008) Is there a role for n-3 long-chain polyunsaturated fatty acids in the regulation of mood and behavior? A review of the evidence to date from epidemiological studies, clinical studies and intervention trials. Nutr Res Rev 21 (1):13–41

Awad R, Levac D, Cybulska P et al (2007) Effects of traditionally used anxiolytic botanicals on enzymes of the gamma-aminobutyric acid (GABA) system. Can J Physiol Pharmacol 85(9):933–942

Bandelow B, Baldwin D, Abelli M et al (2017) Biological markers for anxiety disorders, OCD and PTSD: a consensus statement. Part II: neurochemistry, neurophysiology and neurocognition. World J Biol Psychiatry 18(3):162–214

Barrett E, Ross RP, O'Toole PW et al (2012) γ-Aminobutyric acid production by culturable bacteria from the human intestine. J Appl Microbiol 113:411–417

Beata C, Beaumont-Graff E, Coll V et al (2007a) Effect of alpha-casozepine (Zylkene) on anxiety in cats. J Vet Behav 2:40–46

Beata C, Beaumont-Graff E, Diaz C et al (2007b) Comparison of the effect of alpha-casozepine (Zylkene) versus selegiline hydrochloride on anxiety disorders in dogs. J Vet Behav 2:175–183

Beerda B, Schilder MB, Bernadina W et al (1999) Chronic stress in dogs subjected to social and spatial restriction. II Hormonal and immunological responses. Physiol Behav 66(2):243–254

Benke D, Barberis A, Kopp S et al (2009) GABA A receptors as in vivo substrate for the anxiolytic action of valerenic acid, a major constituent of valerian root extracts. Neuropharmacology 56(1):174–181

Benton D, Cook R (1990) Selenium supplementation improves mood in a double-blind crossover trial. Psychopharmacology 102 (4):549–550

Black PH (2003) The inflammatory response is an integral part of the stress response: implications for atherosclerosis, insulin resistance, type II diabetes and metabolic syndrome X. Brain Behav Immun 17:350–364

Boonstra E, de Kleijn R, Colzato LS et al (2015) Neurotransmitters as food supplements: the effects of GABA on brain and behavior. Front Psychol 6:1520

Bosch G, Beerda B, Hendriks WH et al (2007) Impact of nutrition on canine behavior: current status and possible mechanisms. Nutr Res Rev 20:180–194

Bourin M, Dailly E (2004) Cholecystokinin and panic disorder. Acta Neuropsychiatr 16:85–93

Bukhari IA, Dar A (2013) Behavioral profile of *Hypericum perforatum* (St. John's Wort) extract. A comparison with standard antidepressants in animal models of depression. Eur Rev Med Pharmacol Sci 17(8):1082–1089

Bystritsky A, Kerwin L, Feusner JD (2008) A pilot study of *Rhodiola rosea* (Rhodax) for generalized anxiety disorder (GAD). J Altern Complement Med 14(2):175–180

Carroll D, Ring C, Suter M et al (2000) The effects of an oral multivitamin combination with calcium, magnesium, and zinc on psychological well-being in healthy young male volunteers: a double-blind placebo-controlled trial. Psychopharmacology 150(2):220–225

Centers for Disease Control and Prevention (2003) Nonfatal dog bite-related injuries treated in hospital emergency departments–United States, 2001. MMWR Morb Mortal Wkly Rep 52:605–610

Chagraoui A, Thibaut F, Skiba M et al (2016) 5-HT2C receptors in psychiatric disorders: a review. Prog Neuropsychopharmacol Biol Psychiatry 66:120–135

Choi JY, Woo TS, Yoon SY et al (2011) Red ginseng supplementation more effectively alleviates psychological than physical fatigue. J Ginseng Res 35:331e8

Coccaro EF, Kavoussi RJ, Hauger RL et al (1998) Cerebrospinal fluid vasopressin levels correlates with aggression and serotonin function in personality-disordered subjects. Arch Gen Psychiatry 55:708–714

Cooley K, Szczurko O, Perri D et al (2009) Naturopathic care for anxiety: a randomized controlled trial ISRCTN78958974. PLoS One 4(8):e6628

Deakin J (2013) The origins of '5-HT and mechanisms of defense' by Deakin and Graeff: a personal perspective. J Psychopharmacol 27:1084–1089

DeNapoli JS, Dodman NH, Shuster L et al (2000) Effect of dietary protein content and tryptophan supplementation on dominance aggression, territorial aggression, and hyperactivity in dogs. J Am Vet Med Assoc 217:504–508

Denenberg S, Landsberg G, Gaultier E (2005) Evaluation of DAP's effect on reduction of anxiety in puppies (*Canis familiaris*) as well as its usefulness in improving learning and socialization. In: Mills D, Levine E, Landsberg G (eds) Current issues and research in veterinary behavioral medicine. Purdue University Press, West Lafayette, pp 225–228

Di Cerbo A, Sechi S, Canello S et al (2017) Behavioral disturbances: an innovative approach to monitor the modulatory effects of a nutraceutical diet. J Vis Exp (119):e54878. https://doi.org/10.3791/54878

Farzaei MH, Bahramsoltani R, Rahimi R et al (2016) A systematic review of plant-derived natural compounds for anxiety disorders. Curr Top Med Chem 6(17):1924–1942

Feng M, Sheng G, Li Z et al (2014) Postnatal maternal separation enhances tonic GABA current of cortical layer 5 pyramidal neurons in juvenile rats and promotes genesis of GABAergic neurons in neocortical molecular layer and subventricular zone in adult rats. Behav Brain Res 260:74–82

Fitzgerald KT, Bronstein AC (2013) Selective serotonin reuptake inhibitor exposure. Top Companion Anim Med 28(1):13–17

Gilchrist J, Sacks J, White D et al (2008) Dog bites: still a problem? Inj Prev 14:296–301

Goddard AW (2016) Cortical and subcortical gamma amino acid butyric acid deficits in anxiety and stress disorders: clinical implications. World J Psychiatry 6(1):43–53

Goddard AW, Mason GF, Rothman DL et al (2004) Family psychopathology and magnitude of reductions in occipital cortex GABA levels in panic disorder. Neuropsychopharmacology 29:639–640

Grigg EK, Nibblett BM, Robinson JQ et al (2017) Evaluating pair versus solitary housing in kenneled domestic dogs (*Canis familiaris*) using behavior and hair cortisol: a pilot study. Vet Rec Open 4(1): e000193. https://doi.org/10.1136/vetreco-2016-000193

Gurguis GN, Klein E, Mefford IN et al (1990) Biogenic amines distribution in the brain of nervous and normal pointer dogs. A genetic animal model of anxiety. Neuropsychopharmacology 3(4):297–303

Hattesohl M, Feistel B, Sievers H et al (2008) Extracts of *Valeriana officinalis* L.s.l. show anxiolytic and antidepressant effects but neither sedative nor myorelaxant properties. Phytomedicine 15(1–2):2–15

Head KA, Kelly GS (2009) Nutrients and botanicals for treatment of stress: adrenal fatigue, neurotransmitter imbalance, anxiety, and restless sleep. Altern Med Rev 14(2):114–140

Heinrichs M, von Dawans B, Domes G (2009) Oxytocin, vasopressin, and human social behavior. Front Neuroendocrinol 30:548–557
Heldt SA, Mou L, Ressler KJ (2012) *In vivo* knockdown of GAD67 in the amygdala disrupts fear extinction and the anxiolytic-like effect of diazepam in mice. Transl Psychiatry 2:e181
Herman JP, Cullinan WE (1997) Neurocircuitry of stress: central control of the hypothalamo-pituitary-adrenocortical axis. Trends Neurosci 20(2):78–84
Hughes RN, Lowther CL, van Nobelen M (2011) Prolonged treatment with vitamins C and E separately and together decreases anxiety-related open-field behavior and acoustic startle in hooded rats. Pharmacol Biochem Behav 97(3):494–499
Kennedy DO, Little W, Haskell CF et al (2006) Anxiolytic effects of a combination of Melissa officinalis and *Valeriana officinalis* during laboratory induced stress. Phytother Res 20(2):96–102
Kiecolt-Glaser JK, Belury MA, Andridge R et al (2011) Omega-3 supplementation lowers inflammation and anxiety in medical students: a randomized controlled trial. Brain Behav Immun 25 (8):1725–1734
Kim DH, Moon YS, Jung JS et al (2003) Effects of ginseng saponin administered intraperitoneally on the hypothalamo-pituitary-adrenal axis in mice. Neurosci Lett 343(1):62–66
Kirsch P, Esslinger C, Chen Q et al (2005) Oxytocin modulates neural circuitry for social cognition and fear in humans. J Neurosci 25:11489–11493
Komiya M, Sugiyama A, Tanabe K et al (2009) Evaluation of the effect of topical application of lavender oil on autonomic nerve activity in dogs. Am J Vet Res 70(6):764–769
Kosfeld M, Heinrichs M, Zak PJ et al (2005) Oxytocin increases trust in humans. Nature 435:673–676
Kuribara H, Weintraub ST, Yoshihama T et al (2003) An anxiolytic-like effect of *Ginkgo biloba* extract and its constituent, ginkgolide-A, in mice. J Nat Prod 66(10):1333–1337
Landsberg GM, Mougeot L, Kelly S et al (2015) Assessment of noise-induced fear and anxiety in dogs: modification by a novel fish hydrolysate supplemented diet. J Vet Behav 10:391e398
Lee S, Rhee DK (2017) Effects of ginseng on stress-related depression, anxiety, and the hypothalamic-pituitary-adrenal axis. J Ginseng Res 41(4):589–594
Li H, Ohta H, Izumi H et al (2013) Behavioral and cortical EEG evaluations confirm the roles of both CCKA and CCKB receptors in mouse CCK-induced anxiety. Behav Brain Res 237:325–332
Linde K, Berner MM, Kriston L (2008) St John's wort for major depression. Cochrane Database Syst Rev 4:CD000448
Liu L, Liu C, Wan Y et al (2015) Herbal medicine for anxiety, depression and insomnia. Curr Neuropharmacol 2015(13):481–493
Lloyd JKF (2017) Minimizing stress for patients in the veterinary hospital: why it is important and what can be done about it. Vet Sci 4(2):E22. https://doi.org/10.3390/vetsci4020022
Logan AC, Katzman M (2005) Major depressive disorder: probiotics may be an adjuvant therapy. Med Hypotheses 64:533–538
MacLean EL, Gesquiere LR, Gee NR et al (2017) Effects of affiliative human-animal interaction on dog salivary and plasma oxytocin and vasopressin. Front Psychol 8:1606. https://doi.org/10.3389/fpsyg.2017.01606. eCollection 2017
Maes M, Yirmyia R, Noraberg J et al (2009) The inflammatory and neurodegenerative (I&ND) hypothesis of depression: leads for future research and new drug developments in depression. Metab Brain Dis 24:27–53
Messaoudi M, Lalonde R, Violle N et al (2011) Assessment of psychotropic-like properties of a probiotic formulation (*Lactobacillus helveticus* R0052 and *Bifidobacterium longum* R0175) in rats and human subjects. Br J Nutr 105:755–764
Mills DS, Ramos D, Gandia Estelles M et al (2006) A triple blind placebo-controlled investigation into the assessment of the effect of dog appeasing pheromone (DAP) on anxiety related behavior of problem dogs in the veterinary clinic. Appl Anim Behav Sci 98:114–126
Mishra LC, Singh BB, Dagenais s. (2000) Scientific basis for the therapeutic use of *Withania somnifera* (ashwagandha): a review Altern. Med Rev 5:334–346
Mohan L, Rao US, Gopalakrishna HN et al (2011) Evaluation of the anxiolytic activity of NR-ANX-C (a polyherbal formulation) in ethanol withdrawal-induced anxiety behavior in rats. Evid Based Complement Alternat Med 2011:327160
Mumtaz F, Khan MI, Zubair M et al (2018) Neurobiology and consequences of social isolation stress in animal model—a comprehensive review. Biomed Pharmacother 105:1205–1222
Ortiz JG, Nieves-Natal J, Chavez P (1999) Effects of *Valeriana officinalis* extracts on [3H]fl unitrazepam binding, synaptosomal [3H]GABA uptake, and hippocampal [3H]GABA release. Neurochem Res 24(11):1373–1378
Overall KL (2013) Fear factor: is routine veterinary care contributing to lifelong patient anxiety? Available online: http://veterinarynews.dvm360.com/fear-factor-routine-veterinary-care-contributinglifelong-patient-anxiety. Accessed on 21 Dec 2016
Owen C, Rees AM, Parker G (2008) The role of fatty acids in the development and treatment of mood disorders. Curr Opin Psychiatry 21:19–24
Park JH, Cha HY, Seo JJ et al (2005) Anxiolytic-like effects of ginseng in the elevated plus-maze model: comparison of red ginseng and sun ginseng. Prog Neuropsychopharmacol Biol Psychiatry 29:895e900
Pasquini A, Luchetti E, Cardini G (2010) Evaluation of oxidative stress in hunting dogs during exercise. Res Vet Sci 89:120–123
Passantino A, Quartarone V, Pediliggeri M et al (2014) Possible application of oxidative stress parameters for the evaluation of animal welfare in sheltered dogs subjected to different environmental and health conditions. J Vet Behav 9:290–294
Pisanu C, Squassina A (2017) We are not alone in our body: insights into the involvement of microbiota in the etiopathogenesis and pharmacology of mental illness. Curr Drug Metab 19:688–694
Protopopova A (2016) Effects of sheltering on physiology, immune function, behavior, and the welfare of dogs. Physiol Behav 159:95–103
Ramanathan M, Balaji B, Justin A (2011) Behavioral and neurochemical evaluation of Perment® an herbal formulation in chronic unpredictable mild stress induced depressive model. Indian J Exp Biol 49 (4):269–275
Rao AV, Bested AC, Beaulne TM et al (2009) A randomized, double-blind, placebo-controlled pilot study of a Probiotic in emotional symptoms of chronic fatigue syndrome. Gut Pathog 1:6. https://doi.org/10.1186/1757-4749-1-6
Reichling J, Frater-Schröder M, Herzog K et al (2006) Reduction of behavioral disturbances in elderly dogs supplemented with a standardized Ginkgo leaf extract. Schweiz Arch Tierheilkd 148 (5):257–263
Réus GZ, Fries GR, Stertz L et al (2015) The role of inflammation and microglial activation in the pathophysiology of psychiatric disorders. Neuroscience 300:141–154
Rudolph U, Knoflach F (2011) Beyond classical benzodiazepines: novel therapeutic potential of GABAA receptor subtypes. Nat Rev Drug Discov 10:685–697
Saeed SA, Bloch RM, Antonacci DJ (2007) Herbal and dietary supplements for treatment of anxiety disorders. Am Fam Physician 76(4):549–556
Sarris J, Kavanagh DJ (2009) Kava and St. John's Wort: current evidence for use in mood and anxiety disorders. J Altern Complement Med 15(8):827–836
Sechi S, Chiavolelli F, Spissu N et al (2015) An antioxidant dietary supplement improves brain-derived neurotrophic factor levels in serum of aged dogs: preliminary results. J Vet Med 2015:412501

Sechi S, Di Cerbo A, Canello S et al (2016) Effects in dogs with behavioral disorders of a commercial nutraceutical diet on stress and neuroendocrine parameters. Vet Rec 180(1):18. https://doi.org/10.1136/vr.103865

Sheppard G, Mills DS (2003) Evaluation of dog-appeasing pheromone as a potential treatment for dogs fearful of fireworks. Vet Rec 152:432–436

Tadros MG, Mohamed MR, Youssef AM et al (2009) Involvement of serotoninergic 5-HT1A/2A, alpha-adrenergic and dopaminergic D1 receptors in St. John's wort-induced prepulse inhibition deficit: a possible role of hyperforin. Behav Brain Res 199(2):334–339

Takahashi A, Flanigan ME, McEwen BS et al (2018) Aggression, social stress, and the immune system in humans and animal models. Front Behav Neurosci 12:56

Tasan RO, Bukovac A, Peterschmitt YN et al (2011) Altered GABA transmission in a mouse model of increased trait anxiety. Neuroscience 183:71–80

Thompson R, George K, Walton J et al (2006) Sex-specific influences of vasopressin on human social communication. Proc Natl Acad Sci USA 103:7889–7894

Trofimiuk E, Walesiuk A, Braszko JJ (2006) St John's wort (*Hypericum perforatum*) counteracts deleterious effects of the chronic restraint stress on recall in rats. Acta Neurobiol Exp (Wars) 66(2):129–138

Turcsán B, Range F, Rónai Z et al (2017) Context and individual characteristics modulate the association between oxytocin receptor gene polymorphism and social behavior in border Collies. Front Psychol 8:2232

Uetake K, Okumoto A, Tani N et al (2012) Calming effect of orally administered γ-aminobutyric acid in Shih Tzu dogs. Anim Sci J 83 (12):796–798

Walesiuk A, Braszko JJ (2009) Preventive action of *Ginkgo biloba* in stress- and corticosterone-induced impairment of spatial memory in rats. Phytomedicine 16(1):40–46

Weeks BS (2009) Formulations of dietary supplements and herbal extracts for relaxation and anxiolytic action: relarian. Med Sci Monit 15(11):RA256–RA262

Wisłowska-Stanek A, Lehner M, Skórzewska A et al (2013) Changes in the brain expression of alpha-2 subunits of the GABA-A receptor after chronic restraint stress in low- and high-anxiety rats. Behav Brain Res 253:337–345

Zalcman SS, Siegel A (2006) The neurobiology of aggression and rage: role of cytokines. Brain Behav Immun 20:507–514

Zwanzger P, Domschke K, Bradwejn J (2012) Neuronal network of panic disorder: the role of the neuropeptide cholecystokinin. Depress Anxiety 29:762–774

Nutraceuticals in Cardiovascular Diseases

Csaba K. Zoltani

Abstract

Cardiac dysfunction in animals, under certain conditions, can be alleviated or prevented by the consumption of nutraceuticals. Nutraceuticals, which are naturally occurring non-drug plant components, are able to affect the genetic system and physiological systems of animals following their absorption. This chapter addresses the mode of action of specific phytotherapeutic components in common cardiac diseases of animals.

Keywords

Animal cardiac dysfunction · Cardiac diseases · Nutraceuticals · Mode of action of nutraceuticals

1 Introduction

Cardiovascular diseases in animals emulate their counterparts in humans. Heart failure (HF), the inability of the heart to deliver oxygen and nutrients to cells, is one of the primary diagnoses. There are a large number of possible dysfunctions of the heart including inflammation, blockage of blood flow due to ischemia, atherosclerosis, and disturbance of electrical activity (Francis et al. 1990). In HF, one of the dominant symptoms, reduced ejection fraction, is usually accompanied by several related dysfunctions.

Oxidative stress and disequilibrium between the generation and destruction of reactive oxygen species (ROS) in tissue mark many of these conditions. Lipidemia that results impedes blood flow, causes vascular dysfunction, and changes the nitric oxide (NO) level.

The potential medicinal value of some plants to modify these conditions has been recognized since ancient times. Nutraceuticals, through a number of different processes, provide a means to combat the onset of disease. These actions include antioxidative, anti-inflammatory, anticancer, and lipid controlling aspects. Currently, some feedstock is supplemented with additional ingredients with desirable properties to help animals to overcome potential adverse health conditions. Nutraceuticals (Wildman 2001; Lockwood 2007; Zoltani 2016) have plant ingredients that help to counter cardiac diseases.

Recently, it has been recognized that nutraceuticals may play an important role by inducing desirable signaling pathways controlling cell function and thereby correcting cardiac dysfunctions (Cicero and Colletti 2018; Ferguson et al. 2016). Free radical and platelet-dependent thrombolic activity and hypertension can be particularly influenced. Nutraceuticals such as n-3[1] fatty acids, quercetin (Larson et al. 2012; Choi et al. 2008), flavonoids, and polyphenols are important for antioxidant defenses. Besides being a regular component of feedstock, nutraceuticals are also available as plant food residues (Varzakas et al. 2016) and fruit residues (Babbar et al. 2015) and in nanoscale nutrient delivery systems (McClements 2015).

The introduction of nutraceuticals to confer cardio benefits sometimes requires preliminary transformations of the component. One involves microalgal fermentation for the production of omega fatty acids and subsequent conversion to prostaglandins, which regulate cell activity and cardiovascular function.

Capillary electrophoresis (CE) is used for the selection of the cardiac useful contents of resveratrol, green tea, and phytoestrogens from soy and flax. A large industry now exists supplying feed supplements containing these and other related items.

C. K. Zoltani (✉)
Emeritus US Army Research Lab, Aberdeen Proving Ground, Aberdeen, MD, USA

[1] In the literature n-3, omega-3, as well as Ω-3 are interchangeably used.

R. C. Gupta et al. (eds.), *Nutraceuticals in Veterinary Medicine*, https://doi.org/10.1007/978-3-030-04624-8_28

2 Nutraceuticals Modulate Genetic Expression

Nutraceutical components of plants are bioactive compounds that involve activation of antioxidation, signal transduction, mitochondrial integrity, and genes (Mishra et al. 2009). Anti-inflammatory effects through inhibition of transcription factors and cytotoxic cytokines are notable. Primarily, these components produce endothelial effects and can forestall undesired cardiac outcomes by reducing adhesion of lipids to the walls of blood vessels and loosen deposits and reduce oxidative stress.

When ingested, nutraceuticals induce several signaling pathways that include the transcription factors NF-κB and Nrf2. They exert their anti-inflammatory and antioxidative control through inhibition. The former, nuclear factor kappa, is a light chain enhancer of activated B cells. These are lymphocytes which are not processed by the thymus gland and are responsible for producing antibodies. In addition, it functions as a protein complex that controls the transcription of DNA, cytokine production, and aspects of cell survival and is cardioprotective during hypoxia and reperfusion injury (Brasier 2006). Overproduction of this activator promotes heart failure by promoting inflammation through cytokines that produce an endoplasmic reticulum stress response.

2.1 NF-κB Regulatory Network

In an inactivated state, NF-κB is located in the cytosol complexed with the inhibitory protein 1κBα. The latter blocks the ability of NF-κB transcription factor to bind to DNA. In the diseased heart, NF-κB is increased. It also regulates a number of genetic programs, including hypertrophy, and provides protection from hypoxia and inflammation (Gordon et al. 2011; Hayden and Ghosh 2012). It can be induced via canonical or noncanonical pathways (Valen et al. 2001). As an inductible transcription factor, NF-κB mediates transient changes in gene expression. When dysregulated, it contributes to the pathophysiology of inflammation as a regulatory molecule. Abnormal NF-κB activity and an abnormal level of miRNAs often go together, along with mutual regulation.

2.2 Nrf2 Regulates Antioxidant and Detoxification Genes

Nrf2 is a potent transcriptional activator. It is a master regulator of an antioxidant response to counteract oxidative stress. In response to oxidative stress, it acts on cytoprotective genes. By binding to antioxidant response elements (ARE), it induces antioxidant enzymes that protect against oxidation damage (Chen et al. 2015; Satta et al. 2017). Elevated expressions of Nrf2, marker of oxidative stress, target genes involved with stress resistance. A large number of molecules are Nrf2 inducers which disrupt Keap1-mediated ubiquitination, allowing Nrf2 protein to accumulate and to bind in the promoter of genes (Chen and Maltagliati 2018).

3 Nutraceuticals that Forestall the Development of Cardiac Dysfunction

Numerous nutraceutical ingredients have been identified that counter the development of cardiac dysfunction. These include antioxidants, polyphenols for control of arterial diseases, and flavonoids that block ACE (angiotensin-converting enzyme) thereby regulating arterial blood pressure and strengthening tiny capillaries that carry oxygen to all cells. Also, omega-3 polyunsaturated fatty acids, vitamins of several kinds, minerals for prevention and treatment of CVD (cardiovascular disease), and curcumin that, among other supplements, improves the condition of the arterial wall (Table 1).

3.1 Antioxidants

Antioxidants hinder the oxidation of molecules and the production of free radicals thereby preventing the onset of disease processes. Highly reactive oxygen, such as hydrogen peroxide (H_2O_2) or the hydroxyl radical, due to their instability, reacts with biological molecules oxidizing the DNA and proteins causing mutations. Enzyme inhibitions and protein degradation can ensue.

The production of ROS is part of the process of generating metabolic energy in the electron transport chain. Mitochondria are major producers of ROS. Defects resulting in the decrease of electron transfer increases ROS production and subsequent pathological conditions.

Oxidative stress, through low-density lipoprotein (LDL) oxidation, contributes to atherosclerosis culminating in cardiovascular disease (Lee et al. 2017). In animals, uric acid with a high concentration of antioxidants is catalyzed to allantoin that is beneficial in stimulating the growth of healthy tissue and repairing wounds and also acts as an antihypertensive agent through activation of the imidazoline receptor. This inhibits the sympathetic nervous system thereby also decreasing cardiac contractility.

Nutraceuticals that contain glutathione, a major antioxidant, is also a new biomarker for the detection of oxidative stress (Irino et al. 2016). The level of 2-aminobutyric acid (2-AB) in circulation indicates the metabolism of glutathione. Changes in 2-AB levels reflect the status of oxidative stress.

Table 1 Selected nutraceuticals with therapeutic potential for amelioration of cardiovascular dysfunction

Nutraceutical	Active ingredient	Affects	Effect	References
Turmeric	Curcumin/piperine	Lipid levels	Antioxidant	Aggarwal and Harikumar (2009)
		Attenuate adriamycin-induced cardiotoxicity	Anti-inflammatory	Alwi et al. (2008)
		p300-HAT inhibitory	Mutates cell signaling	
		Effect on cardiac hypertrophy	Lowers LDL	
		Corrects Ca(2+) homeostasis	Blocks NF-κB, VCAM-1	
Quercetin	Polyphenol Flavonoids	Oxidative stress, blood pressure	Inhibits vascular NADPH	Larson et al. (2012)
		Vascular function	Antioxidative	Choi et al. (2008)
			Inhibitor of NF-κB	
Seaweeds	Macroalgae	Dyslipidemia	Reduces blood lipids	Cardoso et al. (2015)
		Oxidative stress	Improves endothelial function, blood pressure	Dousip et al. (2014)
		Vascular inflammation		
Green tea	Catechin Polyphenols	Vascular effects	Maintains NO homeostasis	Grassi et al. (2013)
		Activates endothelial NO	Reduces blood pressure	
		Suppress platelet adhesion	Curtails inflammation	
		Inhibits transcription factor NF-kB		
Resveratrol	Phytoalexin, stilbene provide resistance to infection	Nuclear factor NF-κB, activates SIRT1	Regulates inflammation	Matos et al. (2012)
		Lowers LDL	Deacetylates histones	Araim et al. (2002)
		Decr endothelial cell ICAM	Transcription factors	Berman et al. (2017)
			Antioxidative	
Carotenoids	Lycopene	Endothelial cells, affect lipid metabolism	Antioxidant	Wang et al. (2014)
		Oxidative stress	Curtails free radicals	

Other indicators include levels of vitamin C, vitamin E, and melatonin.

3.2 Polyphenols

Polyphenol micronutrients contained in plants, fruits, and vegetables analogous to phytoalexin play a role in the prevention of cardiovascular dysfunctions. They are very beneficial due to their antioxidant function and their ability to generate detoxication enzymes. Their effect depends on bioavailability in intestinal absorption rate which varies with the class of polyphenols. Cardiovascular diseases are associated with oxidative stress. Polyphenols have abundant antioxidant properties (Galanakis 2017) and, in addition, regulate genes and take part in cellular signaling (Scalbert et al. 2005). The direct antioxidant action of polyphenols is unlikely. Multifaceted bioactivity is more likely (Hollman et al. 2011). Also, polyphenols are metabolized along pathways used by xenobiotics.

Endothelial damage is heavily dependent on ROS (Khurana et al. 2013) generated during physiological processes, but under excessive stress, the antioxidant system is incapable of maintaining equilibrium and ROS binding to DNA and creating pathological conditions. By modulating signaling pathways, polyphenols strengthen antioxidant and anti-inflammatory defenses.

3.3 Flavonoids

Based on the number of phenol rings and structural elements, the phenols are subdivided into several classes. Flavonoids inhibit the angiotensin-converting enzyme (ACE) activity that regulates arterial blood pressure and electrolyte balance. ACE activity depends on the structure of the flavonoid (Guerrero et al. 2012; Li et al. 2010). Wang et al. (2018) have evaluated the structure activity relationship (SAR) of flavonoids with enzyme systems for cardioprotective activity. Flavonoids modulate the activity of enzymes that lower the production of prostaglandins and leukotrienes which are mediators of inflammation (Dabbou et al. 2018).

The antioxidant and chelating properties of flavonoids are significant (Heim et al. 2002; Prochazkova et al. 2011). By inhibiting peroxidation and the attenuating processes of ROS, radical scavenging and chelating activity is curtailed. Chelation (bonding to a metal and thereby removing it from the blood) of copper or iron helps in the prevention of the formation of free radicals.

According to preclinical evidence, soy phytoestrogens (isoflavones) may be beneficial in reducing serum cholesterol levels (Sacks et al. 2006).

3.4 Polyunsaturated Fatty Acids

Essential fatty acids (EFA) Ω-3 (alpha-linoleic acid) and Ω-6 (linoleic acid) are needed for the generation of hormones, anti-inflammatory substances, and cellular membranes (Mozaffarian and Wu 2011). Fatty acids, part of prostaglandins, control inflammation. They are derived mainly from plants since they cannot be synthesized. Ω-3 and its derivatives, EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid), are also derived from oily fish. EFA deficiency is rare and undesirable.

The proper ratio of the omega-related fatty acids is important. If the ratio of Ω-6/Ω-3 is too high, free radical production is increased. EFAs act on DNA by activating or inhibiting transcription factors that may be linked to pro-inflammatory cytokine production (Calder 2004: Rudra et al. 2001).

Grass-fed animals accumulate more omega-3 than grain-fed animals whose omega-6 rises. Since metabolites of omega-6 are more inflammatory, balancing needs to be accommodated (Simopoulos 2002). Omega-3 fatty acids lower blood pressure, and a reduction in blood triglycerides levels has also been observed.

3.5 Vitamins

Calciferol (vitamin D) acts as a hormone and it regulates more than 200 genes. An association between vitamin D deficiency and endothelial dysfunction and lipid peroxidation (Tarcin et al. 2009) has been noted. Low levels of vitamin D are associated with higher incidences of cardiovascular cases. In coronary disease, the amino acid homocysteine is of importance. Hypertension and increased risk of cardiac dysfunction can be ameliorated by lowering homocysteine (HCys) levels and raising vitamin B6 levels through the use of nutraceuticals.

Forage may be deficient in the required minerals or vitamin A. Nutraceuticals can provide needed supplement (Gadberry 2012) for the daily diet.

3.6 Minerals

The association of copper deficiency abnormal cardiovascular anatomy and "falling disease" has been known for some time (Klevay 2000). Copper deficiency also increases cholesterol and contributes to abnormal electrocardiograms. Some forage is seasonally deficient in copper. Mineral levels in livestock forage are often deficient due to the use of pesticides and herbicides. A list of minerals needed for healthy animals and their function is detailed in (Greenpet 2015).

Hypertension as well as hyperlipidemia has been affiliated with low levels of Zinc (Zn^{2+}) in serum measurements. Zn^{2+} inhibition of gene expression has been associated with the NF-κB (Houston 2010).

Manganese (Mn) is known to bind to vascular smooth muscle and enhances vasodilation, thereby reducing blood pressure. In addition, it affects intracellular sodium, potassium, and calcium content.

The digestion of hay in the bovine gut distributes Mn to several organs. It accumulates in mitochondria, disrupts oxidative phosphorylation, and creates ROS. Mn is a cellular toxicant that impairs enzyme activity and receptor functions. Mn affects the autonomic nervous system with an effect on vascular function and myocardial contraction, leading to hypotension (Jiang and Zheng 2005).

High plant uptake of soil selenium can lead to unexpected diseases in animals consuming such feedstock. Excessive selenium produces free radicals causing oxidative tissue damage when the incorporation of selenium instead of sulfur in proteins disrupts cellular function (Raisbeck 2000). Deficiency of selenium in feedstock causes white muscle disease in calves and lambs. The addition of dietary selenium prevents cardiomyopathy as well as cardiovascular disease (Duthie et al. 1989). Selenium, in conjunction with glutathione peroxidase, removes lipid peroxides formed during oxidative stress with an important effect on DNA synthesis and repair (Souyoul et al. 2018).

3.7 Curcumin

Though characterized as displaying low bioavailability, curcumin ameliorates the development of heart failure and the development of cardiac hypertrophy and is beneficial in preventing atrial arrhythmia (Aggarwal and Harikumar 2009; Alwi et al. 2008). In addition, it improves arterial health and enhances nitric oxide (NO) production by activating endothelial nitric oxide synthase. This enhances the relaxation of vascular smooth muscle and the dilation of vessels. Curcumin is beneficial for the functioning of the endothelium (Gupta et al. 2013). Its powerful antioxidant action is supplemented by anti-inflammatory effects, and side effects in animals are few (Shankar et al. 1980).

Nitric oxide (NO) is synthesized from the amino acid L-arginine in endothelial cells (Tousoulis 2012). Vascular homeostasis depends on the availability of sufficient NO. Heart failure is marked by a diminished release of NO due to altered endothelial equilibrium. NO has a number of

important functions in the maintenance of vascular function. These include (a) free radical scavenging, (b) diminishing endothelial permeability, and (c) acting as an anti-inflammatory agent. This is accomplished by inhibiting the synthesis of cytokines and cell adhesion molecules. NO downregulates platelet aggregation and adherence and minimizes the vasoconstrictive effects of free Ca^{2+} in smooth muscle cells.

3.8 Colostrum

Colostrum is secreted by the female mammary glands of animals. The components of the efflux enhance the function of the immune system and its effectiveness against a variety of common pathogens. Bovine colostrum is an effective nutraceutical for the immune function for a large number of animals, including cattle, sheep, horses, cats, and mice (Taillon and Andreasen 2000; Pandey et al. 2011).

Due to the association of the *Chlamydia* bacterium with arterial plaque formation, it has been proposed that in certain cases, heart disease resembles an autoimmune response. Colostrum's proline-rich polypeptide (PRP) moderates the immune system's adverse effectors (IGF-1, IGF-2). In addition, the growth factor of colostrum acts in the regeneration of cardiac tissue.

3.9 Resveratrol

The phytopolyphenol compound resveratrol (3,5,4-′-trihydroxy-*trans*-stilbene), a nutraceutical found in grapes, berries, and peanuts, exerts anti-inflammatory activity and antioxidative effects. It also increases endothelial NO production and ameliorates the development of atherosclerosis (Matos et al. 2012) by decreasing the intercellular adhesion molecule (ICAM), vascular cell adhesion molecules (VCAM), and interleukin (Berman et al. 2017; Araim et al. 2002). Animals ingesting resveratrol benefit from the effect of improved flow-mediated dilation (FMD) (Magyar et al. 2012), which is also instrumental in overcoming the onset of endothelial dysfunction associated with myocardial perfusion defects.

3.10 EGCG (Epigallocatechin Gallate)

Countries where tea is a common beverage report a lower incidence of cardiovascular diseases. Green tea contains bioactive polyphenols including epigallocatechin gallate (EGCG) which has a potent antioxidant ability (Potenza et al. 2007; Grassi et al. 2013). In vascular endothelial cells, it activates endothelial nitric oxide synthase (eNOS) with NO-dependent vasodilator actions. Endothelial dysfunction contributes to ischemic heart failure. EGCG also binds to and breaks down apolipoprotein A-1, a protein that behaves similarly to amyloid plaques. It also attenuates nicotine cardiotoxicity (Nacera et al. 2017).

More importantly, EGCG downregulates GRK2 (Cannavo et al. 2018). Elevated levels and activity of this kinase lead to the dysfunction of cardiac and adrenal pathways. GRK2 inhibition protects the heart from remodeling and allied dysfunctions.

3.11 Coenzyme Q10

A naturally produced antioxidant, CoQ10 (ubiquinone), plays a vital role as an electron carrier in mitochondrial oxidative phosphorylation. It is significantly decreased in congestive heart failure. CoQ10 improves the function of endothelial cells of blood vessels and plays a vital role in the production of ATP, the source of energy for metabolic processes (Ubbink 2001). In ischemic dogs, CoQ10 protects against necrosis in the heart muscle. Older horses who produce increased lactic acid also benefit from CoQ10. In line with its high energy requirement, CoQ10 is concentrated in cells of organs requiring considerable energy for the heart muscle.

3.12 Carotenoids

Plant pigments cannot be synthesized by animals but are potent reactive species scavengers as they inhibit free radical and singlet lipids. Carotenoids are found in leafy vegetables and yellow/orange fruits. For example, β-carotene is a vitamin-A precursor (Cheng et al. 2017) in addition to being a fee radical scavenger.

Lycopene is a natural carotenoid with antioxidant properties and is also an inhibitor of pro-thrombotic and pro-inflammatory factors with CVD prevention characteristics (Wang et al. 2014). Significant reductions in LDL as well a reduction in systolic blood pressure have been noted.

3.13 Seaweed

Seaweeds, also referred to as macroalgae, are multicellular algae belonging to three different taxonomic groups. Their consumption modifies expressions of cardiovascular diseases, including dyslipidemia, vascular inflammation, hypertension, and oxidative stress as well as activation of the sympathetic system. In animal experiments, *Himanthalia elongate*-treated rats showed a remarkable reduction of total

triglycerides in the plasma. Similarly, *Gracilaria changii* powder significantly lowered total cholesterol, LDL, and total triglycerides in Sprague-Dawley rats (Cardoso et al. 2015; Dousip et al. 2014).

Lipids in seaweed contain abundant polyunsaturated omega-3 and omega-6 fatty acids. Macroalgae peptides incorporate amino acid residues that, as bioactive constituents, alleviate cardiovascular symptoms.

4 Soy

Isoflavone-rich soy protein marginally decreases the plasma lipid profile. The effect is caused by binding to estrogen receptors present in the vasculature. An increase in genistein, an isoflavone of soybeans, in plasma dilates constricted vessels. Improved vascular function (Lockwood 2007) helps in the normalization of blood pressure. Atherosclerotic plaque development is also delayed by consumption of soy isoflavones via inhibition of LDL oxidation, binding to estrogen receptors and inhibition of tyrosine kinase. Soy protein is high in polyunsaturated fatty acids (PUFAs), fiber, vitamins, and minerals, making it beneficial for cardiovascular health.

The USFDA is proposing to revoke the health claim that soy protein reduces the risk of heart disease (FDA 2018) but intends to allow the use of a qualified health claim. This requires a lower standard of evidence than an authorized health claim.

5 Cardiac Biomarkers in Animals

The use of biomarkers for animal health status determination, including respiratory disease, lipid peroxidation, and joint disease beside cardiac status, is now widespread (Oyama 2013; Ferreira et al. 2016). Various cardiac dysfunctions provoke neuroendocrine activation. Increases in plasma norephrine (PNE) and atrial natriuretic factor and activation of the renin-angiotensin-aldosterone systems with increased release of pro-inflammatory cytokines occur (Sisson 2004). A large number of markers, besides the conventional and widely used N-terminal prohormone of brain natriuretic peptide (NT-proBNP), troponin (hs-cTnI), and C-reactive protein (CRP), like atrial natriuretic peptide (ANP) and angiotensin-converting enzyme (ACE), are also measured.

The presence of nutraceuticals can modulate signaling pathways. Curcumin's pleiotropic activity (Gupta et al. 2013) is beneficial in improving lipid profiles in cases of acute coronary symptoms. Elevated serum creatine phosphokinase (CPK) level may indicate muscle or heart damage. When the heart is damaged, the enzyme CPK-MB (creatine kinase-muscle/brain) rises. Nutraceuticals can lower it.

Newer markers, more immediate and organ specific, have come into use. With remarkable potential, miRNAs, endogenous noncoding RNAs that can modulate posttranscriptionally gene expression, are being widely promulgated (Ono et al. 2011; Phuah and Nagoor 2014). By binding to complimentary sequences in the coding region of target messenger RNA (mRNA), they have the ability to regulate cellular activity (Small et al. 2010; Vickers et al. 2014). This ensues from mRNA degradation or alternately by attenuating protein translation. In atrial fibrillation (AF) miRNA-29 and miRNA-26 are downregulated with pathological fibrotic atrial remodeling. The inward-rectifying K^+ current increases (Luo et al. 2013). Lowering miRNA-26 promoted atrial fibrillation (AF) in mice.

Secreted from tissue, miRNAs carry markers of their origin; thus they have the attributes of a conventional biomarker (Zoltani 2014; Maegdefessel 2014). The serum level of miRNA changes remarkably subsequent to cardiac events, paralleling troponin, for example, in myocardial infarction (MI). In heart failure, miR-122 and miR-499 are notably increased.

Epigenetic changes of DNA, produced by histone modifications, constitute changes that regulate gene expression (Tokunaga et al. 2013). Nutraceuticals can reverse methylation of miRNAs (Ahmad et al. 2014; Li and Sarkar 2015). Reversal of epigenetic changes can facilitate favorable cardiac processes.

6 Cardiac Issues in Animals

Some cardiac dysfunction in animals may be alleviated with dietary changes involving nutraceuticals. The choice and extent of use to acquire the required changes vary with species.

6.1 Horses and Bovine

A mature, competitive horse requires up to 30,000 calories per day to function at a high level. The usual forage is supplemented by grains. Additional fat in the diet contains considerably more calories than do the grains. The PUFAs, linoleic acid (LA) and alpha-linoleic acid (ALA), need to be in balance. Horses are not routinely affected by coronary artery disease. Heart attack in horses generally occurs from rupture of the aorta or arrhythmia triggered by adrenaline that suddenly stops the heart (Loynachan 2010). Systolic myocardial failure, i.e., reduction in the ability of the heart to contract, causes reduction in normal blood flow. Thoroughbreds experience supraventricular premature contraction but this does not increase the risk of sudden death (Marr and Bowen 2010). Horses with cardiac problems have

elevated aldosterone concentration culminating in compromised ventricular function (Van Der Vekens et al. 2012).

For equine forage, it is known that in the inherited polysaccharide storage myopathy (PSSM), modification of the diet can remove the extra glycogen stored in their muscles. The nutraceutical hawthorn (an evergreen), when digested, dilates peripheral blood vessels improving circulation. It contains a large number of flavonoids which are potent anti-inflammatory agents. It also affects blood pressure and lipids in circulation. Cayenne (*Capsicum annuum*) is an excellent vasodilator, and digested cleavers lowers blood pressure. Omega-3 fatty acids, contained in nutraceuticals such as flax seeds, contain alpha-linolenic acid (ALA) that is converted to EPA and DHA, both important for cardiovascular health (Pomeroy 2011; Jones 1997; National Research Council 2007).

Aside from congenital and high altitude-related cardiac dysfunctions (bovine pulmonary hypertension (BPH)), there are several other diseases that may be of concern (Peek and Divers 2018) in cattle. Bovine principal heart diseases include pericarditis, brisket disease, and right-sided congestive heart failure (RHF) (Buczinski et al. 2010; Neary et al. 2016). The most commonly acquired heart disease in cattle is bacterial endocarditis that causes arrhythmia and cardiomyopathy, but in general, cardiac diseases are not frequent. White muscle disease in cattle, caused by lack of vitamin E, damages cardiac muscle and Purkinje fibers. It can be alleviated with nutraceuticals and sodium selenite that activates antioxidant enzymes (Bhattacharyya and Roy 2015).

6.2 Cats and Dogs

Over 10% of dogs have heart disease. Chronic valvular heart disease (CVHD), affecting some 85% of older dogs, constitutes about 75% of canine heart disease (Atkins et al. 2009; Harker-Murray et al. 2000). It affects the left atrioventricular or mitral valve. Males are affected more than females and it is more prevalent in smaller breeds. Endothelial cell changes are also noted. Mitral valve prolapse is a feature of CVHD.

Coronary heart disease is unusual for cats and dogs (Devi and Jani 2009). Ventricular arrhythmia is frequent in dogs with cardiomyopathy. The Boxer is prone to right ventricular cardiomyopathy (Smith et al. 2007), but when fed an Ω fatty acid dietary supplement, arrhythmia is minimized.

Some of the adverse heart conditions in animals are due to the deposition of LDL. Proprotein convertase subtilisin/kexin type 9 (PCSK9), an enzyme that regulates LDL cholesterol levels, is present in the nutraceuticals (curcumin and berberine) and alleviates this condition.

Cardiomyopathy, where the cardiac muscles are unable to maintain the required blood flow, is the primary heart disease of cats. The mitral valve leaflets are usually affected. Hypertrophic cardiomyopathy (HCM) affects 10–15% of cats. Cardiac walls thicken and become less flexible with reduction in the amount of blood pumped. The left-side build-up surrounds the lungs but it can be affected by the diet. While congestive heart failure and arterial thromboembolism (ATE) are common, arrhythmia is uncommon.

Several nutritional supplements that may be missing from the available animal foodstuffs are essential for cardiac health. Dogs suffering from heart failure are deficient in EPA and DHA which omega-3 fatty acids can provide. Boxers and Doberman Pinschers with dilated cardiomyopathy (DCM) have a carnitine deficiency. L-carnitine is an amino acid used for energy production in the heart. Cases of deficient concentrations of L-carnitine go hand in hand with raised levels of triglycerides, a marker of myocardial disease. Labradors with cardiomyopathy show clinical improvement when supplemented with L-carnitine. Normal heart function also requires taurine, an amino acid usually in high concentration in the heart. Taurine deficiency causes DCM. Cocker Spaniels also suffer from DCM that can be remedied both by L-carnitine and taurine.

Cats also need taurine, but their ability to manufacture it is restricted, and DCM, with proper diet, can be prevented. Taurine, an essential amino acid obtained from animal-based proteins, is critical for normal vision and heart muscle function. "Essential" designation for amino acids means that it must be part of the diet since cats have only a limited capability to manufacture taurine.

CoQ10, the antioxidant and energy production aid for some animals, is only marginally used in older cats and dogs. Nutraceutical nanoemulsions that use a long-chain fatty acid (coenzyme Q10) demonstrated in a rat feeding study the possibilities for the use of nonstandard techniques (Cho et al. 2014).

7 Concluding Remarks and Future Directions

Nutraceuticals are not drugs but components of plants that upon ingestion lessen, and in some cases bar, the development of cardiac dysfunction. In this role, they have attracted a large number of adherents. Cardiac problems have been alleviated by foods containing components that slow the development of processes leading to the development of cardiac disease. Dissenters of this appraisal exist (Aronson 2017). However, contrary arguments emphasize the use of dietary supplements, not their value as desirable components of feedstock. Clinical proofs of the "don't work" hypothesis are unavailable. Overwhelmingly, it is believed that the

choice of nutrients and particular components can play an important role in preventing the development of cardiac problems. The gathering of data is ongoing. Synthetic biology (Nielsen and Keasling 2016) will enhance our understanding of the issue and provide additional answers in the future.

References

Aggarwal BB, Harikumar KB (2009) Potential therapeutic effects of curcumin, the anti-inflammatory agent, against neurodegenerative, cardiovascular, pulmonary, metabolic, autoimmune and neoplastic diseases. Int J Biochem Cell Biol 41(1):40–59

Ahmad A, Li Y, Bao B et al (2014) Epigenetic regulation of miRNA-cancer stem cell nexus by nutraceuticals. Mol Nutr Food Res 58 (1):79–86

Alwi I, Santoso T, Suyomo S et al (2008) The effect of curcumin on lipid level in patients with acute coronary syndrome. Acta Med Indones 40(4):201–210

Araim O, Ballantyne J, Waterhouse AL et al (2002) Inhibition of vascular smooth muscle cell proliferation with red wine and red wine polyphenols. J Vasc Surg 35(6):1226–1231

Aronson JK (2017) Defining 'nutraceuticals': neither nutritious nor pharmaceutical. Br J Clin Pharmacol 83:8–19

Atkins C, Bonagura J, Ettinger P et al (2009) Guidelines for the diagnosis and treatment of canine chronic valvular heart disease. J Vet Intern Med 23:1142–1150

Babbar N, Oberoi HS, Sandhu SK (2015) Therapeutic and nutraceutic potential of bioactive compounds extracted from fruit residues. Crit Rev Food Sci Nutr 55:319–337

Berman AY, Motechin RA, Wiesenfeld MY et al (2017) The therapeutic potential of resveratrol: a review of clinical trials. NPJ Precis Oncol 1 (1):35

Bhattacharyya A, Roy D (2015) Nutraceuticals in livestock and poultry. New India Publishing Agency, New Delhi

Brasier AR (2006) The NF-kappaB regulatory network. Cardiovasc Toxicol 6(2):111–130

Buczinski S, Rezakhani A, Boerboom D (2010) Heart disease in cattle: diagnosis, therapeutic approaches and prognosis. Vet J 184:258–263

Calder PC (2004) n-3 fatty acids and cardiovascular disease: evidence explained and mechanisms explored. Clin Sci (Lond) 107(1):1–11

Cannavo A, Komici K, Bencivenga L et al (2018) GRK2 as a therapeutic target for heart failure. Expert Opin Ther Targets 22(1):75–83

Cardoso SM, Pereira OR, Seca AML et al (2015) Seaweeds as preventive agents for cardiovascular diseases: from nutrients to functional foods. Mar Drugs 13:6838–6865

Chen QM, Maltagliati AJ (2018) Nrf2 at the heart of oxidative stress and cardiac protection. Physiol Genomics 50(2):77–97

Chen B, Lu Y, Chen Y et al (2015) The role of Nrf2 in oxidative stress-induced endothelial injuries. J Endocrinol 225(3):R83–R99

Cheng HM, Koutsidis G, Lodge JK et al (2017) Tomato and lycopene supplementation and cardiovascular risk factors: a systematic review and meta-analysis. Atherosclerosis 257:100–108

Cho HT, Salvia-Trujillo L, Kim J et al (2014) Droplet size and composition of nutraceutical nanoemulsions influences bioavailability of long chain fatty acids and Coenzyme Q10. Food Chem 156:117–122

Choi EJ, Bae SM, Ahn WS (2008) Antiploriferative effects of quercetin through cell cycle arrest and apoptosis in human breast cancer MDA-MB-453 cells. Arch Pharm Res 31:1281–1285

Cicero AFG, Colletti A (2018) Handbook of nutraceuticals for clinical use. Springer, Cham

Dabbou S, Gasco L, Rotolo L et al (2018) Effects of dietary alfalfa flavonoids on the performance, meat quality and lipid oxidation of growing rabbits. Asian-Australas J Anim Sci 31(2):270–277

Devi S, Jani RG (2009) Review on nutritional management of cardiac disorders in canines. Vet World 2(12):482–485

Dousip A, Matanjun P, Sulaiman MR et al (2014) Effect of seaweed mixture intake on plasma lipid and antioxidant profile of hypercholeserolaemic rats. J Appl Physicol 26:999–1008

Duthie GG, Wahle KW, James WP (1989) Oxidants, antioxidants and cardiovascular disease. Nutr Res Rev 2(1):51–62

FDA (2018.) https://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm582744.htm

Ferguson JF, Hooman A, Gerszten RE et al (2016) Nutrigenomics, the microbiome, and gene-environment interactions: new directions in cardiovascular disease research, prevention and treatment. Circ Cardiovasc Genet 9:291–313

Ferreira FS, Barretto FL, Fabres A et al (2016) Cardiac markers in five different breeds of rabbits (Oryctolagus cuniculus Linnaeus, 1758) used for cardiovascular research. Pesq Vet Bras 36(8):737–742

Francis GS, Benedict C, Johnstone DE et al (1990) Comparison of neuroendocrine activation in patients with left ventricular dysfunction with and without congestive heart failure. Circulation 82:1724–1729

Gadberry S (2012) Mineral and vitamin supplementation of beef cows in Arkansas. https://www.uaex.edu/publications/pdf/FSA-3035.pdf

Galanakis C (ed) (2017) Nutraceutical and functional food components. Academic Press, New York

Gordon JW, Shaw JA, Kirshenbaum LA (2011) Multiple facets of NF-kB in the heart: to be or not to NF-kB. Circ Res 108:1122–1132

Grassi D, Desideri G, Di Giosia F et al (2013) Tea, flavonoids, and cardiovascular health: endothelial protection. Am J Clin Nutr 98 (Suppl 6):1660S–1666S

Greenpet (2015) Minerals for healthy animals. https://www.greenpet.com.au/minerals-for-healthy-animals/

Guerrero L, Castillo J, Quinones M et al (2012) Inhibition of angiotensin-converting enzyme activity by flavonoids: structure-activity relationship studies. PLoS One 7(11):1–11

Gupta C, Patchva S, Aggarwal BG (2013) Therapeutic roles of curcumin: lessons learned from clinical trials. AAPS J 15(1):195–218

Harker-Murray AK, Tajik AJ, Ishikura F et al (2000) The role of coenzyme Q_{10} in the pathophysiology and therapy of experimental congestive heart failure in the dog. J Card Fail 6(3):233–242

Hayden MS, Ghosh S (2012) NF-κB, the first quarter-century: remarkable progress and outstanding questions. Genes Dev 26:203–234

Heim KE, Tagliaferro AR, Bobilya DJ (2002) Flavonoid antioxidants: chemistry, metabolism and structure-activity relationships. J Nutr Biochem 13(10):572–584

Hollman PCH, Cassidy A, Comte B et al (2011) The biological relevance of direct antioxidant effects of polyphenols for cardiovascular health in humans is not established. J Nutr 141:989S–1009S

Houston MC (2010) The role of cellular micronutrient analysis, nutraceuticals, vitamins, antioxidants and minerals in the prevention and treatment of hypertension and cardiovascular disease. Ther Adv Cardiovasc Dis 4(3):165–183

Irino Y, Toh R, Nagao M et al (2016) 2-Aminobutyric acid modulates glutathione homeostasis in the myocardium. Sci Rep 6:36749

Jiang Y, Zheng W (2005) Cardiovascular toxicities upon manganese exposure. Cardiovasc Toxicol 5(4):345–354

Jones WE (1997) Nutraceuticals for equine practice. J Equine Vet Sci 17 (11):562–572

Khurana S, Venkataraman K, Hollingsworth A et al (2013) Polyphenols: benefits to the cardiovascular system in health and in aging. Nutrients 5:3779–3827

Klevay LM (2000) Cardiovascular disease from copper deficiency-a history. J Nutr 130:489S–492S

Larson AJ, Symons JD, Jalili T (2012) Therapeutic potential of quercetin to decrease blood pressure: review of efficacy and mechanisms. Adv Nutr 3:39–46

Lee MT, Lin WC, Yu B et al (2017) Antioxidant capacity of phytochemicals and their potential effects on oxidative status in animals. Asian-Australas J Anim Sci 30:299–308

Li Y, Sarkar FH (2015) Targeting epigenetically deregulated miRNA by nutraceuticals: focusing on cancer prevention and treatment. Curr Pharm Rep 1:1–10
Li SH, Liu XX, Bai YY et al (2010) Effect of oral isoflavone supplementation on vascular endothelial function in postmenopausal women: a meta-analysis of randomized placebo-controlled trials. Am J Clin Nutr 91:480–486
Lockwood B (2007) Nutraceuticals, 2nd edn. Pharmaceutical Press, London
Loynachan A (2010) 'Heart attacks' and heart disease in horses. Equine Dis Q 19(4):5–6
Luo X, Pan Z, Shan H et al (2013) MicroRNA-26 governs profibrillatory inward-rectifier potassium current changes in atrial fibrillation. J Clin Invest 123:1939–1951
Maegdefessel L (2014) The emerging role of microRNAs in cardiovascular disease. J Intern Med 275:633–644
Magyar K, Halmosi R, Palfi A et al (2012) Cardioprotection by resveratrol: a human clinical trial in patients with stable coronary artery disease. Clin Hemorheol Microcirc 50(3):179–187
Marr CM, Bowen M (2010) Cardiology of the horse, 2nd edn. Saunders Elsevier, Philadelphia
Matos RS, Baroncini LAV, Precoma LB et al (2012) Resveratrol causes antiatherogenic effects in an animal model of atherosclerosis. Arq Bras Cardiol 98(2):136–142
McClements DJ (2015) Nanoscale nutrient delivery systems for food applications: improving bioactive dispersibility, stability, and bioavailability. J Food Sci 80(7):1602–1611
Mishra S, Singh RB, Dwivedi SP et al (2009) Effects of nutraceuticals on genetic expressions. Open Nutraceuticals J 2:70–80
Mozaffarian D, Wu JHY (2011) Omega-3 fatty acids and cardiovascular disease. J Am Coll Cardiol 58(3):2047–2067
Nacera H, Gregory T, Sihem B et al (2017) Green tea beverage and epigallocatecihin gallate attenuate nicotine cardiotocytotoxicity in rat. Acta Pol Pharm 74(1):277–287
National Research Council (2007) Nutrient requirements of horses, 6th revised edn. National Academic Press, Washington, DC
Neary JM, Booker CW, Wildman BK (2016) Right-sided congestive heart failure in North American feedlot cattle. J Vet Intern Med 30:326–334
Nielsen J, Keasling JD (2016) Engineering cellular metabolism. Cell 164:1185–1197
Ono K, Kuwabara Y, Han J (2011) MicroRNAs and cardiovascular diseases. FEBS J 276(10):1619–1633
Oyama MA (2013) Using cardiac biomarkers in veterinary practice. Vet Clin North Am Small Anim Pract 43(6):1261–1272
Pandey NN, Dar AA, Mondal DB et al (2011) Bovine colostrum: a veterinary nutraceutical. J Vet Med Anim Health 3(3):31–35
Peek SF, Divers TJ (2018) Rebhun's diseases of dairy cattle, 3rd edn. Elsevier, St Louis
Phuah NH, Nagoor NH (2014) Regulation of microRNAs by natural agents: new strategies in cancer therapies. Biomed Res Int 2014:804510
Pomeroy LA (2011) The equine heart: beyond the x-factor. Holistic Horse. https://holistichorse.com/health-care/the-equine-heart-beyond-the-x-factor/
Potenza MA, Marasciulo FL, Tarquinio M et al (2007) EGCG, a green tea polyphenol, improves endothelial function and insulin sensitivity, reduces blood pressure, and protects against myocardial I/R injury in SHR. Am J Physiol Endocrinol Metab 292:E1378–E1387
Prochazkova D, Bousova L, Wilhelmmova N (2011) Antioxidant and prooxidant properties of flavonoids. Fitoterapia 82:513–523
Raisbeck MF (2000) Selenosis. Vet Clin North Am Food Anim Pract 16 (3):465–480
Rudra PK, Nair SSD, Leitch JW et al (2001) Omega-3 polyunsaturated fatty acids and cardiac arrhythmias. In: Wildman REC (ed) Handbook of nutraceuticals and functional foods. CRC Press, Boca Raton, p 331
Sacks FM, Lichtenstein A, Van Horn L et al (2006) Soy protein, isoflavones, and cardiovascular health: an American Heart Association science advisory for professionals from the nutrition committee. Circulation 113:1034–1044
Satta S, Mahmoud AM, Wilkinson FI et al (2017) The role of Nrf2 in cardiovascular function and disease. Oxid Med Cell Long 2017:9237263
Scalbert A, Manach C, Morand C et al (2005) Dietary polyphenols and the prevention of diseases. Crit Rev Food Sci Nutr 45(4):287–306
Shankar TNB, Shantha NV, Ramesh HP (1980) Toxicity studies on tumeric (*Curcuma longa*): acute toxicity studies in rats, guinea pigs & monkeys. Indian J Exp Biol 18:73–75
Simopoulos AP (2002) The importance of the ratio of omega-6/omega-3 essential fatty acids. Biomed Pharmacother 56(8):365–379
Sisson DD (2004) Neuroendocrine evaluation of cardiac disease. Vet Clin North Am Small Anim Pract 34:1105–1126
Small EM, Frost RJA, Olson EN (2010) MicroRNAs add a new dimension to cardiovascular disease. Circulation 121(8):1022–1032
Smith CE, Freeman LM, Rush JE et al (2007) Omega-3 fatty acids in boxer dogs with arrhythmogenic right ventricular cardiomyopathy. J Vet Intern Med 21:265–273
Souyoul SA, Saussy KP, Lupo MP (2018) Nutraceuticals: a review. Dermatol Ther (Heidelb) 8(1):5–16
Taillon C, Andreasen A (2000) Veterinary nutraceutical medicine. Can Vet J 41:231–234
Tarcin O, Yavuz DG, Ozben B et al (2009) Effect of vitamin D deficiency and replacement on endothelial function in asymptomatic subjects. J Clin Endocrinol Metab 94(10):4023–4030
Tokunaga M, Takahashi T, Singh RB (2013) Nutrition and epigenetics. Med Epigenet 1:70–77
Tousoulis D, Kampoli AM, Tentolouris C et al (2012) The role of nitric oxide on endothelial function. Curr Vasc Pharmacol 10(1):4–18
Ubbink JB (2001) Coenzyme Q as a marker of oxidative stress in coronary artery disease. In: Kagan V, Quinn PJ (eds) Coenzyme Q: molecular mechanisms in health and disease (modern nutrition). CRC Press, Boca Raton
Valen G, Yan Z, Hansson GK (2001) Nuclear factor kappa-B and the heart. J Am Coll Cardiol 38(2):307–314
Van Der Vekens N, Decloedt A, De Clercq D et al (2012) The use of cardiac biomarkers in veterinary medicine: the equine perspective. Vlaams Diergeneeskundig Tijdschrift 2012(81):319–327
Varzakas V, Zakynthinos G, Verpoort F (2016) Plant food residues as a source of nutraceuticals and functional foods. Foods 5:88
Vickers KC, Rye KA, Tabet F (2014) MicroRNA in the onset and development of cardiovascular disease. Clin Sci (Lond) 126:183–194
Wang X, Lv H, Gu Y (2014) Protective effect of lycopene on cardiac function and myocardial fibrosis after acute myocardial infarction in rats via the modulation of p38 and MMP-9. J Mol Histol 45:113–120
Wang T, Li Q, Bi K (2018) Bioactive flavonoids in medicinal plants: structure, activity and biological fate. Asian J Pharm Sci 13:12–23
Wildman REC (ed) (2001) Handbook of nutraceuticals and functional foods. CRC Press, Boca Raton
Zoltani CK (2014) Cardiovascular toxicity biomarkers. In: Gupta RC (ed) Biomarkers in toxicology. Elsevier-Academic Press, Amsterdam
Zoltani CK (2016) Nutraceuticals in cardiovascular diseases. In: Gupta RC (ed) Nutraceuticals, efficacy, safety and toxicity. Elsevier-Academic Press, Amsterdam

Nutraceuticals in Hepatic and Pancreatic Diseases

Sharon M. Gwaltney-Brant

Abstract

The unique anatomy and physiology of the liver make it especially susceptible to insult from a variety of metabolic, infectious, immune-mediated, toxic, and carcinogenic sources. Many nutraceutical compounds have been utilized in an attempt to aid in the support or improvement of liver health through antioxidant, anti-inflammatory, antifibrotic, antiproliferative, or antineoplastic mechanisms. Similarly, many nutraceuticals have been investigated for their utility in managing pancreatic disorders, especially diabetes mellitus. This chapter describes the pathophysiology and effects of select nutraceuticals on the liver and pancreas.

Keywords

Antioxidant · Dietary supplement · Hepatoprotective · Hepatotoxicity · Hepatotropic · Herbal · Liver · Nutraceutical

1 Introduction

As the largest internal organ in the body, the liver has essential roles in nutrient homeostasis, filtration of particulates, protein synthesis, bioactivation and detoxification, formation of bile, and biliary secretion and excretion (Jaeschke 2008). Via its extensive portal blood supply, the liver is exposed to virtually all ingested compounds that are absorbed from the gastrointestinal tract, making it susceptible to ingested toxicants. Additionally, the liver's role in the processing, biotransformation, and detoxification can result in exposure to potentially toxic intermediates or metabolites of xenobiotics. In humans, the most common cause of acute liver failure leading to liver transplantation in the United States is hepatic injury secondary to exposure to drugs or chemicals, and liver injury is the leading cause of regulatory action against drugs (Watkins and Seef 2006).

S. M. Gwaltney-Brant (✉)
Veterinary Information Network, Mahomet, IL, USA
e-mail: Sharon@vin.com

2 Liver

2.1 Hepatic Injury and Repair

2.1.1 Mechanisms of Injury

A variety of mechanisms are involved in hepatic injury including damage, triggering of apoptosis (programmed cell death), formation of reactive intermediates, disruption of cell metabolism, cytoskeletal damage, and stimulation of autoimmune responses (Gwaltney-Brant 2016). Direct damage to hepatocellular membranes can occur via mechanical injury such as lysis caused by infectious agents (e.g., viruses, parasites), lipid peroxidation, or disruption/dysfunction of membrane proteins and transporters. Reactive oxygen species (ROS) and other electrophiles produced during inflammation or in biotransformation reactions can trigger lipid peroxidation which, in the absence of antioxidants, can propagate and expand membrane far beyond the site of original injury. Disruption of cellular membranes results in spillage of cytosolic components into the extracellular matrix, which then triggers inflammatory responses which can compound hepatocellular injury. The cause of hepatocellular injury frequently dictates the distribution of injury within the liver. For instance, bacterial hepatitis is frequently multifocal in nature with no particular pattern, while hepatic injury from reactive metabolites formed during biotransformation of xenobiotics tends to occur in centrilobular areas, where biotransforming enzymes are most plentiful (Stalker and Hayes 2007).

The liver is highly vascular with extensive blood supply, but hypoxia can still cause significant hepatocellular injury, death, and fibrosis. Its proximity to the inferior vena cava makes the hepatic vein subject to reduced outflow when abnormal cardiovascular function, such as right heart failure,

R. C. Gupta et al. (eds.), *Nutraceuticals in Veterinary Medicine*, https://doi.org/10.1007/978-3-030-04624-8_29

occurs. The resulting stasis of blood around hepatic central veins can, over time, result in development of centrilobular fibrosis. Because centrilobular hepatocytes are already relatively oxygen deprived, they are more prone to hypoxic injury during situations where oxygen carrying capacity of the blood is compromised (e.g., anemia).

Apoptosis (programmed cell death) is a normal component of cell turnover, but it can also be triggered by a variety of pathologic situations including hepatotoxicosis, ionizing radiation exposure, neoplasia, and infection by hepatotropic viruses (Elmore 2007; Wang 2014). Unlike cellular necrosis, apoptosis is an energy-requiring process that eliminates nonessential or pathologically injured cells. Apoptosis is an ordered, systematic "shutdown" of cellular function culminating in condensation and fragmentation of cytoplasmic and nuclear component without loss of cell membrane integrity. Cellular fragments are quickly phagocytosed by local macrophages, but inflammatory responses are generally not triggered. Compared to cellular necrosis which generally involves multiple adjacent cells and is accompanied by an inflammatory response, apoptosis results in spotty cell loss with minimal to no associated inflammation.

Generation of free radicals within hepatocytes can occur during xenobiotic biotransformation, normal metabolic processes involving redox reaction, inflammatory states mediated by nitric oxide, and exposure to ionizing radiation (Bischoff and Ramaiah 2007). Free radicals have unpaired electrons that make them highly susceptible to binding macromolecules including lipoproteins of cell membranes, enzymatic proteins, and nucleic acids. The process of free radical binding to macromolecules generates additional free radicals, resulting in a snowballing effect of free radical generations. Cells have antioxidant scavenger molecules, particularly glutathione (GSH), that react with and detoxify free radicals, breaking up their propagation; however, excessive free radical formation can deplete cellular antioxidants, resulting in binding of free radicals to vital cellular structures. Free radical damage can alter cell permeability, inactivate membrane-associated proteins, and alter polarity of organelle membranes. DNA damage induced by free radicals can interfere with gene transcription or translation, resulting in decreased protein synthesis. Inactivation of enzymatic proteins can interfere with normal cell metabolism, leading to cellular degeneration, necrosis, or apoptosis. Disruption of proteins that compose the hepatocyte cytoskeleton can result in loss of cell-to-cell adhesion, rounding of hepatocytes, and release of free hepatocytes into the systemic circulation (Stalker and Hayes 2007).

Reactive oxygen species (ROS) are normally formed in the process of mitochondrial cellular respiration, and endogenous antioxidant systems exist to intercept these ROS before they can cause oxidant injury to the mitochondrion. Increased risk of mitochondrial oxidative injury can occur from increased ROS production, impaired electron transport with increased reduced intermediates, increased mitochondrial membrane permeability, and reduced or depleted antioxidant stores. Depending on the nature and severity of mitochondrial injury, cell death due to apoptosis or necrosis may ensue.

2.1.2 Response to Injury

The liver's response to injury depends on the type of insult, extent of insult, duration of insult, cell populations affected, systemic response to insult, and collateral damage. Despite a large list of potential causes of liver injury (e.g., infectious, inflammatory, neoplastic, toxic, metabolic processes), the types of responses of the liver to injury are quite limited. The clinical manifestations of these responses will vary based on the severity and extent of hepatic injury that occurs. The most general response of hepatocytes to nonlethal insult is hepatocellular degeneration, characterized by cellular swelling (Crawford 2005). Degenerate hepatocytes can accumulate a variety of substances including fat, pigment, glycogen, and copper. Oftentimes hepatocellular degeneration is a reversible, transient event; however, with more severe injury, degeneration can progress to cell death via necrosis or apoptosis. With infectious or inflammatory insults, the influx of inflammatory cells can contribute to hepatocellular injury due to ROS or other mediators produced by inflammatory cells. Cholestatic hepatic disease can occur with hepatic injury that interferes with the ability of hepatocytes to excrete bile into canaliculi or that later transport bile through the biliary tree. Cholestasis may be caused by metabolic derangements in hepatocytes, hepatocellular swelling, impediments to canalicular transport, or mechanical obstruction of the biliary tree or gall bladder. Cholestatic disease can result in systemic buildup of biliary solutes within the blood and other tissues, leading to the yellow-orange cast of icterus or jaundice. Steatosis (fatty liver, hepatic lipidosis, hepatosteatosis) is a common marker of moderate to severe hepatocellular insult. Hepatocytes become distended with lipids, a condition that, early on, is generally benign and reversible; however, over time steatosis can progress to steatohepatitis, which can result in chronic progressive hepatic injury, fibrosis, and possibly neoplasia.

The liver has an extensive capacity for repair and regeneration following acute or chronic insults. The extent of regeneration that can occur depends on the balance between a variety of hepatocellular factors including cell cycle regulation, metabolism, angiogenesis, cell adhesion, and extracellular matrix components (Duarte et al. 2015). Deposition of excessive extracellular matrix proteins most commonly begins in the periportal areas, centrilobular areas, or within the space of Disse (the space between apical hepatocyte membrane and adjacent endothelial cells), and it interferes with the exchange of nutrients and oxygen between hepatocytes and the sinusoidal blood, furthering

hepatocellular stress and interfering with normal hepatocellular function (Jaeschke 2008). Hepatic stellate cells produce the majority of hepatic extracellular matrix proteins, whose activation is the initial step in hepatic fibrosis. Activation of hepatic stellate cells occur in the presence of products formed during hepatocellular injury, such as ROS and lipid peroxidation products, or by cytokines released by damaged sinusoidal endothelial cells, Kupffer cells, activated platelets, or inflammatory cells. Hepatic stellate cell activation leads to alteration of the extracellular matrix through the replacement of non-fibrillar basement membrane matrix with fibril-forming collagens. As collagen deposition progresses, disruption of the hepatic architecture by fibrous scars results in islands of regenerating hepatocytes surrounded by bands of dense fibrous connective tissue, a condition known as cirrhosis. Cirrhotic areas are poorly functional, and hepatic failure occurs when sufficient liver tissue is affected. Hepatic cirrhosis is not as commonly identified in veterinary species as it is in humans, where viral hepatitis, alcohol, and nonalcoholic steatosis are common causes (Jaeschke 2008).

Chronic liver injury with inflammation and/or cirrhosis may progress to hepatocellular carcinoma (Jaeschke 2008). In humans, hepatocellular carcinoma has been strongly associated with viral hepatitis, metabolic disorders such as hemochromatosis, nonalcoholic steatosis, and toxicants such as aflatoxin, alcohol, and exogenous androgens, although absolute proof of cause and effect has yet to be discovered.

2.2 Nutraceuticals and the Liver

2.2.1 *S*-Adenosylmethionine

S-Adenosylmethionine (SAMe) is an endogenous compound created through enzymatic conversion of dietary methionine (Center 2004). In health, sufficient SAMe is generated through de novo synthesis via dietary intake of methionine, protein catabolism, and salvage pathways involving trimethylglycine or methyltetrahydrofolate and vitamin B_{12} (Bottiglieri 2002). SAMe functions as a methyl group donor in transmethylation pathways, as a precursor to sulfur containing compounds in transsulfuration pathways, and in production of polyamines via polyamine pathways (Friedel et al. 1989). SAMe is involved in more than 100 reactions catalyzed by methyltransferases including biosynthesis of phospholipids, biosynthesis of L-carnitine, biosynthesis of creatine, formation of neurotransmitters and neural receptors, and reactions involving RNA, DNA, proteins, and other endogenous metabolites (e.g., steroid hormones) (Bottiglieri 2002; Friedel et al. 1989). Transsulfuration pathways in the liver provide endogenous sulfur compounds including sulfates, taurine, and GSH, and hepatic SAMe serves as the major source of hepatic GSH (Center 2004). SAMe therefore is a major contributor to hepatic and systemic redox status, influencing resistance to oxidative injury and protection from toxic electrophiles or adducts produced during xenobiotic metabolism. Polyamine pathways impact cell replication, tissue regeneration and growth, DNA synthesis, and cell response to apoptotic signals. Polyamine pathways generate methylthioadenosine (MTA) via decarboxylation of SAMe; MTA is a major hepatocyte signaling molecule and can be used to regenerate methionine (Mato et al. 2002). SAMe administration is associated with alteration of hepatic disease progression, increase in hepatic GSH stores, improved hepatic mitochondrial function, and reduction in cytokine-mediated detrimental effects (Center 2004; Center et al. 2005a, b). Restoration of hepatic and mitochondrial GSH stores in patients with liver disease improves tolerance to injury from free radicals, cholestasis, and ischemia-reperfusion. SAMe was shown to increase GSH levels and improve redox potential in hepatocytes of normal cats (Center et al. 2005a). Dogs receiving SAMe in conjunction with CCNU (lomustine) therapy for malignancies had improved liver values and were less likely to have therapy discontinued due to CCNU-induced hepatopathy than were dogs that received CCNU alone (Skorupski et al. 2011). SAMe improved oxidative status in dogs on long-term prednisolone but did not prevent vacuolar hepatopathy from developing (Center et al. 2005b). Several case reports have been published on the use of SAMe in dogs with toxicant-induced hepatopathy with the suggestion that SAMe improved outcome, although without a control group, the role of SAMe in these cases is no certain (Schmid and Hovda 2016; Wallace et al. 2002).

SAMe is used in veterinary medicine as an adjunct therapy in the management of necroinflammatory, metabolic, and cholestatic hepatopathies, primarily in dogs and cats (Vandeweerd et al. 2013). SAMe has been shown to aid in restoring hepatocellular function, attenuate free radical production, mitigate cytokine-induced injury, reduce inflammation, enhance detoxification mechanisms and toxicant elimination, improve membrane function, and stimulate hepatocellular repair (Center 2004). Oral dosages of 20 mg/kg of stabilized salt or 53 mg/kg of enteric-coated tablets resulted in significant increases in plasma SAMe concentrations in dogs and cats. SAMe is absorbed across the small intestine and must be administered as enteric-coated formulations in order to be absorbed in pharmacologically relevant oral doses (Center 2004). Maximum plasma levels occur with 1–4 h in dogs and 2–8 h in cats. SAMe appears to be generally well tolerated, with occasional anorexia, nausea, or food refusal occurring within a few hours of administration. Rarely, anxiety may occur which may require discontinuation of SAMe administration. In humans, coadministration of SAMe with a monoamine oxidase inhibitor was associated with development of serotonin syndrome. Toxicity studies in rodents revealed an oral LD_{50} of greater

than 4650 mg/kg; and rats receiving 200 mg/kg body weight per day for 104 weeks showed no adverse effects (Center 2004). Similarly, no toxic effects were noted in cats receiving 40–65 mg/kg per day for over 100 days or in dogs receiving 20 mg/kg daily for 6 months.

2.2.2 *N*-acetylcysteine

N-acetylcysteine (NAC) is the acetylated form of the amino acid cysteine that serves as a thiol donor in metabolic reactions; only the L-isomer of NAC is used therapeutically. NAC can serve as a rapid source of sulfhydryl groups whether administered intravenously or orally. Oral bioavailability is low due to extensive first-pass metabolism in the intestine and liver. NAC is deacetylated to cysteine in enterocytes and hepatocytes, where it stimulates GSH synthesis via enhanced glutathione-S-transferase activity, enhances detoxification of many hepatotoxic agents, and has direct antioxidant activity against free radicals such as HOCl, superoxide, $OH^{\cdot}$, and H_2O_2 (Center 2004). NAC enhances red blood cell GSH, maintains hepatocyte fluidity, maintains antioxidant enzyme activity within cells, promotes GSH synthesis, mitigates formation of toxic adducts, indirectly promotes cytochrome P450 oxidase activity by enhancing NADP and GSH reduction, supports energy production by accelerated recovery of mitochondrial GSH, and protects against hepatic ischemia-reperfusion injury by inhibiting Kupffer cell activation and improving sinusoidal microcirculation (Saito et al. 2010; Vandeweerd et al. 2013). NAC also attenuates neurological effects associated with hepatic failure, including encephalopathy, cerebral edema, and coma (Bémeur et al. 2010).

NAC is generally recommended for situations where severe hepatotoxicosis is imminent or present, such as in acetaminophen toxicosis, and in situations involving oxidative damage to red blood cells, such as Heinz body anemia (Center 2004). In veterinary medicine, NAC has been recommended for hepatotoxicoses associated with acetaminophen, diazepam (cats), NSAIDs (dogs), mushrooms, and hepatic lipidosis syndrome with associated Heinz body anemia and/or hemolysis (dogs) (Avizeh et al. 2010; Center 2004; DeClementi 2018; Puschner 2018; Taboada 2017). Dosages used in veterinary medicine have been derived from human dosages for acetaminophen toxicosis: 140 mg/kg of 5% NAC solution as loading dose followed by 70 mg/kg q 6–8 h for 5–7 treatments (DeClementi 2018; Sellon 2013; Webster and Cooper 2009). Historically, evaluation of hepatic function at the end of the NAC dosing interval was recommended, with continuation of NAC q 6–8 h for up to 17 treatments if evidence of significant hepatic injury is present (DeClementi 2018). However, recent evidence that prolonged NAC treatment may actually impair liver regeneration following acetaminophen-induced injury has caused many to question the benefit of NAC therapy beyond 24 h following toxic insult (Athuraliya et al. 2009; Yang et al. 2009). Both oral and intravenous routes have been used in veterinary medicine. Although an approved intravenous formulation of NAC is available, its cost often makes it impractical for veterinary patients, so nebulization solutions of acetaminophen are frequently utilized; however, such solutions are not sterile, so administration through a millipore filter is required for intravenous administration (DeClementi 2018). Adverse effects of NAC administration include nausea, vomiting, and anaphylactic reactions; gastrointestinal upset can largely be mitigated via careful dilution of NAC solution (Bateman et al. 2014).

2.2.3 Silymarin

Silymarin is an extract of the milk thistle plant (*Silybum marianum*), a plant that has been used medicinally for centuries in Europe. Silymarin comprises 65%–80% of milk thistle extract and is itself composed of the flavonoid taxifolin and a mixture of at least seven flavolignans including silibinin, isosilbinin, silychristin, isosilychristin, and silydianin (DerMarderosian and Beutler 2014). Silybin A and B, stereoisomers of silibinin, are the most biologically active compounds in silymarin. Silymarin has antioxidant effects against ROS and lipid peroxidation, mitigating oxidative damage to hepatocellular and mitochondrial membranes and reducing GSH depletion. Silymarin enhances hepatocellular regeneration by acceleration of DNA synthesis and gene transcription/translation (Center 2004). Silymarin exhibits antifibrotic activity in the liver, inhibiting reactive collagen formation in animal models of hepatotoxic and cholestatic liver injury. Stimulation of bile acid synthesis by silymarin results in enhanced bile flow as well as enhanced levels of the hepatoprotective bile acid UDCA. Silymarin has shown hepatoprotective and hepatotrophic activities in a variety of experimental xenobiotic-induced hepatotoxicoses including those caused by hepatotoxic mushrooms (α-amanitin), acetaminophen, phalloidin, cyanobacteria (microcystin), iron, cisplatin, and methotrexate. Results of clinical trials of silymarin in humans have been equivocal and confounded by methodological flaws that make it difficult to compare studies, although individual studies generally show some improvement in outcome (Center 2004). Evaluation of the few clinical case reports is confounded by the fact that commercial products vary widely in silymarin content with no assurance of purity.

Silymarin is water insoluble with poor oral bioavailability, although bioavailability is reportedly improved by complexing silibinin with phosphatidylcholine (Center 2004). Silymarin is eliminated primarily via bile as glucuronide or sulfate conjugates and undergoes enterohepatic recirculation. Therapeutic dosing recommendations are complicated by the lack of standardization of various milk thistle products. Published dosages for veterinary species

include 50–150 mg/kg silibinin IV in dogs for treatment of amanitin toxicosis and 15–100 mg/kg silymarin IV in dogs, rabbits, and rodents given α-amanitin 10 min prior to silybin administration (Center 2004; Vogel et al. 1984). However, difficulty in extrapolation of these intravenous dosages to oral dosages and lack of standardized milk thistle products complicate dosing recommendations. Dosing comparable to human oral dosing recommendations for chronic hepatitis would be ~7–15 mg/kg per day; dosing up to 40–50 mg/kg per day would be comparable to doses used to control fibrosis in rats with obstructive biliary disease. In humans, adverse reactions reported with silymarin treatment have included mild diarrhea and hypersensitivity reactions (urticaria, pruritis).

2.2.4 Vitamin E

Vitamin E is an essential vitamin that serves as the most important lipid-soluble antioxidant (Center 2004). There are eight enantiomers of vitamin E in nature, but it is α-tocopherol that is most bioavailable and upon which nutritional recommendations should be based. Vitamin E is absorbed by the proximal intestine with the extent of absorption being dependent upon the amount of co-ingested lipid, amount of secreted bile, and presence of digestive esterases. Absorbed vitamin E is transported to the liver as mixed micelles, whereupon it is extracted and shuttled to lysosomes. A specific α-tocopherol transfer protein then sorts the various vitamin E forms and expedites their transfer to lipoproteins.

Vitamin E functions as a component of an antioxidant network that includes GSH, ubiquinol, vitamin C, cysteine, and SAMe. A major role for vitamin E is to mitigate propagation of membrane peroxidation damage through catalytic termination of membrane peroxidation reactions. An oxidized tocopheryl radical is produced that is then reduced back to its functional state via reactions with water- and lipid-soluble antioxidants such as vitamin C and ubiquinol (Center 2004). Non-antioxidant functions of vitamin E are performed via inhibition of protein kinase C, which modulates inflammation, has antiproliferative effects on vascular smooth muscle, inhibits platelet aggregation and adhesion, suppresses inflammatory cells, suppresses injurious immune responses, and reduces free radical production and inhibition of cyclooxygenase and 5-lipoxygenase (Azzi et al. 2002).

Hepatic concentrations of α-tocopherol are decreased in hepatic disease; plasma α-tocopherol levels do not reliably reflect liver concentrations, so should not be used to determine vitamin E status (Center 2004). Vitamin E should be considered in combination with other antioxidants, such as SAMe, for management of hepatobiliary disorders that are likely to involve oxidative membrane injury, such as necroinflammatory and cholestatic disorders. In dogs with chronic hepatic inflammatory disease, dosages of 7 IU/kg daily for 3 months resulted in improved liver enzyme values and hepatic glutathione status (Cantürk et al. 1998). Current dosing recommendation for dogs and cats is 10–15 IU/kg PO daily, with high doses (50–100 IU/kg) reserved for patients with chronic severe cholestatic liver disease; the latter cases may benefit from parenteral vitamin K1 administration (Center 2004; Vandeweerd et al. 2013). Adverse effects are uncommon, although interference in platelet aggregation has been reported. Vitamin E has a relatively low level of toxicity, with acute oral LD_{50}'s of >2 g/kg. In humans, chronic exposure to >5000 IU per day has been associated with alteration in other fat-soluble vitamins resulting in impaired bone mineralization (vitamin A) and/or coagulopathy (vitamin K). Excessive dosing may also promote toxopheroxy radical accumulation if other protective cofactors are out of balance or deficient. Drugs that induce cytochrome p450 enzymes can also potentiate formation of the tocopheryl radical.

2.2.5 Vitamin C

Vitamin C (ascorbic acid) is a hydrophilic vitamin with both antioxidant and prooxidant activities. Under normal physiologic conditions, vitamin C is present as reduced ascorbic acid, interacting with oxidants to be converted to its oxidized form, dehydroascorbic acid, which is readily converted back to ascorbic acid by glutathione (Jacob and Sotoudeh 2002). In excess, vitamin C can have prooxidant activity, especially in the presence of readily oxidized metals such as iron and copper through generation of oxygen-derived free radicals produced via lipid peroxidation (Center 2004). Vitamin C is an important cofactor for at least eight enzymes associated with collagen, amino acid, hormone, and carnitine synthesis and/or metabolism. Vitamin C is also involved in microsomal xenobiotic metabolism, synthesis of corticosteroids and catecholamines, conversion of cholesterol to bile acids, synthesis and modulation of components of the nervous system, and metabolism of tryptophan, tyrosine, and histamine. Vitamin C reduces a variety of ROS, regenerates vitamin E, protects against GSH depletion, and facilitates GSH regeneration. Because of its potential to potentiate certain metal-mediated tissue injury, vitamin C supplementation should be avoided in patients with high hepatic metal concentrations, particularly iron or copper. Similarly, due to its ability to induce collagen formation, vitamin C supplementation is not recommended in patients with hepatic disorders that are associated with hepatic fibrosis. Administration of high doses of vitamin C should be avoided to minimize any prooxidant activity associated with its use. Large overdoses of vitamin C have been associated with stomatitis and gastrointestinal upset in pigs and dogs and anemia and decreased reproductive performance in mink (Leveque 1969; National Research Council 1987).

2.2.6 α-Lipoic Acid

α-Lipoic acid (α-lipoate, thioctic acid, lipoic acid, 1,2-dithiolane-3-pentanoic acid) was originally classified as a vitamin until it was determined that plants and animals are capable of endogenously synthesizing the molecule (Zicker et al. 2002). In plant and animal tissues, α-lipoic acid is covalently bound to lysine residues. It has a key role in energy production by serving as a cofactor of mitochondrial enzymes, catalyzing the decarboxylation of pyruvate, branched-chain α-keto acids and α-ketoglutarate. Its thiol structure makes α-lipoic acid attractive to thiophilic toxicants such as heavy metals.

α-lipoic acid is readily absorbed from the gastrointestinal tract, with a bioavailability of ~30% (Center 2004). Once internalized in cells, α-lipoic acid is reduced to dihydrolipoate (DHLA) by several enzymes; intracellular DHLA and extracellular α-lipoic acid form a redox couple, functioning as antioxidants as they are exchanged across cellular membranes. Lipoamide, another metabolite of α-lipoic acid, serves as an essential cofactor for a number of enzyme systems, primarily within the mitochondria. Free α-lipoic acid interacts with several different biochemical pathways as a substrate, an inhibitor, or an effector (Bustamante et al. 1998).

The use of α-lipoic acid as an antioxidant in the management of liver disease has been extensively investigated in humans and animals (Bustamante et al. 1998). It is thought that α-lipoic acid works synergistically with vitamins A and C and GSH in an "antioxidant network" that enhances recycling of these compounds, and α-lipoic acid may play a role as a conditionally essential nutrient in aging mammals (Ames 1998; Podda et al. 1994). Additionally, α-lipoic acid has been studied for its ability to enhance glucose uptake into skeletal muscles, which makes it of interest to athletes and diabetics. Studies of α-lipoic acid in aging dogs have shown conflicting results in regard to effects on cognition, with some studies showing a positive effect and others showing no effect (Christie et al. 2009; Milgram et al. 2007).

α-lipoic acid has been included as an ingredient in some commercial extruded dog foods (Zicker et al. 2002). At the levels occurring in these foods, α-lipoic acid appears to have a low level of toxicity, with a maximum tolerated oral dosage in laboratory beagles of 126 mg/kg and LD_{50} of 400–500 mg/kg (Hill et al. 2004; Loftin and Herold 2009). However, dogs accidentally ingesting large amounts of α-lipoic acid supplements have been reported to experience hypoglycemia, liver injury, renal failure, and death (Loftin and Herold 2009). Cats appear to be approximately ten times more sensitive than other species, with hepatocellular damage occurring at 30 mg/kg (Hill et al. 2004). Certain disease states, such as thiamine deficiency, can result in enhanced toxicity of α-lipoic acid, significantly lowering toxic dosages (Gal 1965). The American Society for the Prevention of Cruelty to Animals' Animal Poison Control Center considers dosages of α-lipoic acid of >5 mg/kg (cats) and > 50 mg/kg (dogs) to be of concern for toxicosis (Loftin and Herold 2009).

2.2.7 Lecithin

Lecithin is a mixture of phosphatidylcholines, fatty acids, carbohydrates, and other compounds (DerMarderosian and Beutler 2014). Polyenylphosphatidylcholine (PPC) is the substance that is thought to be responsible for the hepatoprotective effects of lecithin, principally through its dilinoleoylphaphatidyl choline (DLPC) moiety (Center 2004; Lee et al. 2015). Both PPC and DLPC have been shown to inhibit hepatic fibrogenesis, attenuate oxidative hepatocellular and mitochondrial membrane injury, reduce accumulation of reactive oxygen species, and conserve hepatocellular and mitochondrial GSH levels. Anti-inflammatory activities of PPC and DLPC occur through inhibition of Kupffer cell activation, while reduced activation of hepatic stellate cells accounts for some of their antifibrotic activities. DLPC has been shown to attenuate oxidative injury from a variety of toxic hepatic insults, with the exception of iron toxicosis where DLPC aids in restoration of GSH levels but does not control oxidative injury caused by iron (Aleynik et al. 2000).

In humans, PPC has been used primarily in alcohol hepatotoxicosis but has also been shown to be beneficial in chronic necroinflammatory liver diseases as well as acute hepatopathy (Center 2004). Although no controlled clinical studies in veterinary species have been performed, general dosing recommendations for veterinary patients are 25–50 mg/kg PPC per day. PPC appears to have a wide margin of safety, with no reported adverse effects in humans receiving therapeutic dosages. Very large ingestions in humans (>25 g/day) have resulted in gastrointestinal distress, sweating, hypersalivation, and anorexia; one study in humans receiving tacrine and lecithin had gastrointestinal distress and hepatitis reported as adverse effects (DerMarderosian and Beutler 2014).

2.2.8 Ubiquinol

Ubiquinones are endogenous lipid soluble benzoquinone antioxidants synthesized in all animal tissues; ubiquinol (coenzyme Q_{10}) is the reduced form of ubiquinone (DerMarderosian and Beutler 2014). Ubiquinones function in oxidation-reduction reactions in the mitochondrial respiratory chain and protect against lipid peroxidation. Ubiquinol acts as an antioxidant that reduces vitamin E and vitamin C following their interactions with oxidants. In hepatic disease, mitochondrial dysfunction results in decreased ubiquinol availability, increasing the risk of oxidative injury (Center 2004). Ubiquinol-mediated support of endothelial cell function may be of benefit in hepatic ischemia-reperfusion injury. Few clinical studies of ubiquinol in veterinary patients exist,

and dosing recommendations are extrapolated from human studies. In general, a dosage of 1–2 mg/kg PO per day could be considered (Center 2004).

2.2.9 Ursodeoxycholic Acid

For centuries, Chinese medicine utilized Yutan, a compound derived from the dried bile of Chinese black bears, to aid in treatment of hepatobiliary disorders (Vandeweerd et al. 2013). Investigation into Yutan identified the major active principle in the bile as ursodeoxycholic acid (UDCA), a hydrophilic bile acid; subsequently, synthetic forms of UDCA are available as FDA-approved human drugs. Beneficial effects of UDCA stem from a variety of activities including replacement of more toxic endogenous bile acids, cytoprotection of hepatocytes and biliary epithelium, antioxidant activity, immunomodulation, suppression of anomalous major histocompatibility foci, enhancement of biliary excretion of toxic compounds, stimulation of bile secretion, mitigation of bile acid-induced mitochondrial injury, and reduction of bile acid-induced hepatocellular apoptosis (Center 2004). The hepatoprotective effects of UDCA are specific for bile acid-induced injury and do not extend to injury from direct or indirect toxic exposure or from ischemia-reperfusion injury. Studies in humans and laboratory animals have failed to show a benefit from use of UDCA in cases of hepatosteatosis; for this reason, UDCA should be used cautiously, if at all, in patients, particularly cats, with severe hepatic lipidosis (Center 2004).

Orally administered UDCA is absorbed from large and small intestines, and $>$ 60% of an absorbed dose is taken up by the liver. As with other bile acids, UDCA is conjugated with glycine or taurine in a species-dependent fashion and is excreted in the bile. In patients with cholestasis, UDCA becomes the predominant bile acid in both the liver and systemic circulation. In general, UDCA may be of benefit in patients with necroinflammatory or cholestatic liver disorders where cholestasis is a concern, although published studies showing efficacy in veterinary patients are lacking. Dosages of 10–15 mg/kg PO with food in one or two divided doses have been recommended for veterinary patients (Center 2004).

3 Pancreas

3.1 Pancreatic Dysfunction

The pancreas has both endocrine and exocrine functions. The main function of the acinar cells of the exocrine pancreas is the synthesis and secretion of digestive enzymes including the inactive proenzymes of chymotrypsin, trypsin, collagenase, phospholipases, elastases, and carboxypeptidases, as well as active lipase and amylase (Jubb and Stent 2016). The endocrine pancreas islet cells secrete the hormones insulin, glucagon, somatostatin, pancreatic polypeptide, adrenomedullin, and ghrelin. Broadly speaking, the exocrine pancreas mediates digestion, while the endocrine pancreas regulates blood glucose concentrations. Exocrine pancreatic dysfunction results in a maldigestion syndrome termed exocrine pancreatic insufficiency (EPI), resulting in diarrhea, weight loss, and proliferation of small intestinal microflora. Pancreatic islet dysfunction most commonly causes decreased or absent insulin secretion resulting in diabetes mellitus, characterized by hyperglycemia, glucosuria, polydipsia, polyuria, and weight loss. Pancreatitis, an inflammation of the pancreas, can result in damage to acinar and islet cells, predisposing to diabetes or exocrine pancreatic insufficiency, or, in severe cases, both.

3.2 Nutraceuticals and the Pancreas

3.2.1 Enzymes

For most veterinary patients with EPI, the use of commercial pancreatic enzyme extracts derived from beef or pork pancreas will provide the degree of digestion of food needed to resolve most clinical signs (Steiner 2010). Plant-derived digestive enzymes that have been recommended as substitutes for pancreatic enzymes include papain, bromelain, and ficin; however, there are no studies to demonstrate efficacy comparable to pancreatic-derived enzymes for EPI (Silver 2012). Supplementation with fat-soluble vitamins (A, D, E, K) is also recommended in EPI patients (Steiner 2010).

3.2.2 Nutraceuticals and Diabetes

A large number of nutraceuticals have been investigated for their ability to alter blood glucose levels in humans, but extrapolation to veterinary species is complicated by the fact that insulin and blood glucose responses can vary between species. For instance, xylitol is a 5-carbon sugar alcohol derived from willow bark that is used as a sweetener by human diabetics because it has no impact on blood insulin or glucose levels; in contrast, xylitol administration to dogs can cause an intense and prolonged spike in insulin secretion from the pancreas, leading to hypoglycemia that can be potentially life threatening (Dunayer 2006). Extrapolating between veterinary species is also difficult with regard to diabetes mellitus, since the type of diabetes tends to differ: diabetic dogs generally have an absolute lack of insulin due to islet cell destruction (human Type 1 diabetes), while diabetes mellitus in cats is more similar to human Type 2 diabetes, i.e., β-cell dysfunction and impaired tissue response to insulin (Reusch 2010). Table 1 highlights some nutraceutical compounds that have been investigated for human diabetes mellitus; no studies in veterinary species exist, and extrapolation of results to veterinary patients should be done with

Table 1 Nutraceutical effects in diabetes

Nutraceutical	Active constituent	Proposed mechanism of action	Adverse effects	Reference
α-Lipoic acid	α-Lipoic acid	↓ insulin resistance	Hypoglycemia, hepatotoxicity	Hill et al. (2001), Jacob et al. (1995), Loftin and Herold (2009)
Chromium	Chromium	↑ insulin binding, ↑ insulin receptor numbers, ↑ insulin sensitivity of cells	Renal injury at high doses	Anderson (2000)
Garlic	S-allyl cysteine sulfoxide	↓ fasting glucose	Can cause Heinz body anemia in cat and dog	Sheela and Augusti (1992), Tang et al. (2008)
Green tea	Polyphenols	↑ insulin sensitivity of cells	Caffeine can have adverse effects; vitamin K may interfere with therapeutic anticoagulants	Potenza et al. (2007)
Ubiquinol (coenzyme Q_{10})	Ubiquinol	Improves long-term glycemic control in Type 2 diabetes	None found	Afolayan and Olubunmi (2014)

extreme caution. For details on nutraceuticals/plant extracts in canines and felines, readers are referred to Chapter "Nutraceuticals in Gastrointestinal Conditions" in this book.

4 Concluding Remarks and Future Directions

Hundreds of nutraceuticals have been investigated for their potential benefit or harm in hepatic or pancreatic disease, but further studies are needed for most to determine clinical efficacy of the individual agents. Although research has shown that many nutraceuticals possess potential medicinal activities that are measurable at the cellular and/or molecular level, demonstrating clinical efficacy can be much more problematic. Reports of positive clinical response of veterinary patients to nutraceuticals are largely no more than anecdotal evidence or individual case reports where confounding factors such as coadministered compounds (e.g., drugs, herbals, other nutraceuticals), questionable nutraceutical purity, or lack of effective control populations make it difficult to determine any real effect from nutraceuticals. Commercially available nutraceuticals frequently vary considerably between brands (and often between lots within a brand) in quality and content of active components; many manufacturers consider their formulations "proprietary" and refuse to divulge contents, and researchers frequently use purified and/or extracts of individual constituents of nutraceuticals rather than the products as available for use, making it difficult to make comparisons between studies. Herbal, supplement, and nutraceutical products are poorly regulated in many countries, leaving the consumer at the mercy of the manufacturer in terms of product quality and safety (Seeff et al. 2015).

Nutraceutical compounds that work well in vitro may have poor bioavailability or rapid elimination that interferes with their ability to reach plasma concentrations required for them to have any therapeutic effect. Compounds that achieve good plasma levels may be unable to distribute to target tissues due to inability to pass through physiological barriers due to physiochemical properties (e.g., lipophilicity, pH) or due to the presence or absence of appropriate transporter molecules (e.g., p-glycoprotein transporters). Other nutraceuticals may have beneficial effects on some tissues but may be harmful to other tissues within the body. For instance, the hepatoprotective compound silymarin has been shown to enhance the growth of mammary tumors (Malewicz et al. 2006); similarly, silibinin has hepatoprotective properties but can promote progression of ethanol-dependent hepatocellular carcinoma when coadministered with ethanol (Brandon-Warner et al. 2012).

Nutraceuticals demonstrating antioxidant, anti-inflammatory, and/or antifibrotic activities in the liver may inhibit or minimize acute injury induced by endogenous and exogenous hepatotoxic agents or may prevent or slow progression of chronic liver disorders such as hepatic lipidosis, chronic active hepatitis, liver storage diseases, and hepatic carcinogenesis. However, because alteration of hepatic metabolic enzymes, decreased inflammatory/immune responses, and stimulation of hepatocellular proliferation can cause unfavorable consequences such as interference with therapeutic agents, diminished hepatic immune surveillance, or increased risk of hepatic neoplasia, potential adverse effects of nutraceuticals must be researched and documented. Most nutraceuticals lack long-term safety and carcinogenicity data

and lack the regulatory requirement to track adverse effects. Illness and death in humans and animals have been reported to be associated with the contamination or intentional adulteration of nutraceuticals with biotoxins, heavy metals, mycotoxins, pesticide residues, pharmaceuticals, toxic plants, and other potentially hazardous substances (Seeff et al. 2015; Mittelman et al. 2016; Brown 2017; Gupta et al. 2018). These issues will need to be addressed through further research before the safety and efficacy of nutraceutical products can be determined.

References

Afolayan AJ, Olubunmi AW (2014) Dietary supplements in the management of hypertension and diabetes – a review. Afr J Tradit Complement Altern Med 11(3):248–258

Aleynik SI, Leo SM, Aleynik MK et al (2000) Polyenylphosphatidylcholine protects against alcohol but not iron-induced oxidative stress in the liver. Alcohol Clin Exp Res 24(2):196–206

Ames BN (1998) Micronutrients prevent cancer and delay aging. Toxicol Lett 102–103:5–18

Anderson RA (2000) Chromium in the prevention and control of diabetes. Diabetes Metab 26(1):22–27

Athuraliya T, Nimmi C, Jones AL (2009) Prolonged N-acetylcysteine therapy in late acetaminophen poisoning associated with acute liver failure--a need to be more cautious? Crit Care 13(3):144

Avizeh R, Najafzadeh H, Razi Jalali M et al (2010) Evaluation of prophylactic and therapeutic effects of silymarin and N-acetylcysteine in acetaminophen-induced hepatotoxicity in cats. J Vet Pharmacol Ther 33(1):95–99

Azzi A, Ricciarelli R, Zingg M (2002) Non-antioxidant molecular functions of alpha-tocopherol (Vitamin E). FEBS Lett 519 (1–3):8–10

Bateman D, Dear JW, Thanacoody HK et al (2014) Reduction of adverse effects from intravenous acetylcysteine treatment for paracetamol poisoning: a randomised controlled trial. Lancet 383 (9918):697–704

Bémeur C, Vaquero J, Desjardins P et al (2010) N-acetylcysteine attenuates cerebral complications of non-acetaminophen-induced acute liver failure in mice: antioxidant and anti-inflammatory mechanisms. Metab Brain Dis 25(2):241–249

Bischoff K, Ramaiah SA (2007) Liver toxicity. In: Gupta R (ed) Veterinary toxicology: basic and clinical principles. Academic Press, Amsterdam, pp 145–160

Bottiglieri T (2002) S-Adenosyl-L-Methionine (SAMe): from the bench to the bedside--molecular basis of a pleiotropic molecule. Am J Clin Nutr 76(5):1151S–1157S

Brandon-Warner EE, Ashley L et al (2012) Silibinin (Milk Thistle) potentiates ethanol-dependent hepatocellular carcinoma progression in male mice. Cancer Lett 326(1):88–95

Brown AC (2017) Liver toxicity related to herbs and dietary supplements: online table of case reports. Part 2 of 5 series. Food Chem Toxicol 107:472–501

Bustamante J, Lodge J, Marcocci L et al (1998) Alpha-lipoic acid in liver metabolism and disease. Free Radic Biol Med 24 (6):1023–1039

Cantürk NZ, Cantürk Z, Utan N et al (1998) Cytoprotective effects of alpha tocopherol against liver injury induced by extrahepatic biliary obstruction. East Afr Med J 75(2):77–80

Center SA (2004) Metabolic, antioxidant, nutraceutical, probiotic, and herbal therapies relating to the management of hepatobiliary disorders. Vet Clin North Am Small Anim Pract 34(1):67–172

Center SA, Randolph J, Warner K et al (2005a) The effects of S-adenosylmethionine on clinical pathology and redox potential in the red blood cell, liver, and bile of clinically normal cats. J Vet Int Med 19(3):303–314

Center SA, Warner K, McCabe J et al (2005b) Evaluation of the influence of S-adenosylmethionine on systemic and hepatic effects of prednisolone in dogs. Am J Vet Res 66(2):330–341

Christie L, Opii W, Head E et al (2009) Short-term supplementation with acetyl-L-carnitine and lipoic acid alters plasma protein carbonyl levels but does not improve cognition in aged beagles. Exp Gerontol 44(12):752–759

Crawford JM (2005) Liver and biliary tract. In: Kumar VA, Abbas V, Fausto N (eds) Robbins and Cotran's pathologic basis of disease. Saunders, St. Louis, MO, pp 877–937

DeClementi C (2018) Prevention and treatment of poisoning. In: Gupta R (ed) Veterinary toxicology: basic and clinical principles. Academic Press, Amsterdam, pp 1141–1159

DerMarderosian A, Beutler JA (2014) The review of natural products, 8th edn. Clinical Drug Information, LLC, St. Louis, MO

Duarte S, Baber J, Fujii T, Coito AJ (2015) Matrix metalloproteinases in liver injury, repair and fibrosis. Matrix Biol 44–46:147–156

Dunayer EK (2006) New findings on the effects of xylitol ingestion in dogs. Vet Med 101(12):791–796

Elmore S (2007) Apoptosis: a review of programmed cell death. Toxicol Pathol 35(4):495–516

Friedel HA, Goa KL, Benfield P (1989) S-adenosyl-L-methionine. A review of its pharmacological properties and therapeutic potential in liver dysfunction and affective disorders in relation to its physiological role in cell metabolism. Drugs 38(3):389–416

Gal EM (1965) Reversal of selective toxicity of (-)-alpha-lipoic acid by thiamine in thiamine-deficient rats. Nature 207(996):535

Gupta RC, Srivastava A, Lall R (2018) Toxicity potential of nutraceuticals. In: Nicolotti O (ed) Computational toxicology: methods and protocol. Springer, New York, NY, pp 367–394

Gwaltney-Brant SM (2016) Nutraceuticals in hepatic diseases. In: Gupta R (ed) Nutraceuticals: efficacy, safety and toxicity. Academic Press, Amsterdam, pp 87–99

Hill AS, O'Neill S, Rogers QR et al (2001) Antioxidant prevention of Heinz body formation and oxidative injury in cats. Am J Vet Res 62 (3):370–374

Hill AS, Werner JA, Rogers QR et al (2004) Lipoic acid is 10 times more toxic in cats than reported in humans, dogs or rats. J Anim Physiol Anim Nutr 88(3–4):150–156

Jacob RA, Sotoudeh G (2002) Vitamin C function and status in chronic disease. Nutr Clin Care 5(2):66–74

Jacob S, Henriksen JE, Schiemann A et al (1995) Enhancement of glucose disposal in patients with Type 2 diabetes by alpha-lipoic acid. Arzneimittelforschung 45(8):872–874

Jaeschke H (2008) Toxic responses of the liver. In: Klaasen CD (ed) Casarett and Doul's toxicology, the basic science of poisons. McGraw-Hill, New York, pp 557–582

Jubb KV, Stent AW (2016) Pancreas. In: Maxie MG (ed) Jubb, Kennedy, and Palmer's pathology of domestic animals. Elsevier, St. Louis, MO, pp 353–375

Lee W, Weng SH, Su N (2015) Individual phosphatidylcholine species analysis by RP-HPLC-ELSD for determination of polyenylphosphatidylcholine in lecithins. J Agric Food Chem 63(15):3851–3858

Leveque JI (1969) Ascorbic acid in treatment of the canine distemper complex. Vet Med Small Anim Clin 64(11):997–999

Loftin EG, Herold LV (2009) Therapy and outcome of suspected alpha lipoic acid toxicity in two dogs. J Vet Emerg Crit Care 19 (5):501–506

Malewicz B, Wang Z, Jiang C et al (2006) Enhancement of mammary carcinogenesis in two rodent models by silymarin dietary supplements. Carcinogenesis 27(9):1739–1747

Mato JM, Corrales FJ, Lu SC et al (2002) S-adenosylmethionine: a control switch that regulates liver function. FASEB J 16(1):15–26

Milgram NW, Araujo JA, Hagen TM et al (2007) Acetyl-L-carnitine and alpha-lipoic acid supplementation of aged beagle dogs improves learning in two landmark discrimination tests. FASEB J 21 (13):3756–3762

Mittelman NS, Engiles JB, Murphy L et al (2016) Presumptive iatrogenic microcystin-associated liver failure and encephalopathy in a Holsteiner gelding. J Vet Intern Med 30(5):1747–1751

National Research Council (1987) Vitamin tolerance of animals. National Academy Press, Washington, DC

Podda M, Tritschler HJ, Ulrich H et al (1994) Alpha-lipoic acid supplementation prevents symptoms of Vitamin E deficiency. Biochem Biophys Res Commun 204(1):98–104

Potenza MA, Marasciulo M, Tarquinio M et al (2007) EGCG, a green tea polyphenol, improves endothelial function and insulin sensitivity, reduces blood pressure, and protects against myocardial I/R injury in SHR. Am J Physiol Endocrinol Metab 292(5):E1378–E1387

Puschner B (2018) Mushroom toxins. In: Gupta R (ed) Veterinary toxicology: basic and clinical principles. Academic Press, Amsterdam, pp 955–966

Reusch C (2010) Feline diabetes mellitus. In: Ettinger SJ, Feldman EC (eds) Textbook of veterinary internal medicine. St. Louis, MO, Saunders, pp 1796–1816

Saito C, Zwingmann C, Jaeschke H (2010) Novel mechanisms of protection against acetaminophen hepatotoxicity in mice by glutathione and N-acetylcysteine. Hepatology 51(1):246–254

Schmid RD, Hovda LR (2016) Acute hepatic failure in a dog after xylitol ingestion. J Med Toxicol 12(2):201–205

Seeff LB, Bonkovsky HL, Navarro VJ et al (2015) Herbal products and the liver: a review of adverse effects and mechanisms. Gastroenterology 148(3):517–532.e3

Sellon RK (2013) Acetaminophen. In: Peterson ME, Talcott PA (eds) Small animal toxicology. Saunders, St. Louis, MO, pp 423–429

Sheela CG, Augusti KT (1992) Antidiabetic effects of S-allyl cysteine sulphoxide isolated from garlic *Allium sativum* Linn. Indian J Exp Biol 30(6):523–526

Silver RJ (2012) Clinical applications of nutraceutical and botanical compounds in companion animals. Proceedings of the Wild West Veterinary Conference. Reno, NV

Skorupski KA, Hammond GM, Irish AM et al (2011) Prospective randomized clinical trial assessing the efficacy of denamarin for prevention of CCNU-induced hepatopathy in tumor-bearing dogs. J Vet Int Med 25(4):838–845

Stalker MJ, Hayes MA (2007) Liver and biliary system. In: Maxie MG (ed) Jubb, Kennedy, and Palmer's pathology of domestic animals. Elsevier, St. Louis, MO, pp 297–388

Steiner JM (2010) Canine pancreatic disease. In: Ettinger SJ, Feldman EC (eds) Textbook of veterinary internal medicine. Saunders, St. Louis, MO, pp 1695–1704

Taboada J (2017) Clinical update on the use of nutraceuticals in the management of canine and feline liver disease. Proceedings of the Southwest Veterinary Symposium

Tang X, Xia Z, Yu J (2008) An experimental study of hemolysis induced by onion (*Allium Cepa*) poisoning in dogs. J Vet Pharmacol Ther 31(2):143–149

Vandeweerd JM, Cambier C, Gustin P (2013) Nutraceuticals for canine liver disease: assessing the evidence. Vet Clin North Am Small Anim Pract 43(5):1171–1179

Vogel GB, Tuchweber T, Trost W, Mengs U (1984) Protection by silibinin against amanita phalloides intoxication in beagles. Toxicol Appl Pharmacol 73(3):355–362

Wallace KP, Center SA, Hickford SF et al (2002) S-adenosyl-L-methionine (SAMe) for the treatment of acetaminophen toxicity in a dog. J Am Anim Hosp Assoc 38(3):246–254

Wang K (2014) Molecular mechanisms of hepatic apoptosis. Cell Death Dis 5(1):e996–e910

Watkins PB, Seef LB (2006) Drug-induced liver injury: summary of a single topic clinical research conference. Hepatology 43:618–631

Webster CRL, Cooper J (2009) Therapeutic use of cytoprotective agents in canine and feline hepatobiliary disease. Vet Clin North Am Small Anim Pract 39(3):631–652

Yang R, Miki K, He S, Kileen ME et al (2009) Prolonged treatment with N-acetylcysteine delays liver recovery from acetaminophen hepatotoxicity. Crit Care 13(2):R55

Zicker SC, Hagen TM, Golder C et al (2002) Safety of long-term feeding of Dl- α -lipoic acid and its effect on reduced glutathione: oxidized glutathione ratios in beagles. Vet Ther 3(2):167–176

Nutraceuticals in Periodontal Health and Diseases in Dogs and Cats

Ramesh C. Gupta, Denise M. Gupta, Rajiv Lall, Ajay Srivastava, and Anita Sinha

Abstract

Periodontal diseases are the most common diseases of domestic dogs and cats. Conventional treatment of periodontal diseases rests with broad-spectrum antibiotics and antiseptics. Due to rising healthcare costs, severe side effects, and drug resistance, complementary and alternative medicines are a preferable choice for patients with periodontal diseases. This chapter describes several nutraceuticals and herbal complementary medicines that can be used singly or in combination for prevention and treatment of periodontitis in dogs and cats.

Keywords

Nutraceuticals · Veterinary nutraceuticals · Oral health · Periodontal diseases

1 Introduction

Currently, about 3.9 billion people suffer from periodontal diseases associated with gum and tooth decay. Periodontal diseases are more frequently encountered in dogs and cats than in humans due to lack of oral health hygiene. According to the American Veterinary Dental College (AVDC), 80% of dogs and 70% of cats suffer from periodontal diseases. Periodontal disease affects dogs and cats of all ages, although it is more common in older animals. It can include sore, swollen, bleeding gums, infected teeth, caries, plaque buildup, and tartar. In animals, periodontal disease is an often overlooked health problem which may be directly or indirectly linked to an increased risk of cardiovascular disease, arthritis, diabetes, and even neurodegenerative disease (chronic cognitive dysfunction syndrome in dogs and cats).

In a number of studies, the influence of diet on oral health in dogs and cats has been reported (Gawor et al. 2006; Logan 2006; Chandler 2014; Oba et al. 2018). Plant products have been used to improve dental health and promote oral hygiene for a long time (reviewed in Kumar et al. 2015). Ganesan (2008) provided a list of 114 plant species belonging to 51 families which have potential for use in periodontal diseases. Ganesan (2008) reported that leaves were the dominant plant part used in oral care (25.44%), followed by root (20.17%), seed/nut/fruits (18.42%), bark (14.03%), stem (12.28%), whole plant (9.65%), and gum/latex (8.77%). Most of the plant extracts and products are used, in various forms such as mouthwash, paste, or powder, to relieve toothache, gingivitis, stomatitis, ulcers, gum bleeding, and other disorders.

In conventional treatment of periodontal diseases, various antimicrobial or antiseptic agents are applied locally in the form of fibers, films, gels, microparticulate systems, or nanoparticulate systems (Puri and Puri 2013). Based on required duration of action, drug or phytochemical agents can be applied locally using sustained release devices [(a) drug delivery for less than 24 h, (b) require multiple applications, and (c) follow first-order kinetics] or controlled delivery devices [(a) duration of drug release exceeds 24 h, (b) administered once, and (c) follow zero-order kinetics]. Due to rising healthcare costs, side effects, and drug resistance, complementary and alternative medicines are preferred over modern medicines. This chapter describes some nutraceuticals and plant extracts that can be used for various periodontal diseases in dogs and cats.

R. C. Gupta (✉)
Toxicology Department, Breathitt Veterinary Center, Murray State University, Hopkinsville, KY, USA
e-mail: rgupta@murraystate.edu

D. M. Gupta
Hopkinsville, KY, USA

R. Lall · A. Srivastava · A. Sinha
Vets Plus Inc., Menomonie, WI, USA

R. C. Gupta et al. (eds.), *Nutraceuticals in Veterinary Medicine*, https://doi.org/10.1007/978-3-030-04624-8_30

2 Pathophysiology of Periodontal Diseases

Pathophysiology of periodontal diseases is complex due to multifactorial etiologies. The oral cavity consists of the teeth, gums, hard palate, and soft tissue. Periodontium consists of four tissues (free and attached gingiva, periodontal ligament, alveolar bone, and root cementum) and protects the teeth. Dental problems may include plaque, tartar, tooth decay, gingivitis, and periodontal diseases (Muhammad and Lawal 2010). Gum deterioration, often characterized by swelling, bleeding and pain, is the leading cause of tooth loss. Periodontitis is considered the second most important cause of tooth loss in humans and animals. Clinical features that may be useful in the early detection of onset of periodontitis include overt gingival inflammation, dental calculus and a high rate of caries, restorations, and tooth loss (Albandar et al. 1997).

Periodontitis can be defined as an inflammatory process, initiated by the formation of plaque biofilm, which leads to loss of periodontal attachment to the root surface and adjacent alveolar bone and may ultimately result in tooth loss. In both gingivitis and periodontitis, inflammation is the result of microorganisms present in dental plaque (Newman et al. 2006). Initially, the bacteria in plaque are predominately non-motile, Gram-positive aerobes, including *Staphylococcus* spp. and *Streptococcus* spp., but many others are also present. As the plaque biofilm thickens and matures, Gram-negative anaerobes become predominant. Bacteria in subgingival plaque secrete toxins and metabolic by-products, initiating gingivitis and the inflammatory process. Organisms most often associated with periodontal disease include *Bacteroides fragilis*, *Peptostreptococcus*, *Porphyromonas gulae*, *Porphyromonas salivosa*, *Porphyromonas denticanis*, *Prevotella intermedia*, *Treponema* spp., *Bacteroides splanchnicus*, and many others. Recently Singh et al. (2016) emphasized the role of periopathogens (*P. gingivalis*, *A. actinomycetemcomitans*, *P. intermedia*, *F. nucleatum*, etc.) in periodontal diseases.

Periodontal pathogens can induce reactive oxygen species (ROS) overproduction and thus may cause collagen and periodontal cell breakdown (Soni et al. 2012). In fact, periodontitis is associated with reduced antioxidant capacity and increased oxidative damage (Lima et al. 2017). Oxidative stress induces inflammation and bone loss contributing to the pathological progression of periodontal diseases. During periodontitis, the inflammatory process is marked by neutrophils that invade periodontium and induces the release of proteolytic enzymes and production of ROS (D'Aiuto et al. 2010). An imbalance between pro-inflammatory and anti-inflammatory response results in periodontal breakdown (Preshaw 2008). The continuous exacerbation of inflammation culminates with collagen fiber destruction and bone resorption (Zheng et al. 2015).

In the field of periodontal health and diseases, various diagnostic markers are used such as plaque accumulation (PLA), plaque index (PI), gingival index (GI), bleeding on probing (BOP), sulcus bleeding index (SBI), periodontal probing depth (PD), clinical attachment level (CAL), and oral hygiene index-simplified (OHI-S) (Pannuti et al. 2003; Nagata et al. 2008; Khairnar et al. 2013). Recently, Kajiura et al. (2016) reported that soluble form of IL-6 reporter (sIL-6R) and calprotectin concentrations in gingival crevicular fluid are useful biomarkers in the evaluation of periodontitis. Figure 1 shows dental tartar in the dog.

According to Dr. Alexander M. Reiter (School of Veterinary Medicine, University of Pennsylvania), periodontitis in dogs and cats is classified in four stages:

Stage 1: There is gingivitis only, without attachment loss; the height and architecture of the alveolar margin are normal.

Stage 2: There is early periodontitis with <25% of attachment loss or, at most, there is a stage 1 furcation involvement in multirooted teeth. There are early radiographic signs of periodontitis. The loss of periodontal attachment

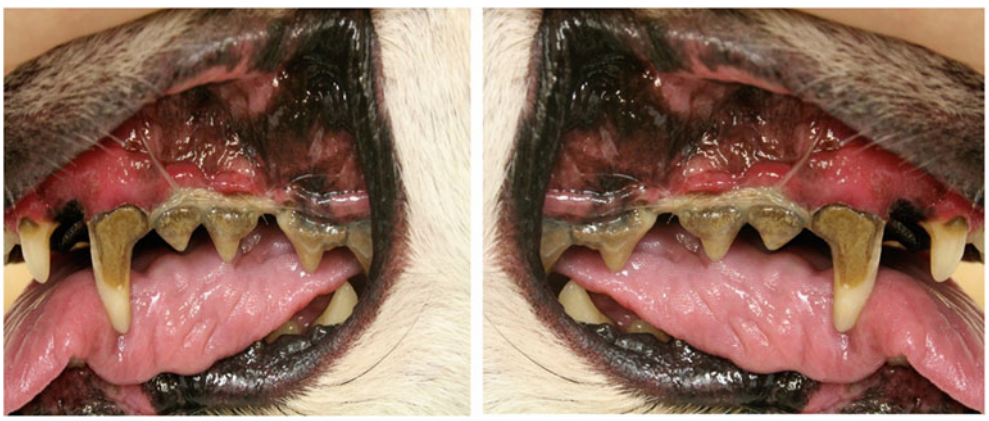


Fig. 1 Tartar in dog teeth

is <25% as measured by probing of the clinical attachment level or by radiographic determination of the distance of the alveolar margin from the cementoenamel junction relative to the length of the root.

Stage 3: There is moderate periodontitis, with 25–50% of attachment loss as measured by probing of the clinical attachment level or by radiographic determination of the distance of the alveolar margin from the cementoenamel junction relative to the length of the root, or there is a stage 2 furcation involvement in multirooted teeth.

Stage 4: There is advanced periodontitis, with >50% of attachment loss as measured by probing of the clinical attachment level or by radiographic determination of the distance of the alveolar margin from the cementoenamel junction relative to the length of the root, or there is a stage 3 furcation involvement in multirooted teeth.

In advanced periodontitis, teeth may fall out and are sometimes swallowed by dogs and cats (Fig. 2).

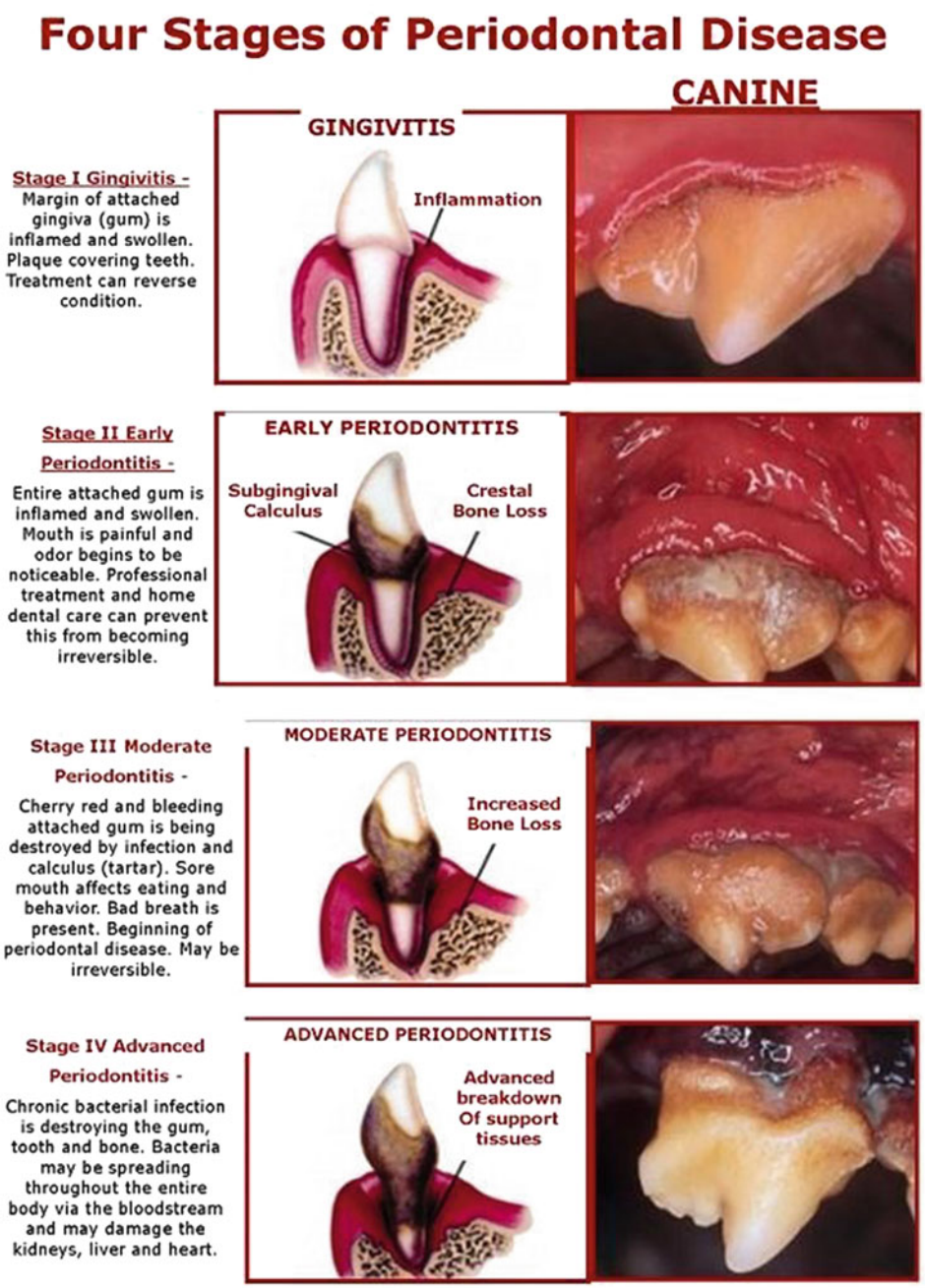


Fig. 2 Four stages of periodontal disease in canine

3 Nutraceuticals in Periodontal Diseases

As in humans, periodontal diseases in dogs and cats can be treated with four objectives: (1) remove built-up plaque and tartar, (2) fight bacteria which cause infection, (3) reduce inflammation, and (4) stop bleeding.

Some commonly used nutraceuticals are discussed here in brief, and the rest are listed in Table 1.

3.1 *Calendula Officinalis*

Calendula officinalis (*C. officinalis*), commonly known as pot marigold, is an annual medicinal herb. More than 100 phytoconstituents have been identified in different parts of this plant. The flowers of *Calendula officinalis* contain flavonol glycosides, triterpene oligoglycosides, oleanane-type triterpene glycosides, saponins, and a sesquiterpene glucoside. The petals and pollen contain triterpenoid esters and the carotenoids flavoxanthin and auroxanthin (antioxidant). The leaves and stems contain other carotenoids, mostly lutein (80%), zeaxanthin (5%), and beta-carotene. In essence, the plant is very rich in quercetin, carotenoids, lutein, lycopene, rutin, ubiquinone, xanthophylls, and other antioxidants. The flavonoid quercetin is considered to be of great importance for treating periodontitis due to its antioxidative and anti-inflammatory properties (Napimoga et al. 2013; Li et al. 2016).

By having these many phytoconstituents, *C. officinalis* extract exerts antioxidative, anti-inflammatory, antibacterial, antiviral, antifungal, and hemostatic activities (Preethi et al. 2009; Preethi and Utta 2006; Parente et al. 2012). In an experimentally induced periodontitis in rats, Lima et al. (2017) demonstrated that 11 days of ligature caused bone loss, breakdown of collagen fibers, and increased immunostaining for DKK-1 while reducing WNT 10b and β-catechin expressions. Periodontitis reduced glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT) and increased malondialdehyde (MDA). All findings were reversed by 90 mg/kg of *C. officinalis*. Lima et al. (2017) suggested that *C. officinalis* reduced oxidative stress and bone loss and preserved collagen fibers in rats with experimental periodontitis involving the WNT signaling pathway. In a clinical trial, Khairnar et al. (2013) reported that patients with gingivitis receiving *C. officinalis* (2 ml tincture with 6 ml of distilled water) showed reduction in dental plaque and gingivitis. In a number of other experimental and clinical studies, *C. officinalis* (in gel, tincture, or mouthwash) has shown positive effects against gingivitis and periodontitis (Lauten et al. 2005; Yusoffs and Kamin 2006; Preethi and Utta 2006; Machado 2010; Saini et al. 2012; Khairnar et al. 2013; Tanideh et al. 2013).

C. officinalis extract appears to be a promising nutraceutical for treating periodontitis in dogs and cats after achieving positive effects in multicentered, long-term clinical trials.

3.2 Pau d'Arco

Pau d'Arco (*Tabebuia impetiginosa*), also called "ipê roxo" or "purple lapacho," is a huge canopy tree native to the Amazon rainforest and other tropical parts of South America and Latin America. There are indications that its use may actually predate the Incas. The Guarani and Tupi Indians call the tree "Tajy," which means "to have strength and vigor." In South American herbal medicines, Pau d'Arco has been indicated for many health conditions, including malaria, anemia, fever, colitis, arthritis, respiratory problems, colds, cough, flu, fungal infections, rheumatism, snakebite, poor circulation, boils, syphilis, and cancer.

The bark of Pau d'Arco contains many phytochemicals, including quinoids (anthraquinones, furanonaphthoquinones, lapachones, and naphthoquinones), benzenoids, and flavonoids. Pau d'Arco's beneficial health effects stem from its lapachol and β-lapachone content (Lagrota et al. 1983; Giuraud et al. 1994). The structural formula of lapachol and β-lapachone are shown in Fig. 3. Typically, Pau d'Arco bark contains 2–7% lapachol. A good-quality Pau d'Arco bark contains approximately 4% lapachol. These phytoconstituents exert antimicrobial, antifungal, antiviral, antitumorus, antileukemic, and anti-inflammatory activities.

Pau d'Arco increases blood flow to tissues, helping to detoxify them while providing antibacterial, anti-inflammatory, antifungal, antioxidative, and antiviral benefits. Due to its many biological/pharmacological properties, Pau d'Arco currently takes center stage in herbal medicines commonly used in South and Central America for treating ulcerations and other inflammatory conditions. Pau d'Arco appears to be an excellent nutraceutical for oral hygiene and periodontitis in dogs and cats after establishing proper dosage and clinical trials.

Studies revealed that Pau d'Arco is safe for prevention and treatment of periodontal treatment, since its reported LD_{50} for lapachol is 1.2–2.4 g/kg body wt in rats and 487–621 mg/kg in mice.

3.3 *Echinacea purpurea* (Coneflowers)

Echinacea purpurea plant contains several chemical compounds, including polysaccharides, caffeic acid derivatives (including chicoric acid), alkylamides, and glycoproteins. Phytoconstituents of *E. purpurea* exert anti-inflammatory, antimicrobial, and analgesic effects. Kumar

Table 1 Nutraceuticals/plant extracts having potential for prevention/treatment of periodontal diseases

Nutraceuticals/plant extracts	Active ingredients	Pharmacological effects	References
Abrus precatorius Linn.	Abrusogenin, stigmasterol, β-sitosterol, monoglycerides, triglycerides	Strengthening gum and teeth, dentifrice, antibacterial, antifungal	Ganesan (2008) and Ragasa et al. (2013)
Acasia arabica Wild.	Polyphenolics, gallic acid, catechin, chlorogenic acid, robidandiol	Antioxidative, antiviral, antimicrobial, antifungal, anti-plaque, anti-gingivitis	Ganesan (2008) and Pradeep et al. (2010)
Acacia farnesiana Linn.	Phenolic compounds, galloylglycoside	Antioxidative, antibacterial, dentifrice, anti-gingivitis	Ganesan (2008) and Ramli et al. (2011)
Acacia nilotica L. (babool)	Androstene, D-pinitol, catechins, gallic acid, rutin	Antioxidative, antibacterial, antifungal, anti-gingivitis, anti-plaque	Amos et al. (1999), Gilani et al. (1999), Shekhawat and Batra (2006), Bushra et al. (2007), Bachayaa et al. (2009), and Umaru et al. (2016)
Acalypha indica L. (Indian copper leaf)	Hexanedioic acid, bis(2-ethylhexyl) ester	Anti-toothache	Siddamallayya et al. (2010) and Chaichoowong et al. (2017)
Achyranthes aspera L. (devil's horsewhip)	Triterpenoid saponins, ecdysterone, achyranthine	Anti-toothache	Mahmood et al. (2005)
Allium sativum L.	Allicin (diallyl disulfide sulfoxide), alliin (*S*-allylcysteine sulfoxide), ajoene	Anti-toothache	Gupta (2006)
Aloe ferox Mill.	3,6 Octatriene, 3-cyclohexane-1-heptanol, bornylene, 1,3-cyclopentadiene, 1,3-cyclopentadiene	Anti-toothache	Gupta (2006) and Magwa et al. (2010)
Aloe vera L.	Salicylic acid, enzymes, vitamins, minerals, fatty acids	Dentifrice, anti-plaque, anti-gingivitis	Scherer et al. (1998), Wynn (2005), De Oliveira et al. (2008), Bhat et al. (2011), Sajjad and Subhani Sajjad (2014), Subhash et al. (2014), and Petrovic et al. (2015)
Althea officinalis L. (marshmallow)	Polysaccharides, flavonoids, coumarins, phenolic acid, sterols	Antimicrobial, anti-inflammatory, anti-periodontitis	Iauk et al. (2003)
Anacardium occidentale L.	B-Phellandrene, limonene, methyl chavicol, germacrene, linalool, β-cadinol, bisabolene	Sore gum, toothache	Kayode and Omotoynibo (2009)
Areca catechu Linn.	Arecoline, guvacoline, procyanidins, catechin, epicatechin	Dentifrice	Ganesan (2008) and Patil et al. (2009)
Arnica montana L.	Thymol, pseudoguaianolide, sesquiterpene lactones, flavanone glycosides	Antimicrobial, anti-periodontal	Koo and Jeon (2009) and Iauk et al. (2003)
Berberis vulgaris (barberry)	Berberine, berbamine, jateorrhizine, palmatine, oxycanthine	Antioxidative, anti-inflammatory, antibacterial, anti-gingivitis	Makarem et al. (2007), Palombo (2011), and Tu et al. (2013)
Azadirachta indica (neem)	Gallotannins	Glucosyl transferase inhibitory, antibacterial, anti-plaque	Wolinsky et al. (1996), Pai et al. (2004), Bhambal et al. (2011), and Singh et al. (2016)
Blood root (*Sanguinaria canadensis*)	Alkaloids	Bacteriostatic, anti-inflammatory, anti-bone loss, anti-tooth loss	Pai et al. (2004)
Cajanus cajan (Linn.) (pigeon pea)	Cajanstilbene acid, pinostrobin, vitexin, orientin	Antioxidative, anti-gingivitis	Ganesan (2008) and Wu et al. (2009)
Calendula officinalis L. (pot marigold)	Triterpine, oligoglycosides, saponins, quercetin, sesquiterpene glucoside, lutein, flavoxanthin, rutin, auroxanthin, zeaxanthin, carotenoids, lycopene	Antioxidant, anti-inflammatory, immunostimulating, anti-plaque, anti-gingivitis, wound healing, antimicrobial, antifungal	Iauk et al. (2003), Chakraborthy (2008), Preethi et al. (2009), Machado (2010), Parente et al. (2012), Khairnar et al. (2013), Li et al. (2016), and Lima et al. (2017)
β-Caryophyllene (*Origanum vulgare* L.; *Cinnamomum* spp.; *Piper nigrum* L.; *Cannabis sativa* L.)	β-Caryophyllene	Antibacterial, anti-plaque	Gertsch et al. (2008) and Pieri et al. (2016)

(continued)

Table 1 (continued)

Nutraceuticals/plant extracts	Active ingredients	Pharmacological effects	References
Chitosan	Chitosan	Antibacterial, antifungal, anti-plaque, anti-gingivitis	Ikinci et al. (2002), Uraz et al. (2012), Costa et al. (2014), and Husain et al. (2017)
Clove (*Syzygium aromaticum*)	Eugenol, eugenin, eugenitin, acetyl eugenol, β-caryophyllene, vanillin, crategolic acid, bicornin, caempesterol, sesquiterpenes	Antioxidative, anti-inflammatory, antibacterial, antiviral, immunostimulatory, anti-gingivitis, anti-toothache	Pinto and Santos (2017), Nagababu and Lakshmaiah (1992), Kumaravelu et al. (1996), and Rodrigues et al. (2009)
Colgate herbal toothpaste	Calcium carbonate, chamomile, sage, myrrh, eucalyptus, sodium monofluorophosphate	Anti-plaque, anti-gingivitis	George et al. (2009)
Commiphora myrrha (myrrh)	Sesquiterpenes (furanoeudesma-1,3-diene, curzarene)	Antibacterial, ant-inflammatory, anti-pyorrhea, astringent, analgesic, anti-gum diseases and mouth ulcers, antispasmodic	Dolara et al. (2000), Zhu et al. (2003), and Tipton et al. (2006)
Ceylon cinnamon Powder	Cinnamaldehyde, cinnamyl acetate, cinnamyl alcohol	Antimicrobial, anti-inflammatory	–
Citrus sinensis (orange peel extract)	Alkaloids, anthraquinones, tannins, phenolics, triterpenoids, saponins, flavonoids	Antimicrobial, Anti-caries	Shetty et al. (2016)
Coenzyme Q10	Coenzyme Q10	Antioxidative, anti-inflammatory, anti-periodontitis, anti-gingivitis	Nakamura et al. (1974), Hanioka et al. (1994), Pradeep et al. (2010), Soni et al. (2012), and Singh et al. (2016)
Echinacea purpurea (purple coneflowers)	Chicoric acid, alkylamides, caffeic acid, glycoproteins, polysaccharides	Antimicrobial, anti-inflammatory, immunomodulatory, analgesic, anti-gingivitis, anti-periodontitis	Kumar et al. (2009) and Modarai et al. (2011)
Eucalyptus extract/ eucalyptol oil	Borneol, 1,8-Cineol, globulol, *p*-cymene, limonene, α-pinene, cryptone, α-terpineol	Antibacterial, anti-gingivitis, anti-gum bleeding, anti-plaque	Nagata et al. (2008), Sebei et al. (2015), and Singh et al. (2016)
Folate	Folate	Anti-gingivitis	Vogel et al. (1978) and Pack (1984)
Fragaria vesca	Myrthenol, nonal, linalool, geraniol	Anti-plaque, anti-tartar	–
Grapefruit seed extract (GSE) (*Vitis vinifera*)	Proanthocyanidins	Antibacterial, antioxidative, anti-inflammatory, immunostimulatory, antifungal	Garlet et al. (2004), Houde et al. (2006), Baydar et al. (2006), La et al. (2009), Govindaraj et al. (2010), and Özden et al. (2017)
Green tea	Epicatechin, epicatechin gallate, epigallocatechin, (−)-epigallocatechin-3-gallate	Antioxidative, anti-inflammatory, antibacterial, preventing bone resorption, anticariogenic	Yu et al. (1992), Hirasawa et al. (2002), Yun et al. (2004), Coimbra et al. (2006), Kushiyama et al. (2009) and Chatterjee et al. (2012)
Hamamelis virginiana L.	Hamamelitannins, catechins, proanthocyanin, quercetin, saponins	Anti-inflammatory, astringent, antimicrobial, anti-periodontitis	Erdelmeier et al. (1996) and Iauk et al. (2003)
Hyaluronic acid	Hyaluronic acid	Periodontal regeneration, bacteriostatic	Pirnazar et al. (1999) and Bansal et al. (2010)
Hydrastis canadensis (goldenseal)	Isoquinolone alkaloids (hydrastine, hydrastinine, berberine, berberastine, canadine, canalidine, tetrahydroberberastine	Antibacterial, anti-inflammatory, immunostimulating	Baghele (2017)
Illicium verum Hook (Chinese star anise)	A-pinene, farnesol, α-terpineol, safrol, anisaldehyde, β-phellandrene, p-cumicaldehyde, limonene, shikimic acid	Antioxidative, antimicrobial, antibacterial, antifungal, anti-periodontitis	Iauk et al. (2003), Chouksey et al. (2010)
Matricaria chamomilla L. (chamomile)	Terpenoids, flavonoids, chamazulene, α-bisabolol, umbelliferone	Antioxidative, anti-inflammatory, antiseptic, fungistatic, anti-periodontitis, anti-sore gum and teeth	Singh et al. (2011)
Mellissa officinalis L.	Citronellal, geraniol	Antimicrobial, anti-periodontitis	Iauk et al. (2003), Abdellatif et al. (2014)

(continued)

Table 1 (continued)

Nutraceuticals/plant extracts	Active ingredients	Pharmacological effects	References
Mentha piperita L. (peppermint)	Menthol, menthone, menthyl acetate, 1,8-cineole, limonene, β-pinene, β-caryophyllene, flavonoids	Antioxidative, anti-inflammatory, analgesic, antiulcer, cytoprotective, chemopreventive, anti-gingivitis	Blumenthal et al. (1998), Kumar et al. (2009), and Taheri et al. (2011)
Origanum vulgare (oregano oil)	Polyphenols, flavones	Antioxidants	Petrovic et al. (2015)
Parodontax®	Sodium bicarbonate, sodium fluoride, chamomile, echinacea, sage, myrrh, rhatany, peppermint oil	Anti-gingivitis, anti-plaque, anti-periodontitis	Pannuti et al. (2003) and Ozaki et al. (2006)
Pau D'Arco	Quinods, quercetin, benzenoids, flavonoids, lapachol, lapachenol, deoxylapachol, α-lapachone, β-lapachone, tabebuin, anisic acid, anthraquinones, etc.	Anti-infective, anticancer, anti-inflammatory, antimicrobial, antiviral, antifungal, anti-stomatitis, anti-abscess	Morrison et al. (1970) and Giuraud et al. (1994)
Propolis extract (*Apis mellifera* L.)	Flavonoids, phenolic acids, terpenes, aromatic acids, chalcones	Antioxidative, antimicrobial, cavity cleaning, anti-tartar, anti-plaque, wound healing	Koo and Jeon (2009), Gebaraa et al. (2003), Coutinho (2012), Campos et al. (2014), Srivastava et al. (2016), de Moraes Porto et al. (2018), and Kubiliene et al. (2017)
Quercetin	Quercetin	Antioxidative, anti-inflammatory, immunomodulatory	Liang et al. (2011), Napimoga et al. (2013), and Li et al. (2016)
Red clover (*Trifolium pretenase* L.)	Formononetin, biochanin A, daidzein, genistein	Antioxidative, anti-inflammatory, antibacterial, anticancer	Ramos et al. (2012) and Petrovic et al. (2015)
Red thyme oil (*Thymus vulgaris*)	Terpinene, thymol	Antibacterial, antifungal	Borugă et al. (2014)
Rosemary (*Rosmarinus officinalis* L.)	Borneol, 1,8-cineol, α-pinene, limonene, rosmarinic acid, linalool, camphor, camphene, verbenone, linalool	Antioxidative, anti-inflammatory, antibacterial, antifungal, antimutagenic, chemopreventive	Bozin et al. (2007), Santoyo et al. (2005), Hussain et al. (2010), Lee et al. (2015), and Petrovic et al. (2015)
Royal jelly (Rojelexin™, Vets Plus, Inc.)	10-Hydroxy-δ2-decenoic acid, apismin, royalactin, royalisin, jelleines, apolipophorin III	Antibacterial, antifungal, anti-plaque	Shiga et al. (2010), Eick et al. (2014), Pasupuleti et al. (2017), and Lin et al. (2018)
Salvadora persica (miswak)	*N*-benzylbenzamide, stigmasterol, β-sitosterol, spiculesporic acid, methyl hexadecanoate	Antimicrobial, anti-plaque, anti-gingivitis, anticarcinogenic	Almas (2001), Almas and Almas (2014), and El-Hefny et al. (2017)
Salvia officinalis L. (sage)	α-Thujone, β-thujone, camphor, α-pineno, β-pineno, borneol, tannic acid, cornsole, cornsolic acid, chlorogenic acid	Antibacterial, antifungal, antiviral, anticandidal, anti-stomatitis, anticaries, anti-plaque, anti-gingivitis, astringent	Delamare et al. (2007), Pierozan et al. (2009), George et al. (2009), Porte et al. (2013), Ghorbani and Esmaeilizadeh (2014), Narayanan and Thangavelu (2015), and Petrovic et al. (2015)
Sodium hexametaphosphate (SHMP)	Hexametaphosphate	Anti-calculus	Stookey et al. (1995, 1996), White and Gerlach (2000), and Oba et al. (2018)
Tea tree oil (*Melaleuca alternifolia*)	Terpine-4-ol, γ-terpinene, 1,8-cineole, α-cymene, δ-cadinene, globulol	Antimicrobial	Carson et al. (2006)
Terminalia chebula	Hydrolysable tannins (gallic acid, chebulic acid, punicalagin, chebulanin, corilagin, chebulinic acid), triterpenoids, phenolic compounds	Antibacterial	Muhammad et al. (2012) and Nayak et al. (2014)
Turmeric/curcumin	Curcuminoids	Antioxidative, anti-inflammatory	Chaturvedi (2009)
Vitamin C	Vitamin C	Antioxidative	Turesky et al. (1970)

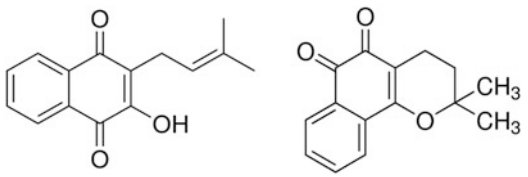


Fig. 3 Structural formula of lapachol (left) and β-lapachone (right)

et al. (2009) reported anti-inflammatory and antibacterial effects of *E. purpurea*. *E. purpurea* extract fights against gingivitis and periodontal disease by stimulating the immune system against bacterial infections (Modarai et al. 2011). Its analgesic property soothes aching gums and teeth. In humans, a mouth rinse with *Echinacea*, sage, mint oil, and chamomile is used in gingivitis and periodontal treatment (Modarai et al. 2011). Unlike many other plant products, *E. purpurea* extract is safe for use in pregnant dogs and cats.

3.4 Barberry

Barberry (*Berberis vulgaris*) plant contains two classes of alkaloids: protoberberines (berberine, berbamine, jateorrhizine, and palmatine) and bisbenzylisoquinolines (oxycanthine). Berberine is the main active compound and most studied alkaloid for its multiple pharmacological actions, including anti-inflammatory, anti-cyclooxygenase, and anti-inducible nitric oxide synthase (for details, refer to chapter "Glucosinolates and Organosulfur Compounds"). Barberry extract and berberine alkaloids are used for many health conditions throughout the world. It has been suggested that berberine may decrease periodontal tissue degradation through the regulation of matrix metalloproteinases (MMPs) during the progression of periodontal diseases (Tu et al. 2013). Berberine gel has been found to reduce the oral biofilm by 56% and gingival index by 33%. (Makarem et al. 2007). These authors also assessed the effect of berberine gel on periodontal inflammation. The only significant finding was reduction in the number of inflammatory cells infiltrating the oral mucosa. Palombo (2011) demonstrated that berberine was found to be more effective against bacteria (*Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis*) by inhibiting their collagenase activity. Berberine appears to be a promising nutraceutical for periodontal diseases in canines and felines. For details on berberine, refer to chapter "Glucosinolates and Organosulfur Compounds."

3.5 Rosemary

Rosemary (*Rosmarinus officinalis* L.) is an evergreen perennial plant which has needle-like leaves. Its phytoconstituents include borneol, 1,8-cineol, α-pinene, limonene, camphor, camphene, verbenone, rosmarinic acid, and linalool (Santoyo et al. 2005; Hussain et al. 2010). These phytochemicals exert powerful antioxidative, anti-inflammatory, antibacterial, antifungal, antimutagenic, and chemopreventive properties (Huhtanen 1980; Bozin et al. 2007; Hussain et al. 2010; Petrovic et al. 2015). Santoyo et al. (2005) reported that antimicrobial activity in rosemary oil is due primarily to borneol, followed by camphor and verbenone. Significant activation of alkaline phosphatase by rosmarinic acid may induce mineralization in osteoblasts which could prevent bone metabolic diseases (Lee et al. 2015). It can be suggested that rosemary oil has a nutraceutical potential for prevention and treatment of periodontitis.

3.6 Babool

Babool (*Acacia nilotica*) is a tropical tree native to Indian and African subcontinents. Other names for babool tree are babul, booni, Egyptian thorn, thorn mimosa, thorny acacia, prickly *Acacia*, and gum Arabic tree. The leaves, roots, bark, fruit, and gum extracts of babool have been used in various Ayurvedic herbal preparations for centuries. Babool extract has an anti-inflammatory compound, androstene steroid. The extract also has other chemical compounds such as D-pinitol, kaempferol, gallic acid, ellagic acid, (+/−) catechin, (−)-epigallocatechin, and rutin. In addition, the extract has cyclitols, fatty acids, seed oils, nonprotein amino acids, terpenes, saponins, hydrolyzable tannins, flavonoids, and niloticane (Malviya et al. 2011). Its extract contains a total phenolic content ranging from 9.2 to 16.5% (Bushra et al. 2007) and tannins and gallic acid from 24 to 42% (Rahaman 2010).

By having these phytochemicals, babool extract is known to exert hemostatic, antioxidative, anti-inflammatory, antispasmodic, analgesic, immunostimulating, antibacterial, antifungal, anti-platelet, antiparasitic, and galactogogue activities (Amos et al. 1999; Gilani et al. 1999; Bushra et al. 2007; Bachayaa et al. 2009; Umaru et al. 2016). In addition to its use as an antihypertensive, antidiabetic, antimicrobial, anti-inflammatory, and antiprotozoal (*Entamoeba histolytica* in dogs and cats), babool stick and toothpaste have been used for oral health. In an in vitro study, Sharma et al. (2014) demonstrated that an aqueous babool extract, in a dose (5%, 10%, and 50%)-dependent manner, exhibited antimicrobial activity against *Streptococcus mutans*. In another in vitro study, Sunitha et al. (2015) reported that toothpaste having babool extract provided greater inhibitory effect against *S. mutans* than other commonly used toothpastes. It has been suggested that babool extract has inhibitory effect against *S. mutans* equal to that of chlorhexidine. These findings suggest that babool-based toothpaste can be

indicated in dogs and cats for oral health and periodontal diseases.

Based on acute toxicity in mice, aqueous extract of babool root extract is safe up to a dose of 2000 mg/kg; and its NOEL in rats is reported to be <250 mg/kg/day.

3.7 Chitosan

Chitosan is an anti-abrasive fiber obtained from crustaceans (shrimp, lobsters, and crabs) exoskeleton. Its structural formula is shown in Fig. 4. Chitosan with a molecular weight of >100 kDa is poorly soluble in water, and its bioavailability is about 1–3%.

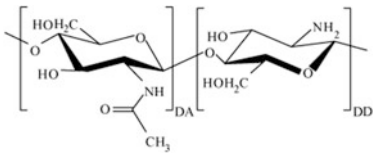


Fig. 4 Structural formula of chitosan

Chitosan has emerged as a potential material for biodental applications due to its unique properties such as bioactivity, antimicrobial activity, and biocompatibility (Husain et al. 2017). For details of its applications in dentistry, see Fig. 5.

Chitosan is also directly applied to gums to treat inflammation and to prevent dental caries. Ikinci et al. (2002) reported that chitosan in combination with chlorhexidine gluconate showed a greater activity compared to chlorhexidine alone. In combination, chlorhexidine is needed at a lower concentration, thereby avoiding its unwanted side effects. Chitosan is known to exert antimicrobial activity against *P. gingivalis*, especially with high molecular weight chitosan. In other studies, chitosan proved to be an effective antibacterial and antifungal agent (Uraz et al. 2012; Costa et al. 2014). Costa et al. (2014) reported that chitosan-containing mouthwash was capable of interfering with all microorganisms' adherence and biofilms with superior activity to other commercially available mouthwashes. By having antimicrobial, antifungal, and adhesive properties, chitosan in a mouthwash, film, or gel appears to be an ideal for local delivery systems for prevention or therapy of periodontal diseases.

Fig. 5 Potential applications of chitosan materials in dentistry (Courtesy of Dr. Shehriar Husain and his co-investigators 2017)

3.8 Royal Jelly

Royal jelly (RJ) is secreted by honeybees from their hypopharyngeal and mandibular glands. RJ consists of water (50–60%), proteins (18%), carbohydrates (15%), lipids (3–6%), mineral salts (1.5%), and vitamins (Pasupuleti et al. 2017). RJ proteins contain major royal jelly proteins (MRJPs), apismin, royalactin, royalisin, jelleines, and apolipophorin III, free amino acids, glucose oxidase, sugars, lipids, vitamins, etc. (Lin et al. 2018). MRJPs may contain nine types of water-soluble proteins with a molecular weight of 49–87 kDa. Additionally, there are some signature peptides that could have potential applications in many health conditions, including periodontal diseases.

Honey, having RJ proteins, has been used for the treatment of many oral diseases, including periodontitis, stomatitis, and halitosis. In addition, it has been applied for the prevention of dental plaque, gingivitis, and mouth ulcers. The antibacterial and anti-inflammatory properties of honey can stimulate the growth of granulation tissue, leading to the repair of damaged tissue cells. Antibacterial activity against *P. gingivalis* has been well documented (Eick et al. 2014; Pasupuleti et al. 2017). Shiga et al. (2010) reported that honey consumption ameliorates halitosis due to its strong antibacterial activity resulting from its methylglyoxal component.

Royal jelly-based product Rojelexin™ (Vets Plus, Inc., Menomonie, WI), by containing royalisin, 10-hydroxy-δ2-decenoic acid, and jelleines, exerts an antimicrobial effect. 10-Hydroxy-δ2-decenoic acid, which is a major component of the lipid fraction of Rojelexin™, exhibits antibacterial and antifungal activities. The structural formula of 10-hydroxy-δ 2-decenoic acid is shown in Fig. 6.

This bioactive component of RJ has been found less than one-fourth as active as penicillin against *Micrococcus pyogenes* and less than one-fifth as active as chlortetracycline against *E. coli*. A dental paste consisting Rojelexin™ and honey showed a potent synergistic effects against oral pathogenic bacteria, such as *S. mutans* and *P. gingivalis*.

3.9 Propolis

Bees (*Apis mellifera* L.) collect a resinous exudate called propolis or bee glue from different plant sources to protect them from bee pathogens. Analysis of propolis has revealed more than 600 chemicals, including flavonoids, phenolic acids, terpenes, and aromatic acids. The majority of these compounds are lipophilic and are therefore easily dissolved in ethanol or methanol (Dantas Silva et al. 2017; Kubiliene et al. 2017). Recent reports suggest that Brazilian red propolis with the sap of *Dalbergia ecastophyllum* has unique components (such as isoflavonoids, propolones/guttiferones, terpenes, chalcones, and phenolic compounds) (Machado et al. 2016; Rufatto et al. 2017), which differ from other propolis produced in Brazil and around the world. The chemical composition of propolis varies significantly depending upon the flora of a particular location (reviewed in detail by de Moraes Porto et al. 2018). Propolis has been used in folk medicine and complementary therapies since ancient times. During the past decade, propolis has been shown to exert multiple biological and pharmacological effects, such as antioxidative, anti-inflammatory, immunomodulatory, antiseptic, antibacterial, antifungal, antineoplastic, cardioprotective, and hepatoprotective properties (Cornara et al. 2017; Dantas Silva et al. 2017).

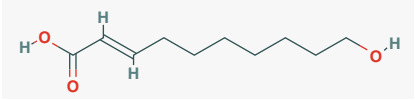


Fig. 6 Structural formula of 10-hydroxy-δ2-decenoic acid

Propolis products, containing microemulsions or nanostructured lipids, have been used for wound healing (Zilius et al. 2016; Rosseto et al. 2017). Recently, Cao et al. (2017) explained the underlying mechanism of propolis in wound healing. An ethanol extract of Chinese propolis (EECP) induced the expression of antioxidant-related genes, such as HO-1, GCLM, and GCLC, which has great implications for the potential of propolis to alleviate oxidative stress in wound tissues, and therefore it can be a promising nutraceutical for wound healing.

In a number of clinical studies, propolis has been indicated for treatment of periodontal diseases. Gebaraa et al. (2003) evaluated the effect of subgingival irrigation with propolis extract based on clinical and microbiological parameters. Findings revealed that propolis treatment (irrigation with a hydroalcoholic solution of propolis extract twice/week) for 2 weeks caused a decrease in total viable counts of anaerobic bacteria, an increase in the proportion of sites with low levels of *Porphyromonas gingivalis*, and a decrease in the number of sites with detectable presence of yeasts. In a similar clinical study, Coutinho (2012) also reported that subgingival irrigation, with propolis extract as an adjuvant to periodontal treatment, was more effective than scaling and root planing as assessed by clinical and microbiological parameters.

Dental caries is considered a chronic and multifactorial disease that occurs as a result of the dissolution of tooth mineral by acids derived from bacterial fermentation of dietary carbohydrates, mainly by *Streptococcus* and *Lactobacillus* ssp., which are involved in the initiation and progression of the lesions, respectively (Karpinski and Szkaradkiewicz 2013). Recently, de Moraes Porto et al. (2018) reported that ethyl acetate extract of Brazilian red propolis (EARP) and micellar nanocomposites loaded with EARP (MNRP) showed antimicrobial activity for the main agents causing

dental caries (*Streptococcus mutans* and *Lactobacillus acidophilus*) and for *Candida albicans*. MNRP at concentrations of 0.3 and 0.6% used as a cavity cleaner do not compromise the aesthetics or microtensile bond strengths of the dentin/resin interface. Propolis extract can be applied topically as a gel or toothpaste.

Srivastava et al. (2016) reported that Proclean (Vets Plus Inc., Menomonie, WI, USA), which contains the bioactive compound propolis, exhibited antibacterial activity in an in vitro study and anti-inflammatory, anti-tartar, anti-plaque, and anti-halitosis effects in dogs.

Based on all these findings, it can be suggested that propolis extract can be used as a nutraceutical for preventing and treating periodontal diseases in dogs and cats.

3.10 Cinnamon Powder

The healing properties of Ceylon cinnamon come primarily from three essential oils (cinnamaldehyde, cinnamyl acetate, and cinnamyl alcohol). Cinnamon phytoconstituents exert antimicrobial (antiseptic), anti-inflammatory, carminative, cholesterol-lowering, and mild anesthetic properties. Additionally, cinnamon is a source of calcium, manganese, and fiber.

In periodontal diseases, the daily recommended dose of cinnamon is: small dogs and cats-1/8th tsp a day, medium dogs-1/4th tsp a day, and large dogs-1/2 tsp a day.

3.11 *Hydrastis canadensis* (Goldenseal)

Hydrastis canadensis, also known as goldenseal, orange root, or yellow puccoon, consists of isoquinoline alkaloids (hydrastine, berberine, berberastine, hydrastinine, tetrahydroberberastine, canadine, and canalidine). Pharmacological effects come from berberine and hydrastine. Antibacterial activity is from berberine (Baghele 2017). Other properties include anti-inflammatory and immune-stimulating activities. It needs to be mentioned that this herb may not be as safe as others and is contraindicated in pregnant dogs and cats.

3.12 Oregano

Oil of *Origanum vulgare* contains polyphenols, including numerous flavones, which are antioxidants. It exerts antibacterial, antiviral, and antifungal activities. In 2005, the US Federal Trade Commission brought legal action against a firm that had claimed oregano oil treated colds and flu or relieved bacterial and viral infections. Herbal products having oregano essential oil were found to have numerous anti-disease effects and so were being sold as unauthorized, misbranded drugs subject to seizure and federal penalties. Oregano oil is safe for dogs, but not for cats.

3.13 Sage

Sage (*Salvia officinalis* L.) is a perennial low shrub native to the Middle East and Mediterranean regions. Presently, it is cultivated throughout the world. Essential oils of sage leaves contain α-thujone, β-thujone, α-pineno, β-pineno, and camphor. In folk medicine, *S. officinalis* extract has been used for the treatment of many diseases and conditions, including seizure, tremor, ulcers, gout, rheumatism, inflammation, dizziness, paralysis, diarrhea, and hyperglycemia. In recent years, phytochemicals in *S. officinalis* have been found to exert antioxidative, anti-inflammatory, antibacterial, antiviral, antifungal, anticaries, anti-plaque, antinociceptive, spasmolytic, carminative, antiseptic, astringent, hypoglycemic, antihidrotic, and anti-dementic properties (Bozin et al. 2007; Delamare et al. 2007; Pierozan et al. 2009; Sookto et al. 2013; Ghorbani and Esmaeilizadeh 2014; Petrovic et al. 2015). *S. officinalis* is used in dental practices for the management of periodontal diseases and to prevent halitosis (Smullen et al. 2012; Narayanan and Thangavelu 2015). The thujones and phenolic acids exert antiseptic and antibiotic action, and tannins exert astringent action. In an in vitro study, Smullen et al. (2012) found that the extracts of *S. officinalis* and *Rosmarinus officinalis* inhibited glucosyl transferase activity, glucon production, and plaque formation and suggested that the extract of these plants can be used as an anti-plaque agent. Sookto et al. (2013) reported anticandidal activity of *S. officinalis* against *Candida albicans* and indicated that it can be used as an antifungal agent in periodontal diseases. Sage extract is often used in toothpaste. It can also be indicated for treatment of periodontal diseases in dogs and cats after clinical studies.

3.14 *Aloe vera*

Aloe vera contains salicylic acid, enzymes, vitamins, minerals, fatty acids, and sugars. *Aloe vera* has strong antioxidative, anti-inflammatory, analgesic, immunostimulating, antibacterial, antiviral, and antifungal properties (Scherer et al. 1998; Bhat et al. 2011; Sajjad and Subhani Sajjad 2014). Sajjad and Subhani Sajjad (2014) reported anti-inflammatory activity of *Aloe vera* by providing evidence of its inhibitory action on the cyclooxygenase pathway, thereby reducing prostaglandin E_2 production.

Scherer et al. (1998) found that a mouth rinse containing *Aloe vera* reduced gingival inflammation, lesions, and bleeding. In another study, Bhatt et al. (2011) demonstrated that *Aloe vera* gel, when applied sublingually, significantly

decreased pocket depth and showed a relative decrease in gingival index and plaque index (reviewed in Petrovic et al. 2015). In a number of studies, *Aloe vera* (gel, toothpaste, or mouthwash) is reported to prevent periodontal diseases and caries (Wynn 2005; Subhash et al. 2014).

3.15 Peppermint

The herb peppermint (*Mentha piperita* L.) is a cross between two types of mint (watermint and spearmint) which grows throughout Europe and North America. Mint has been used for a variety of health purposes (irritable bowel syndrome, headache, common cold, muscle aches, etc.) for thousands of years (McKay and Blumberg 2006; Ford et al. 2008). Peppermint oil contains several phytoconstituents of medicinal importance, including menthol (40.7%), menthone (23.4%), menthyl acetate, 1,8-cineole, limonene, β-pinene, β-caryophyllene, and flavonoids. Phytoconstituents of peppermint exert antioxidative, anti-inflammatory, analgesic, antiulcer, cytoprotective, and chemopreventive activities. Peppermint oil, when applied locally, or leaf extract given orally, produces an analgesic effect (Blumenthal et al. 1998) and reduces toothache and gingival inflammation (Taheri et al. 2011). Essential oil and leaf extract of peppermint reduce periodontal bacteria (Kumar et al. 2009).

3.16 Grape Seed Extract

Grape seed extract (GSE), obtained from *Vitis vinifera* or other *Vitis* spp., is very rich in proanthocyanidins. Proanthocyanidin A (polyhydroxyflavan-3-ol) is a 20–50 times more potent antioxidant than vitamin C or vitamin E. The structural formula of proanthocyanidin A is shown in Fig. 7.

GSE exerts antioxidative, anti-inflammatory, antibacterial, antifungal, and antiparasitic activities. Govindaraj et al. (2010) demonstrated the antioxidative effect of proanthocyanidin (40 mg/kg body wt) in an experimental periodontitis rat model that was induced by injecting *E. coli* endotoxin. Recently, Özden et al. (2017) reported that rats with ligature-induced periodontitis receiving GSE (200 mg/kg body wt, orally) showed an anti-inflammatory effect (lower inflammatory cell number), higher connective tissue attachment level, and lower osteoclast density. Since microbial dental biofilm accumulation is the primary etiologic factor for periodontal disease and the biofilms are highly resistant to antibiotics, GSE appears to be useful due to its antibacterial action. Also, in several other studies, GSE has been found to ameliorate periodontitis by suppressing oxidative stress and inflammation, matrix metalloproteinases, osteoclast factors, immunostimulating, and antibacterial effects (Nuttall et al. 1998; Li et al. 2001; Garlet et al. 2004; Baydar et al. 2006; Houde et al. 2006; Furiga et al. 2009; La et al. 2009). All these findings suggest that GSE can be used as a nutraceutical for the prevention and treatment of periodontitis in canines and felines.

3.17 Myrrh (*Commiphora myrrha*)

Myrrh (*Commiphora myrrha*) or Myrrha (Latin) gets its name from the daughter of Thesis, king of Syria. The tree is native to Saudi Arabia, Oman, Yemen, Somalia, Eritrea, and Ethiopia. Its use has been described in Roman literature, the Bible, the Hebrew Bible, the Koran, and Ayurvedic, Unani, and Chinese medicine. Myrrh is used as a fragrance in cosmetics and as a flavoring agent in food and beverages. Myrrh contains chemical constituents such as sesquiterpenes (furanoeudesma-1,3-diene and curzarene), sterols, and steroids (Zhu et al. 2003). Myrrh oil is extracted from the tree trunk, stem, and branches by steam distillation. Myrrh's gum resin and its main chemical constituent (furanoeudesma-1,3-diene) are shown in Fig. 8.

Resin and oil of myrrh have been used for many centuries to treat swollen gums, mouth ulcers, etc. Phytoconstituents of myrrh exert antibacterial, antiviral, antifungal, antiseptic, astringent, antispasmodic, amoebocidal, anti-inflammatory, analgesic, and hypoglycemic properties (Dolara et al. 2000; Tipton et al. 2006). In Greek culture, when soldiers went to battle, myrrh was an essential part of their combat gear because of its extremely high antiseptic and

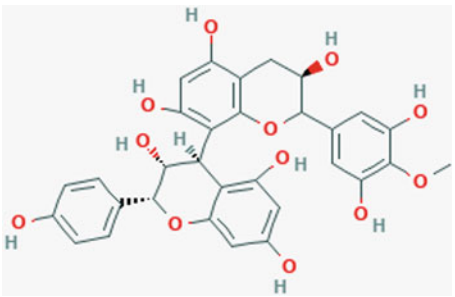


Fig. 7 Structural formula of proanthocyanidin A

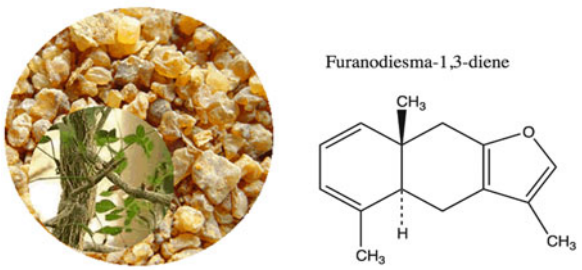


Fig. 8 Myrrh's gum resin and active principles (furanoeudesma-1,3-diene)

anti-inflammatory properties. It was used to clean wounds and to prevent infection and gangrene. Myrrh is an excellent antiseptic in the mouth for sore throat, gum sores, toothache pain, and pyorrhea. Currently, myrrh is used in toothpastes, dental powders, gargles, and mouthwashes. It is noteworthy that myrrh is considered safe in pregnant dogs when used in small amounts. It is not recommended for cats.

3.18 Green Tea

Green tea (*Camellia sinensis*) is a shrub native to Tibet, Western China, and Northern India. The active compounds present in green tea are polyphenols called catechins (epicatechin, epicatechin gallate, epigallocatechin, and (−)-epigallocatechin-3-gallate). Among all catechins, (−)-epigallocatechin-3-gallate (EGCG) is the principal catechin with maximal biological activity. Its structural formula is shown in Fig. 9.

Green tea leaves may contain flavonoids, including catechins and their derivatives, up to 30% on a dry weight basis. Of course, green tea also contains many other compounds, such as carotenoids, tocopherols, ascorbic acid, caffeine, L-theanine, etc. Green tea has been used to rinse and brush the teeth. *Streptococcus mutans* could be inhibited completely after contact with green tea extract for 5 min. There is no drug resistance after repeat cultures. The clinical effects showed that the plaque index and gingival index decreased significantly after green tea extract was used. In a number of studies, local application of green tea has been shown to promote periodontal health by antioxidative and anti-inflammatory effects, preventing bone resorption and dental caries, and limiting the growth of certain bacteria (*Porphyromonas gingivalis*, *Prevotella intermedia*, *Prevotella nigrescens*, *Streptococcus mutans*) associated with periodontal diseases (Sakanaka et al. 1996; Hirasawa et al. 2002; Coimbra et al. 2006; Kushiyama et al. 2009; Chatterjee et al. 2012). *P. gingivalis* has been reported to stimulate the activity and expression of several groups of matrix metalloproteinases (MMPs), and EGCG has inhibitory effects on the activity and expression of MMPs (Chatterjee et al. (2012). EGCG prevents bone resorption that occurs in periodontal diseases by inhibiting the expression of MMP-9 in osteoblasts and formation of osteoclasts (Yun et al. 2004). The anticariogenic effect of green tea is also well established (Yu et al. 1992).

3.19 Clove

The clove (*Syzygium aromaticum*) is an evergreen tropical tree native to India and Indonesia's Spice Islands (Aka and Maluku). Dried flower buds are commonly used as a spice in the cuisines of India, Africa, the Middle East, and other parts

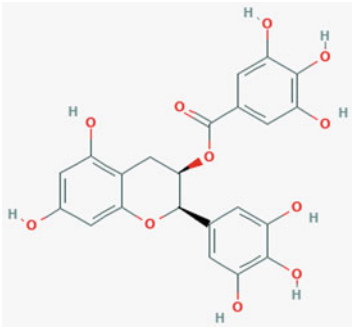


Fig. 9 Structural formula of (−)-epigallocatechin-3-gallate (EGCG)

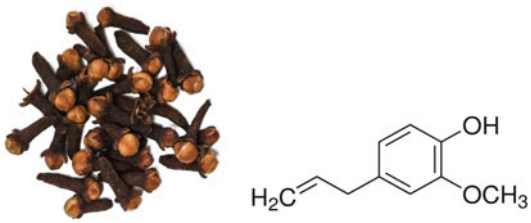


Fig. 10 Clove (left) and its active principle eugenol (right)

of the world. Its use dates back to the fourth century BC. In China (200 BC), clove was used as "mouth perfume." In both Ayurvedic and Chinese medicines, clove has been used for many human ailments. In the tenth century, the Arab dentist Al-Gazzar used clove for controlling mouth odors and pain. The clove and the structural formula of its active principle eugenol are shown in Fig. 10.

Essential oil of clove, consisting of 80–95% eugenol, exerts antibacterial, antiviral, and immunostimulatory activities. Eugenol also has analgesic and antiseptic properties. Essential oil of clove also contains other chemical constituents, such as eugenin, eugenitin, acetyl eugenol, β-caryophyllene, vanillin, crategolic acid, bicornin, caempesterol, and sesquiterpenes (Table 1). Clove oil is known to reduce the bacteria that cause gum diseases and promotes the growth of good bacteria, thereby creating a balanced oral biome. Furthermore, pathogenic bacteria do not develop resistance to clove oil, as they do against antibiotics. Clove oil has proven to be effective against *P. gingivalis*. Eugenol also fights the growth of many fungi, including *Candida albicans* and *C. tropicalis*.

Clove is reported to have the highest antioxidant value (15,188.2 mg (poly)phenols/100 g fresh weight) compared to any other natural plant product (Pinto and Santos 2017), thereby exerting a strong anti-inflammatory effect. Rodrigues et al. (2009) reported that mice treated with water extract of clove showed inhibition of macrophages which produce pro-inflammatory cytokines (IL-1βand IL-6). In an in vitro study, these investigators demonstrated that eugenol in essential oil of clove also inhibited the production of these cytokines. Findings of this study and of others confirmed

that eugenol exerts antioxidative and anti-inflammatory effects (Nagababu and Lakshmaiah 1992; Kumaravelu et al. 1996), and it may be useful to prevent or treat periodontal diseases in dogs and cats. Clove can be applied topically in the form of a gel, toothpaste, or oil.

3.20 *Eucalyptus*

There are more than 700 species of *Eucalyptus*, and most are native to Australia. Presently, *Eucalyptus* are widely cultivated in the tropical and temperate world, including the Americas, Europe, Africa, the Mediterranean Basin, the Middle East, China, and the Indian subcontinent. Eucalyptus leaves contain many phytoconstituents, including borneol, 1,8-cineol, globulol,

p-cymene, limonene, α-pinene, cryptone, α-terpineol, and many others (Sebei et al. 2015). Nagata et al. (2008) reported that *Eucalyptus* extract chewing gum (60–90 mg/day) had significant effects on plaque accumulation, gingival index, bleeding on probing, and periodontal probing depth. Findings revealed that the use of *Eucalyptus* extract chewing gum may promote periodontal health. Also, *Eucalyptus* extract/oil possesses antibacterial activity against cariogenic and periodontopathic bacteria, and therefore it is used in toothpastes.

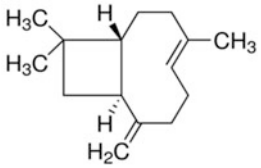
3.21 Chamomile

Chamomile (*Matricaria chamomilla* L.), also known as "star among medicinal species," is native to Southern and Eastern Europe. Currently, the plant is grown in Germany, France, Hungary, Brazil, Russia, India, North and South America, Australia, New Zealand, and many other countries. Phytoconstituents present in *M. chamomilla* L. include 28 terpenoids, 36 flavonoids, and 52 additional compounds with potential pharmacological activity. Chamomile oil (blue oil) also consists of sesquiterpene derivatives and traces of monoterpenes. Components such as α-bisabolol and cyclic ethers are antimicrobial, umbelliferone is fungistatic, and chamazulene and α-bisabolol are antiseptic (Singh et al. 2011). Some of these phytoconstituents exert antioxidative, anti-inflammatory, antiseptic, antispasmodic, anti-periodontitis, and anti-sore gum activities. Chamomile is a nutraceutical which can be used for many periodontal diseases.

3.22 β-Caryophyllene

The sesquiterpene β-caryophyllene (BCP) is found in large amounts in the essential oils of many different spice and food plants, such as oregano (*Origanum vulgare* L.), cinnamon (*Cinnamomum* spp.), and black pepper (*Piper nigrum* L.).

Fig. 11 Structural formula of caryophyllene (right)



BCP is also present in large amounts in essential oil (up to 35%) of *Cannabis sativa* L (Gertsch et al. 2008). The structural formula of β-caryophyllene is shown in Fig. 11.

In both lipopolysaccharide (LPS) and carrageenan models of inflammation, BCP induced anti-inflammatory effect (Gertsch et al. 2008). Upon binding to the cannabinoid 2 (CB2) receptor, BCP inhibits adenylate cyclase, leads to intracellular calcium transients, and weakly activates the mitogen-activated kinases, Erk ½ and p^{38} in primary human monocytes, thereby exerting anti-inflammatory effect.

Recently, Pieri et al. (2016) evaluated the antimicrobial activity of BCP against bacteria from canine's dental plaque in vitro and in vivo. Bacterial adherence of three *Enterococcus* sp., one *Streptococcus* sp., one *Haemophilus* sp., one *Aerococcus* sp., one *Bacillus* sp., and one *Lactococcus* sp. was inhibited by subinhibitory concentration. An in vivo assay showed reduction in dental plaque formation by BCP, with final plaque coverage of 23.3±2.6% of the total area of the teeth, a significant difference compared to chlorhexidine (37.5±3.7%).

The study concluded that BCP has antimicrobial activity against the proliferation of dog's dental plaque-forming bacteria representing a suitable alternative to the use of chlorhexidine in prophylaxis and treatment of periodontal diseases of dogs.

3.23 Thyme

Thyme oil is obtained from *Thymus vulgaris*. Orally, thyme has been indicated in many health conditions, such as bronchitis, pertussis, sore throat, colic, arthritis, dyspepsia, gastritis, diarrhea, enuresis, flatulence, skin disorders, urinary disinfectant, and as an appetite stimulant. Topically, thyme oil is used as a counterirritant, an antiseptic in mouthwashes and liniments. Thymol has been shown to be effective in reducing plaque formation, gingivitis, and cavities due to its antimicrobial and antioxidant properties. It has also been used in combination with chlorhexidine as a dental varnish to prevent caries. By having antibacterial and antifungal activities, thyme oil is used in toothpastes (Meeker and Linke 1998; Borugă et al. 2014).

3.24 Folic Acid

Folic acid has been clinically tested in mouthwash solutions to treat gingivitis and maintain normal gum tissue. In a

number of clinical studies, folic acid has been found to be effective in gingivitis via multiple mechanisms including anti-inflammatory effects (Pack 1984). Folic acid binds with bacterial endotoxins and renders them neutral. However, when taken as a dietary supplement, folic acid does not produce positive results. Therefore, topical application of folic acid to the gum is advised.

3.25 Coenzyme Q

Coenzyme Q10 (CoQ10) is a naturally occurring compound present in the mitochondria. Deficiencies of CoQ10 have been recognized in many health conditions, including periodontal diseases. It serves as an endogenous antioxidant, and initially its concentration is increased as a compensatory mechanism in the diseased gingiva to suppress periodontal inflammation. CoQ10 can ameliorate collagen degradation (Soni et al. 2012). Clinical investigators and dentists recommend CoQ10 supplementation, particularly in diabetic patients and others who are at risk for periodontal diseases (Hanioka et al. 1994; Pradeep et al. 2010; also reviewed in Soni et al. 2012). Topical administration of CoQ10 (Perio-Q®) to the gingiva as a sole treatment has been shown to decrease gingival crevicular fluid (GCF) flow and probing depths and improve clinical gingival attachment (Hanioka et al. 1994; Singh et al. 2016).

3.26 Hyaluronic Acid

Hyaluronic acid (HA) is a mucopolysaccharide present in all living organisms. Studies have shown that HA exerts antioxidative, anti-inflammatory, anti-edematous, and antibacterial activities and thereby may prevent or ameliorate periodontal diseases in humans and animals (Cristina et al. 2013). Bansal et al. (2010) emphasized that HA may (1) act as an antimicrobial agent adjunct to scaling and root planing, (2) accelerate bone regeneration in periodontal bony defects, (3) act as an autologous cell HA graft in mucogingival surgeries, and (4) act as a carrier for platelet-derived growth factor (PDGF) and bone morphogenetic protein (BMP-2) in regenerative therapies. HA has been shown to have a bacteriostatic effect against *Prevotella oris*, *Staphylococcus aureus*, and *Aggregatibacter actinomycetemcomitans*, which are commonly found in periodontal wounds (Pirnazar et al. 1999). Currently, HA is available in the form of a gel (Gingigel®), sponge, or membrane.

3.27 Sodium Hexametaphosphate (SHMP)

One of the most used supplements to control periodontal diseases in the pet food industry is phosphate salts, among them the sodium polyphosphate or hexametaphosphate (Oba et al. 2018). SHMP is an AAFCO-approved mineral sequestrant for tartar control. SHMP binds salivary calcium making it less available for precipitation as dental calculus (Stookey et al. 1996; White Gerlach 2000). The nutritional safety of SHMP rests with the fact that this compound is known to be converted to orthophosphate in the presence of strong acids, such as hydrochloric acid in the stomach, and orthophosphate can be utilized by the host. Stookey et al. (1995) reported that feeding a single daily snack of two HMP-coated plain biscuits (0.6% HMP) decreased calculus formation by nearly 80%. It is suggested that the coating of dry dog chow or plain dog biscuits with HMP is an effective means of reducing calculus formation in dogs.

3.28 Parodontax®

Parodontax® is an herbal toothpaste (Glaxo Smith Kline, Middlesex, UK) which consists of sodium bicarbonate, sodium fluoride, chamomile, echinacea, sage, myrrh, rhatany, and peppermint oil. Although Parodontax® provided significant improvement in oral health and can be used as an alternative to regularly used standard toothpastes, it presented no significant clinical advantage over conventional toothpastes with fluoride (Pannuti et al. 2003). In another study, Ozaki et al. (2006) compared the efficacy of Parodontax® with Colgate Total (Colgate-Palmolive Co, New York, NY, USA) containing 0.3% triclosan, 2.0% copolymer, and 0.243% sodium fluoride. Although both types of toothpaste reduced plaque and gingivitis, no additional benefit of the Parodontax® could be observed over Colgate Total.

3.29 Colgate Herbal Toothpaste

George et al. (2009) assessed the effects of Colgate herbal toothpaste (Colgate-Palmolive India, Mumbai, India) containing calcium carbonate, chamomile, sage, myrrh, eucalyptus, and sodium monofluorophosphate and compared the effects with a conventional Colgate toothpaste containing calcium carbonate, sodium monofluorophosphate, triclosan, and silicate. Based on parameters used in this study (plaque index, gingival index, etc.), findings revealed no significant difference in effectiveness in these two types of toothpaste.

3.30 Probiotics

Probiotics are nonpathogenic living bacteria (including *Lactobacillus acidophilus*, *Lactobacillus casei*, *Bifidobacterium infantis*, and *Streptococcus thermophilus*) which are given orally to activate the immune system. The application of

these bacteria as an adjunct to scaling and root planing appears to inhibit the periopathogenic recolonization of the periodontal pocket, thereby achieving and maintaining periodontal health (Lagdive and Lagdive 2014). The use of probiotics in prevention and treatment of periodontal diseases has been indicated for decades (Meurman 2005; Wim et al. 2008; Yanine et al. 2013; Lagdive and Lagdive 2014). Yanine et al. (2013) reported that for the primary outcome, probing pocket depth, there was no clinical beneficial effect of probiotics. For secondary outcomes, probiotics have shown small benefits on plaque index and gingival inflammation. Vanderpool (2009) used replacement therapy for regeneration of periodontal osseous defects in Beagles by local application of *Streptococcus sanguinis*, *Streptococcus salivarius*, and *Streptococcus mitis*. There is currently insufficient evidence demonstrating the benefits of systematic preventative use of probiotics in patients with periodontal diseases (Yanine et al. 2013). Therefore, more studies need to be done before their use can be justified in periodontal diseases. No negative effects of probiotics use on oral health have been reported to date.

4 Concluding Remarks and Future Directions

Phytochemicals such as alkaloids, tannins, essential oils, and flavonoids exert antimicrobial, antiseptic, antiviral, antifungal, antioxidative, anti-inflammatory, and immunomodulatory activities, thereby improving oral hygiene and preventing gum disease, tooth decay, and periodontitis. This chapter describes the pathophysiology of periodontal diseases in brief and common nutraceuticals that can be used to prevent or treat periodontal diseases in canines and felines. Future investigations need to be conducted on clinical studies in canines and felines for judicious use of nutraceuticals in periodontal diseases.

References

Abdellatif F, Boudjella H, Zitouni A et al (2014) Chemical composition and antimicrobial activity of the essential oil from leaves of Algerian *Melissa officinalis* L. EXCLI J 13:772–781

Albandar JM, Brown LJ, Loe H (1997) Clinical features of early-onset periodontitis. J Am Dent Assoc 128(10):1393–1399

Almas K (2001) The effects of extracts of chewing sticks (*Salvadora persica*) on healthy and periodontally involved human dentine: a SEM study. Indian J Dent Res 12(3):127–132

Almas AK, Almas K (2014) Miswak (*Salvadora persica* chewing stick): the natural toothbrush revisited. Odontostomatol Trop 37 (145):27–39

Amos S, Akah CJ, Odukwe KS et al (1999) The pharmacological effects of an aqueous extract from *Acacia nilotica* seeds. Phytother Res 13:683–685

Bachayaa HA, Iqbala Z, Khana MN et al (2009) Anthelmintic activity of *Ziziphus nummularia* (bark) and *Acacia nilotica* (fruit) against *Trichostrongylid* nematodes of sheep. J Ethnopharmacol 123:325–329

Baghele ON (2017) Antibacterial activity of *Hydrastis canadensis* extract on selected periodontal pathogens: an *in vitro* study. Bhavnagar Univ J Dent 7(1):12–15

Bansal J, Keige SD, Anand S et al (2010) Hyaluronic acid: a promising mediator for periodontal regeneration. Indian J Dent Res 21 (4):575–578

Baydar NG, Sagdic O, Ozkan G et al (2006) Determination of antibacterial effects and total phenolic contents of grape (*Vitis vinifera* L.) seed extracts. Int J Food Sci Technol 41:799–804

Bhambal A, Kothari S, Saxena S et al (2011) Comparative effect of neemstick and toothbrush on plaque removal and gingival health—a clinical trial. J Adv Oral Res 2(3):51–55

Bhat G, Kudva P, Dodwad V (2011) *Aloe vera*: nature's soothing healer to periodontal disease. J Indian Soc Periodontol 15:205–209

Blumenthal M, Busse WR, Goldberg A et al (1998) The complete German Commission E monographs: therapeutic guide to herbal medicines. American Botanical Council and, Integrative Medicine Communications, Austin, Boston, pp 180–182

Borugă O, Jianu C, Mişcă C et al (2014) *Thymus vulgaris* essential oil: chemical composition and antimicrobial activity. J Med Life 7 (3):56–60

Bozin B, Mimica-Dukic N, Samojlik I et al (2007) Antimicrobial and antioxidant properties of rosemary and sage (*Rosemarinus officinalis L.* and *Salvia officinalis L.*, *Lamiaceae*) essential oils. J Agric Food Chem 5:7879–7885

Bushra S, Farooq A, Roman P (2007) Antioxidant activity of phenolic components present in barks of *Azarichta indica*, *Terminalia arjuna*, and *Acacia nilotica*, and *Eugenia jambolana* Lam trees. Food Chem 104(3):148–161

Campos JF, dos Santos UP, Macorini LF et al (2014) Antimicrobial, antioxidant and cytotoxic activities of propolis from *Melipona orbignyi* (Hymenoptera, Apidae). Food Chem Toxicol 65:374–380

Cao XP, Chen YF, Zhang JL et al (2017) Mechanisms underlying the wound healing potential of propolis based on its *in vitro* antioxidant activity. Phytomedicine 34:76–84

Carson CF, Hammer KA, Riley TV (2006) *Melaleuca alternifolia* (tea tree) oil: a review of antimicrobial and other medicinal properties. Clin Microbiol Rev 19(1):50–62

Chaichoowong S, Bol JB, Bol P et al (2017) Chemical profiling of *Acalypha indica* obtained from supercritical carbon dioxide extraction and Soxhlet extraction methods. Orient J Chem 33(1):66–73

Chakraborthy GS (2008) Antimicrobial activity of leaf extract of *Calendula officinalis* Linn. J Herb Med Toxicol 2:65–66

Chandler M (2014) Impact of nutrition on dental issues in companion animals. Vet Times:1–8

Chatterjee A, Saluja M, Agarwal G et al (2012) Green tea: a boon for periodontal and general health. J Indian Soc Periodontol 16 (2):161–167

Chaturvedi TP (2009) Use of turmeric in dentistry: an update. Indian J Dental Res 20:107–109

Chouksey D, Sharma P, Pawar RS (2010) Biological activities and chemical constituents of *Illicium verum* hook fruits (Chinese star anise). Der Pharmac Sinica 1(3):1–10

Coimbra S, Castro E, Rocha-Pereira P et al (2006) The effect of green tea in oxidative stress. Clin Nutr 25:790–796

Cornara L, Biagi M, Xiao J et al (2017) Therapeutic properties of bioactive compounds from different honeybee products. Front Pharmacol 8:412

Costa EM, Silva S, Madureira AR et al (2014) A comprehensive study into the impact of a chitosan mouthwash upon microorganism's biofilm formation *in vitro*. Carbohydr Polym 101:1081–1086

Coutinho A (2012) Honeybee propolis extract in periodontal treatment: a clinical and microbiological study of propolis in periodontal treatment. Indian J Dent Res 23:294

Cristina GM, Stana P, Maniu G et al (2013) Biotechnological value of the hyaluronic acid in periodontal treatment. Rom Biotechnol Lett 18 (4):8551–8558

D'Aiuto F, Nibali L, Parkar M et al (2010) Oxidative stress, systemic inflammation, and severe periodontitis. J Dent Res 89:1241–1246

Dantas Silva RP, Machado BA, Barreto GA et al (2017) Antioxidant, antimicrobial, antiparasitic, and cytotoxic properties of various Brazilian propolis extracts. PLoS One 12(3):e172585

de Moraes Porto ICC, de Almeida DCC, de Oliveira Costa GVC et al (2018) Mechanical and aesthetic compatibility of Brazilian red propolis micellar nanocomposite as a cavity cleansing agent. BMC Complement Altern Med 18:219

De Oliveira SM, Torres TC, Pereira SL et al (2008) Effects of a dentifrice containing *Aloe vera* on plaque and gingivitis control: a double-blind clinical study in humans. J Appl Oral Sci 16 (4):293–296

Delamare APL, Moschen-Pistorello IT, Artico L et al (2007) Antibacterial activity of the essential oil of *Salvia officinalis* L. and *Salvia triloba* L. cultivated in South Brazil. Food Chem 100:603–608

Dolara P, Corte B, Ghelardini C et al (2000) Local anesthetic, antibacterial and antifungal properties of sesquiterpenes from myrrh. Planta Med 66(4):356–358

Eick S, Schäfer G, Kwiecinski J et al (2014) Honey-a potential agent against *Porphyromonas gingivalis*: an *in vitro* study. BMC Oral Health 14(1):14

El-Hefny M, Ali HM, Ashmawy NA et al (2017) Chemical composition and bioactivity of *Salvadora persica* extracts against some potato bacterial pathogens. Bioresources 12(1):1835–1849

Erdelmeier CAJ, Cinati J, Rabenau H et al (1996) Antiviral and antiparasitic activity of *Hamamelis virginiana* bark. Planta Med 62:241–245

Ford AC, Talley NJ, Spiegel BM et al (2008) Effect of fiber, antispasmodics, and peppermint oil in the treatment of irritable bowel syndrome: systematic review and meta-analysis. Br Med J 337:a2313

Furiga A, Lonvaud-Funel A, Badet C (2009) *In vitro* study of antioxidant capacity and antibacterial activity on oral anaerobes of a grape seed extract. Food Chem 113:1037–1040

Ganesan S (2008) Traditional oral care medicinal plants survey of Tamil Nadu. Nat Prod Radiance 7(2):166–172

Garlet GP, Martins W Jr, Fonseca BA et al (2004) Matrix metalloproteinases, their physiological inhibitors and osteoclast factors are differently regulated by the cytokine profile in human periodontal disease. J Clin Periodontol 31:671–679

Gawor JP, Reiter AM, Jodkowska K et al (2006) Influence of diet on oral health in cats and dogs. J Nutr 136(7 Suppl):2021S–2023S

Gebaraa EC, Pustiglioni AN, de Lima LA et al (2003) Propolis extract as an adjuvant to periodontal treatment. Oral Health Prev Dent 1 (1):29–35

George J, Hegde S, Rajesh KS et al (2009) The efficacy of a herbal-based toothpaste in the control of plaque and gingivitis: a clinico-biochemical study. Indian J Dent Res 20:480–482

Gertsch J, Leonti M, Raduner S et al (2008) Beta-caryophyllene is a dietary cannabinoid. Proc Natl Acad Sci USA 105(26):9099–9104

Ghorbani A, Esmaeilizadeh M (2014) Pharmacological properties of salvia officinalis and its components. J Tradit Complement Med 7 (4):433–440

Gilani AH, Shaheen F, Zaman M et al (1999) Studies on antihypertensive and antispasmodic activities of methanol extract of *Acacia nilotica* pods. Phytother Res 13:665–669

Giuraud P, Steiman R, Campos-Takaki GM et al (1994) Comparison of antibacterial and antifungal activities of lapachol and β-lapachone. Planta Med 60:373–374

Govindaraj J, Emmedi P, Lakshmi D et al (2010) Protective effect proanthocyanidins on endotoxin induced experimental periodontitis in rats. Indian J Exp Biol 48:133–142

Gupta MP (2006) The future of products of the Andean high plateau and central valleys: report on medicinal plants originating in the Andean high plateau and central valleys region of Bolivia, Acuador and Peru. United Nations Industrial Development Organization Investment and Technology Promotion Branch

Hanioka T, Tanaka M, Ojima M et al (1994) Effect of topical application of coenzyme Q10 on adult periodontitis. Mol Asp Med 15:241–248

Hirasawa M, Takada K, Makimura M et al (2002) Improvement of periodontal status by green tea catechin using a local delivery system: a clinical pilot study. J Periodontal Res 37:433–438

Houde V, Grenier D, Chandad F (2006) Protective effects of grape seed proanthocyanidins against oxidative stress induced by lipopolysaccharides of periodontopathogens. J Periodontol 77:1371–1379

Huhtanen C (1980) Inhibition of *Clostridium botulinum* by spice extract and aliphatic alcohols. J Food Prot 43:195–196

Husain S, Al-Samadani KH, Najeeb S et al (2017) Chitosan biomaterials for current and potential dental applications. Materials 10:602. https://doi.org/10.3390/ma10060602

Hussain AI, Anwar F, Chatha SAS et al (2010) *Rosmarinus officinalis* essential oil: antiproliferative, antioxidant and antibacterial activities. Braz J Microbiol 41:1070–1078

Iauk L, Lo Bue AM, Milazzo I et al (2003) Antibacterial activity of medicinal plant extracts against periodontopathic bacteria. Phytother Res 17:599–604

Ikinci G, Şenel S, Akincibay H et al (2002) Effect of chitosan on a periodontal pathogen *Porphyromonas gingivalis*. Int J Pharm 235 (1–2):121–127

Kajiura Y, Lew J-H, Ikuta T et al (2016) Clinical significance of GCF sIL-6R and calprotectin to evaluate the periodontal inflammation. Ann Clin Biochem 54(6):664–670

Karpinski TM, Szkaradkiewicz AK (2013) Microbiology of dental caries. J Biol Earth Sci 3:M21–M24

Kayode J, Omotoynibo MA (2009) Ethnobotanical utilization and conservation of chewing sticks plants in Ekiti state, Nigeria. Res J Bot 4 (1):1–9

Khairnar MS, Pawar B, Marawar PP et al (2013) Evaluation of *Calendula officinalis* as an anti-plaque and atigingivitis agent. J Indian Soc Periodontol 17(6):741–747

Koo H, Jeon JG (2009) Naturally occurring molecules as alternative therapeutic agents against cariogenic biofilms. Adv Dent Res 21:63–68

Kubiliene L, Jekabsone A, Zilius M et al (2017) Comparison of aqueous, polyethylene glycol-aqueous and ethanolic propolis extracts: antioxidant and mitochondria modulating properties. BMC Complement Altern Med 18:165

Kumar P, Ansari SH, Ali J (2009) Herbal remedies for the treatment of periodontal disease—a patent review. Recent Pat Drug Deliv Formul 3:221–228

Kumar A, Lather A, Kumar V et al (2015) Pharmacological potential of plant used in dental care: a review. J Herb Drug 5(4):179–186

Kumaravelu P, Subramaniyam S, Dakshinamoorthy DP et al (1996) The antioxidant effect of eugenol on CCl4-induced erythrocyte damage in rats. J Nutr Biochem 7:23–28

Kushiyama M, Shimazaki Y, Murakami M et al (2009) Relationship between intake of green tea and periodontal disease. J Periodontol 80:372–377

La VD, Bergeron C, Gafner S et al (2009) Grape seed extract suppresses lipopolysaccharide-induced matrix metalloproteinase (MMP)

secretion by macrophages and inhibits human MMP-1 and MMP-9 activities. J Periodontol 80:1875–1882
Lagdive SS, Lagdive SB (2014) Role of probiotics in periodontal health and diseases. Asian J Biosci (1):01, 20–23
Lagrota MHC, Wigg MD, Pereira LOB et al (1983) Antiviral activity of lapachol. Rev Microbiol 14:21–26
Lauten JD, Boyd L, Hanson MB et al (2005) A clinical study: *Melaleuca*, *Manuka*, *Calendula* and green tea mouth rinse. Phytother Res 19:951–957
Lee JW, Asai M, Jeon SK et al (2015) Rosmarinic acid exerts an antiosteoporotic effect in the RANKL-induced mouse model of bone loss by promotion of osteoblastic differentiation and inhibition of osteoclastic differentiation. Mol Nutr Food Res 59:386–400
Li WG, Zhang XY, Wu YJ et al (2001) Anti-inflammatory effect and mechanism of proanthocyanidins from grape seeds. Acta Pharmacol Sin 22:1117–1120
Li Y, Yao J, Han C et al (2016) Quercetin, inflammation and immunity. Nutrients 8:167
Liang W, Luo Z, Ge S et al (2011) Oral administration of quercetin inhibits bone loss in rat model of diabetic osteopenia. Eur J Pharmacol 670:317–324
Lima MDR, Lopes AP, Martins C et al (2017) The effect of *Calendula officinalis* on oxidative stress and bone loss in experimental periodontitis. Front Physiol 8:440
Lin N, Chen S, Zhang H et al (2018) Quantification of major royal jelly protein 1 in fresh royal jelly by ultraperformance liquid chromatography-Tandem mass spectrometry. J Agric Food Chem 66:1270–1278
Logan EI (2006) Dietary influences on periodontal health in dogs and cats. Vet Clin North Am Small Anim Pract 36(6):1385–1401
Machado MA (2010) Management of two cases of desquamative gingivitis with clobetasol and *Calendula officinalis* gel. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub 154:335–338
Machado CS, Mokochinski JB, Lira TOD et al (2016) Comparative study of chemical composition and biological activity of yellow, green, brown, and red Brazilian propolis. Evid Based Complement Alternat Med 2016:6057650. https://doi.org/10.1155/2016/6157650
Magwa ML, Gundidza M, Coopoosamy R et al (2010) Chemical composition of volatile constituents from the leave of *Aloe ferox*. Afr J Biotechnol 5(18):1652–1654
Mahmood A, Ahmad M, Jabeen A et al (2005) Pharmacognostic studies of some indigenous medicinal plants of Pakistan. Ethnobot Leaflets 9:1
Makarem A, Khalili N, Asodeh R (2007) Efficacy of barberry aqueous extracts dental gel on control of plaque and gingivitis. Acta Med Iran 45:91–94
Malviya S, Rawat S, Kharia A et al (2011) Medicinal attributes of *Acacia nilotica* Linn. A comprehensive review on ethnopharmacological claims. Int J Pharm Life Sci 2(6):830–837
McKay DL, Blumberg JB (2006) A review of the bioactivity and potential benefits of peppermint tea (*Mentha piperita* L.). Phytother Res 20(8):619–633
Meeker HG, Linke HAB (1998) The antibacterial action of eugenol, thyme oil, and related essential oils used in dentistry. Compendium 9:32–40
Meurman JH (2005) Probiotics: do they have a role in oral medicine and dentistry. Eur J Oral Sci 113:188–196
Modarai M, Silva E, Suter A et al (2011) Safety of herbal medicinal products: *Echinacea* and selected alkylamides do not induce CYP3A4 mRNA expression. Evid Based Complement Alternat Med 2011:213021
Morrison RK, Brown D et al (1970) Oral toxicology studies with lapachol. Toxicol Appl Pharmacol 17(1):1–11
Muhammad S, Lawal MT (2010) Oral hygiene and the use of plants. Sci Res Essays 5(14):1788–1795
Muhammad S, Khan BA, Akhtar N et al (2012) The morphology, extractions, chemical constituents and uses of *Terminalia chebula*: a review. J Med Plant Res 6(33):4772–4775
Nagababu E, Lakshmaiah M (1992) Inhibitory effect of eugenol on non-enzymatic lipid peroxidation in rat liver mitochondria. Biochem Pharmacol 43:2393–2400
Nagata H, Inagaki Y, Tanaka M (2008) Effect of *Eucalyptus* extracts chewing gum on periodontal health: a double-masked, randomized trial. J Periodontol 79:1378–1385
Nakamura R, Littarru GP, Folkers K et al (1974) Study of CoQ10 in gingiva from a patient with periodontal disease and evidence for deficiency of coenzyme Q10. Proc Natl Acad Sci USA 71:1456–1460
Napimoga MH, Clemente-Napimoga JT, Macedo CG et al (2013) Quercetin inhibits inflammatory bone resorption in a mouse periodontitis model. J Nat Prod 76:2316–2321
Narayanan N, Thangavelu L (2015) Salvia officinalis in dentistry. Dent Hypotheses 6:27–30
Nayak SS, Ankola AV, Metgud SC et al (2014) An *in vitro* study to determine the effect of *Terminalia chebula* extract and its formulation on *Streptococcus mutans*. J Contemp Dent Pract 15:278–282
Newman MG, Takai HH, Carranza FA (2006) Carranza's clinical periodontology, 10th edn. WB Saunders Co, Philadelphia, PA
Nuttall SL, Kendall MJ, Bombardelli E et al (1998) An evaluation of the antioxidant activity of a standardized grape seed extract, Leucoselect. J Clin Pharm Ther 23:385–389
Oba PM, Rentas MF, Vendramini TH et al (2018) Nutrition as a tool to control periodontal diseases in dogs and cats. Nutr Food Sci Int J 4 (4):1–3
Ozaki F, Pannuti CM, Imbronito AV et al (2006) Efficacy of a herbal toothpaste on patients with established gingivitis-a randomized controlled trial. Braz Oral Res 20:172–177
Özden FO, Sakkalioğlu EE, Sakallioğlu U et al (2017) Effects of grape seed extract on periodontal disease: an experimental study in rats. J Appl Oral Sci 25(2):121–129
Pack ARC (1984) Folate mouth wash: effects on established gingivitis in periodontal patients. J Clin Periodontol 11:619–628
Pai MR, Acharya LD, Udupa N (2004) Evaluation of antiplaque activity of *Azadirachta indica* leaf extract gel- a 6-week clinical study. J Ethnopharmacol 90(1):99–103
Palombo EA (2011) Traditional medicinal plant extracts and natural products with activity against oral bacteria: potential application in the prevention and treatment of oral diseases. Evid Based Complement Alternat Med 2011:680354
Pannuti CM, de Mattos JP, Ranoya PN et al (2003) Clinical effect of herbal dentifrice on the control of plaque and gingivitis: a double-blind study. Pesqui Odontol Bras 17(4):314–318
Parente LM, Lino Junior R, Trevenzol LM et al (2012) Wound healing and anti-inflammatory effect in animal models of *Calendula officinalis* L. growing in Brazil. Evid Based Complement Alternat Med 2012:357671
Pasupuleti VR, Sammugam L, Ramesh N et al (2017) Honey, propolis, and Royal Jelly: a comprehensive review of their biological actions and health benefits. Oxidat Med Cell Longev 2017:1259510
Patil PR, Rakesh SU, Dhabale PN et al (2009) Pharmacological activities of *Areca catechu* Linn.—a review. J Pharm Res 2 (4):683–687
Petrovic MS, Kesic LG, Kitic DV et al (2015) Periodontal disease and phytotherapy. J Oral Hyg Health 3:172
Pieri FA, de Castro Souza MC et al (2016) Use of β-caryophyllene to combat bacterial dental plaque formation in dogs. BMC Vet Res 12:216
Pierozan MK, Pauletti GF, Rota L et al (2009) Chemical characterization and antimicrobial activity of essential oils of *Salvia* L. species. Cienc Technol Aliment 29:764–770

Pinto P, Santos CN (2017) Worldwide (poly)phenol intake: assessment methods and identified gaps. Eur J Nutr 56:1393–1408
Pirnazar P, Wolinski L, Nachnani S et al (1999) Bacteriostatic effects of hyaluronic acid. J Periodontol 70:370–374
Porte A, Godoy RLO, Maia-Porte LH (2013) Chemical composition of sage (*Salvia officinalis* L.) essential oil from the Rio de Janeiro State (Brazil). Rev Bras Plantas Med 15(3):438–441
Pradeep AR, Happy D, Garg G (2010) Short-term clinical effects of commercially available gel containing *Acacia arabica*: a randomized controlled clinical trial. Aust Dent J 55:65–69
Preethi KC, Utta GK (2006) Antioxidant potential of an extract of *Calendula officinalis in vivo* and *in vitro*. Pharm Biol 44:691–697
Preethi KC, Kuttan G, Kuttan R (2009) Anti-inflammatory activity of flower extract of *Calendula officinalis* Linn. and its possible mechanism of action. Indian J Exp Biol 47:113–120
Preshaw PM (2008) Host response modulation in periodontics. Periodontol 2000 48:92–110
Puri K, Puri N (2013) Local drug delivery agents as adjuncts to endodontic and periodontal therapy. J Med Life 6(4):414–419
Ragasa C, Lorena GS, Mandia E et al (2013) Chemical constituents of *Abrus precatorius*. Am J Essent Oils Nat Prod 1(2):1–10
Rahaman O (2010) A review of uses *Acacia nilotica* (Booni) in alternative medicine. SearchWarp.com
Ramli S, Harada K-I, Ruangrungsi N (2011) Antioxidant, antimicrobial and cytotoxicity activities of *Acacia farnesiana* (L.) Wild. Leaves ethanolic extract. Pharmacogn J 3(23):50–58
Ramos GP, Apel MA, Morais CBD et al (2012) *In vivo* and *in vitro* anti-inflammatory activity of red clover *Trifolium pratense* dry extract. Rev Bras Farmacogn 22:176–180
Rodrigues TG, Fernandes A Jr, Sousa JP et al (2009) *In vitro* and *in vivo* effects of clove on pro-inflammatory cytokines production by macrophages. Nat Prod Res 23(4):319–326
Rosseto HC, Toledo LAS, Francisco LMB et al (2017) Nanostructured lipid systems modified with waste material of propolis for wound healing: design, *in vitro* and *in vivo* evaluation. Colloids Surf B Biointerfaces 158:441–452
Rufatto LC, dos Santos DA, Marinho F et al (2017) Red propolis: chemical composition and pharmacological activity. Asian Pac J Trop Med 7:591–598
Saini P, Al-Shibani N, Sun J et al (2012) Effects of *Calendula officinalis* on human gingival fibroblasts. Homeopathy 101:92–98
Sajjad A, Subhani Sajjad S (2014) *Aloe vera*: an ancient herb for modern dentistry—a literature review. J Dent Surg 2014:210463
Sakanaka S, Aizawa M, Kim M et al (1996) Inhibitory effects of green tea polyphenols on growth and cellular adherence of an oral bacterium, *Porphyromonas gingivalis*. Biosci Biotechnol Biochem 60:745–749
Santoyo S, Cavero S, Jaime L et al (2005) Chemical composition and antimicrobial activity of *Rosmarinus officinalis* L. essential oil obtained via supercritical fluid extraction. J Food Prot 68:790–795
Scherer W, Gultz J, Lee SS et al (1998) The ability of an herbal mouth rinse to reduce gingival bleeding. J Clin Dent 9:97–100
Sebei K, Sakouhi F, Herchi W et al (2015) Chemical composition and antibacterial activities of seven *Eucalyptus* species essential oils leaves. Biol Res 48(1):7
Sharma A, Sankhla B, Parkar S et al (2014) Effect of traditionally used neem and babool chewing stick (Datum) on Streptococcus mutans: an in vitro study. J Clin Diagn Res 8(7):ZC15–ZC17
Shekhawat D, Batra A (2006) Household remedies of Keshavraipatan tehsil in Bundi district Rajasthan. Indian J Tradit Knowl 5 (3):362–367
Shetty SB, Mahin-Syed-Ismail P, Varghese S et al (2016) Antimicrobial effects of *Citrus sinensis* peel extracts against dental caries bacteria: an *in vitro* study. J Clin Exp Dent 8(1):e70–e77
Shiga H, Jo A, Terao K et al (2010) Decrease of halitosis by intake of Manuka honey, vol 14. IADR General Session, Barcelona
Siddamallayya N, Yasmeen A, Gopakumar K (2010) Hundred common medicinal plants of Karnataka in primary health care. Indian J Tradit Knowl 9(1):90–95
Singh O, Khanam Z, Misra N et al (2011) Chamomile (*Matricaria chamomilla* L.): an overview. Pharmacogn Rev 5(9):82–95
Singh J, Singh R, Gambhir RS et al (2016) Local drug delivery system in treatment of periodontitis: a review. J Periodontal Med Clin Pract 3 (3):153–160
Smullen J, Finney M, Storey DM et al (2012) Prevention of artificial dental plaque formation *in vitro* by plant extracts. J Appl Microbiol 113:964–973
Soni S, Aggarwal PK, Sharma N et al (2012) Coenzyme Q10 and periodontal health: a review. Int J Oral Maxillofac Pathol 3(2):21–26
Sookto T, Srithavaj T, Thaweboon B et al (2013) *In vitro* effects of *Salvia officinalis* L. essential oil on *Candida albicans*. Asian Pac J Trop Biomed 3:376–380
Srivastava A, DuBourdieu D, Deshpande M, Lall R (2016) Effect of novel natural substitute of chlorhexidine on halitosis in canine: a preliminary report. Proceedings of the 19th American Dental Congress. Phoenix
Stookey GK, Warrick JM, Miller LL (1995) Effect of sodium hexametaphosphate on dental calculus in dogs. Am J Vet Res 56 (7):913–918
Stookey GK, Warrick JM, Miller LL et al (1996) Hexametaphosphate-coated snack biscuits significantly reduce calculus formation in dogs. J Vet Dent 13(1):27–30
Subhash AV, Suneela S, Anuradha C et al (2014) The role of *Aloe vera* in various fields of medicine and dentistry. J Orafac Sci 6:5–9
Sunitha J, Ananthalakshmi R, Sathiya Jeeva J et al (2015) Antimicrobial effect of herbal dentifrice: an *in vitro* study. J Pharm Bioallied Sci 7 (2):S628–S631
Taheri JB, Azimi S, Rafieian N et al (2011) Herbs in dentistry. Int Dent J 61:287–296
Tanideh N, Tavakoli P, Saghiri MA et al (2013) Healing acceleration in hamsters of oral mucositis induced by 5-uracil with topical Calendula officinalis. Oral Surg Oral Med Oral Pathol Oral Radiol 115:332–338
Tipton DA, Hamman NR, Dabbous MKH (2006) Effect of myrrh oil on IL-1beta stimulation of NF-kappaB activation and PGE_2 production in human gingival fibroblasts and epithelial cells. Toxicol in Vitro 20 (2):248–255
Tu HP, Fu MM, Kuo PJ et al (2013) Berberine's effect on periodontal tissue degradation by matrix metalloproteinases: an *in vitro* and *in vivo* experiment. Phytomedicine 20:1203–1210
Turesky S, Gilmore ND, Glickman I (1970) Reduced plaque formation by chloromethyl analogue of vitamin C. J Periodontol 41:41–43
Umaru B, Mahre S, Dogo HM et al (2016) Effects of aqueous pod extract of *Acacia nilotica* on white blood cells, platelets and clotting time in albino rats. Am J Pharmacol Pharmacother 3(3):1–6
Uraz A, Boynueğri D, Özcan G et al (2012) Two percent chitosan mouthwash: a microbiological and clinical comparative study. J Dent Sci 7(4):342–349
Vanderpool C (2009) Mechanisms of probiotics action: implication for therapeutic application in inflammatory bowel disease. Inflamm Bowel Dis 15:300–310
Vogel RI, Fink RA, Frank O et al (1978) The effect of topical application of folic acid on gingival health. J Oral Med 33(1):20–22
White DJ, Gerlach RW (2000) Anticalculus effects of a novel, dual-phase polypyrophosphate dentifrice: chemical basis, mechanism, and clinical response. J Contemp Dent Pract 1(4):1–12
Wim W, Teughel S, Van Esche M (2008) Probiotics in oral health care. Periodontology 48:111–147

Wolinsky LE, Mania S, Nachnani S et al (1996) The inhibiting effect of aqueous *Azadirachta indica* (Neem) extract upon bacterial properties influencing *in vitro* plaque formation. J Dent Res 75 (2):816–822

Wu N, Fu K, Fu Y-J et al (2009) Antioxidant activities of extracts and main components of Pigeon pea [*Cajanus cajan* (L.) Millsp.] leaves. Molecules 14:1032–1043

Wynn RL (2005) *Aloe vera* gel: update for dentistry. Gen Dent 53:6–9

Yanine N, Araya I, Brignardello-Petersen R et al (2013) Effects of probiotics in periodontal diseases: a systematic review. Clin Oral Investig 17:1627–1634

Yu H, Oho T, Tagomori S et al (1992) Anticariogenic effects of green tea. Fukuoka Igaku Zasshi 83:174–178

Yun JH, Pang EK, Kim CS et al (2004) Inhibitory effects of green tea polyphenol (-)-epigallocatechin gallate on the expression of matrix metalloproteinase-9 and on the formation of osteoclasts. J Periodontal Res 39:300–307

Yusoffs S, Kamin S (2006) The effect of a mouthwash containing extracts of *Calendula officinalis* on plaque and gingivitis. J Clin Periodontol 33:118

Zheng W, Wang S, Wang J et al (2015) Periodontitis promotes the proliferation and suppresses the differentiation potential of human periodontal ligament stem cells. Int J Mol Med 36:915–922

Zhu N, Sheng S, Sang S et al (2003) Isolation and characterization of several aromatic sesquiterpenes from *Commiphora myrrha*. Flavour Fragr J 18:282–285

Zilius M, Ramanauskiene K, Juskaite V et al (2016) Formulation of propolis phenolic acids containing microemulsions and their biopharmaceutical characterization. Evid Based Complement Alternat Med 2016:1–7. https://doi.org/10.1155/2016/8175265

Nutraceuticals in Gastrointestinal Conditions

Jamil Talukder

Abstract

The health of the digestive system is important in order for it to perform its physiological functions appropriately. The physiological parameters of the digestive system depend on the types of food ingested and the presence of bioactive compounds therein. Ingested foodborne bioactive compounds, or phytochemicals, can play an important role in the mediation of the pathophysiology of the digestive system. Pathological conditions are mostly treated with different drugs, and almost all the drugs have undesirable effects. Therefore, adverse effects and toxicity of drugs led scientists to consider alternatives, functional foods, safer bioactive compounds, or nutraceutical-based approaches for the management of gastrointestinal (GI) conditions. In the last few decades, many nutraceuticals with antioxidative, anti-inflammatory, fatty acid, probiotic, and prebiotic properties have been discovered that ameliorate the signs of GI disease. More preclinical and clinical studies will lead us into a new era of nutraceuticals for the management of gut health and conditions in animals.

Keywords

Nutraceuticals · Veterinary nutraceuticals · Gastrointestinal diseases

1 Introduction

The digestive system is primarily responsible for the digestion of food, absorption of nutrients and water, and expelling wastes from the body. The feeding of commercial food has increased the number of conditions caused due to improper nutrition. Adequacy and safety of the food supply are of great interest to consumers (Buchanan et al. 2011). Generally, pet owners do not refuse to provide foods that can support the health and wellness of their animals, but at the same time, they doubt their safety. For instance, the incorporation of corn and wheat which have documented antioxidant and anticancer activities (Wood et al. 1994) into pet foods has been perceived as negative by a subgroup of pet owners. These owners believe that they are of lower quality or of poor nutritional value for dogs and cats, despite them matching the Association of American Feed Control Officials (AAFCO) standards (Carter et al. 2014). Pet owners have shown an increased interest in holistic natural diets.

Obesity is recognized as a one of the most important health issues. Heart disease continues to be a primary cause of death in most developing countries worldwide, followed by cancer, osteoporosis, arthritis, and many others. Thus, appropriate food is an integral part for normal body functions as well as for the digestive system that helps in the absorption of nutrients, prevention of nutritional deficiencies and malnutrition, repair of damaged intestinal epithelium, restoration of normal luminal bacterial populations, promotion of normal gastrointestinal (GI) motility, and maintenance of normal immune functions (Zoran 2003). In addition, the GI tract contains some multifaceted microorganisms, including both pathogenic and nonpathogenic bacteria. It has been reported that more than 400 bacterial species inhabit the GI tract (Eckburg et al. 2005). Normal bacterial species and their populations are altered by ingesting different kinds of foods including drugs such as antibiotics. Despite undesirable effects, veterinarians have to prescribe antibiotics to treat animals for bacterial diseases. Further, pet owners who are frustrated with the expensive, high-tech disease-treatment approach in modern medicines are seeking complementary or alternative beneficial products. Functional foods provide health benefits if they are consumed on a regular basis as part of a varied diet. They provide health benefits beyond the provision of essential nutrients, such as vitamins, minerals, water, proteins, carbohydrates, and fats (Hasler 2000).

J. Talukder (✉)
Vets Plus, Inc., Menomonie, WI, USA
e-mail: Jamilt@vets-plus.com

R. C. Gupta et al. (eds.), *Nutraceuticals in Veterinary Medicine*, https://doi.org/10.1007/978-3-030-04624-8_31

Medicinal plants and naturally available ingredients have been used for centuries for the prevention, control, and treatment of several conditions. Recently, there is an increasing interest in examining potential of novel compounds which may prevent or treat different ailments including increased growth, immunomodulator, or antimicrobial actions. Plants contain several bioactive compounds, including phytosterols, phytoestrogens, polyphenols, and polyunsaturated fatty acids (PUFAs), which are used as nutraceuticals or functional foods and are appealing options for different ailments (Foster et al. 2005). Therefore, scientists are now focusing more on the examination of nutraceuticals for their protective and disease preventing potential with little or no side effects (Nicoli et al. 1999; Kaur and Kapoor 2001; Adelaja and Schilling 1999). This chapter describes the benefits and rationale for the use of nutraceuticals in the GI disorders.

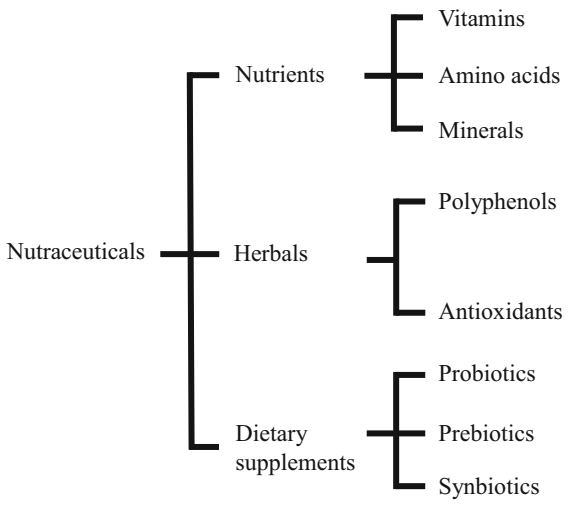


Fig. 1 Categories of nutraceuticals and representative compounds. Adopted from Larussa et al. (2017)

2 Phytochemicals for Gastrointestinal Conditions

Plants that contain many phytochemicals are bioactive in nature and provide many health benefits. These are known as nutraceuticals. They induce anti-inflammatory responses, scavenge free radicals, maintain a homeostatic regulation of the gut microbiota, and activate the intestinal T regulatory cells (Saxena et al. 2014). Phytochemicals contain phytoestrogens, phenolic compounds, and secondary metabolites and exhibit their effects on intestinal inflammation (Mosele et al. 2015; Jarosová et al. 2015). These compounds are classified as flavonoids, isoflavonoids, lignans, ellagitannins, coumestans, and stilbenes (Bilal et al. 2014; Gaya et al. 2016). A single plant can contain many types of phytoestrogens, and different parts of the plant can contain different amounts of phytoestrogens (Jarosová et al. 2015). The main sources of these phytoestrogens are fruits, vegetables, and whole grains (Sirotkin and Harrath 2014). Flavonoids are transformed by bacterial species in the intestine, but they can also represent a substrate for the human gut microbiota. This may influence intestinal absorption (Fig. 1).

Flavonoids influence and regulate the intestinal barrier and its permeability. It has been demonstrated that flavonoids have a direct trophic influence on *Akkermansia* but not on mucin production (Cassidy and Minihane 2017). Flavonoids provide an antimicrobial effect, offering protection against pathogenic bacteria, fungi, and viruses. In this era of antimicrobial resistance, flavonoids may be considered suitable alternatives to antibiotics, especially in mild/moderate infections or in their prevention (Iranshahi et al. 2015).

Aloe vera is one of the common herbal remedies that has been used in GI conditions such as diarrhea, including inflammatory bowel disease (IBD), for centuries in several cultures around the world. It contains several compounds (anthraquinones, carbohydrates, vitamins, minerals, enzymes, and amino acids) with many beneficial effects. Moreover, it is considered an important and natural source of prebiotics. It was observed that the leaf gel, due to oxidation, leads to fermentation and regulates bacterial growth. Several research groups have reported the presence of lactic acid bacteria after the fermentation of *Aloe vera* pulp, laying the groundwork for studying its probiotic effects. Furthermore, Langmead et al. (2004) also used *Aloe vera* in the treatment of ulcerative colitis (UC).

Turmeric (*Curcuma longa*) is commonly used as a spice, food preservative, and a coloring agent in foods. It contains a class of phenols, known as curcuminoids. In a clinical study, oral administration of curcumin proved to be a therapeutic agent in the maintenance of UC (Hanai et al. 2006; Lang et al. 2015). Curcumin can also be used as enema (Singla et al. 2014) and was demonstrated to be very effective in chronic enteritis such as IBD, without raising concerns regarding its safety (Suskind et al. 2013). IBS is the most common functional GI disorder, and treatment focuses primarily on the relief of symptoms (Lacy and Lee 2005). Those symptoms classically include abdominal discomfort or pain, bloating, flatulence, and fecal urgency. Phytochemicals such as peppermint oil, artichoke leaf extract, and turmeric have been used in small uncontrolled trials and have shown clinical benefit (Bundy et al. 2004). Curcuminoids act as an anti-inflammatory agents. It suppresses the nuclear factor kappa-light-chain-enhancer of the activated B cells (NF-κB)-related inflammatory pathway. It subsequently inhibits TNF-α, IL-12, and IL-2 (Fig. 2, adopted from Larussa et al. 2017). Thus, curcumin modulates the immune response as a safe and promising agent for the treatment of chronic diarrhea (Vecchi Brumatti et al. 2014) without producing adverse effects (Holt et al. 2005; Hsu and Cheng 2007; Langhorst et al. 2013).

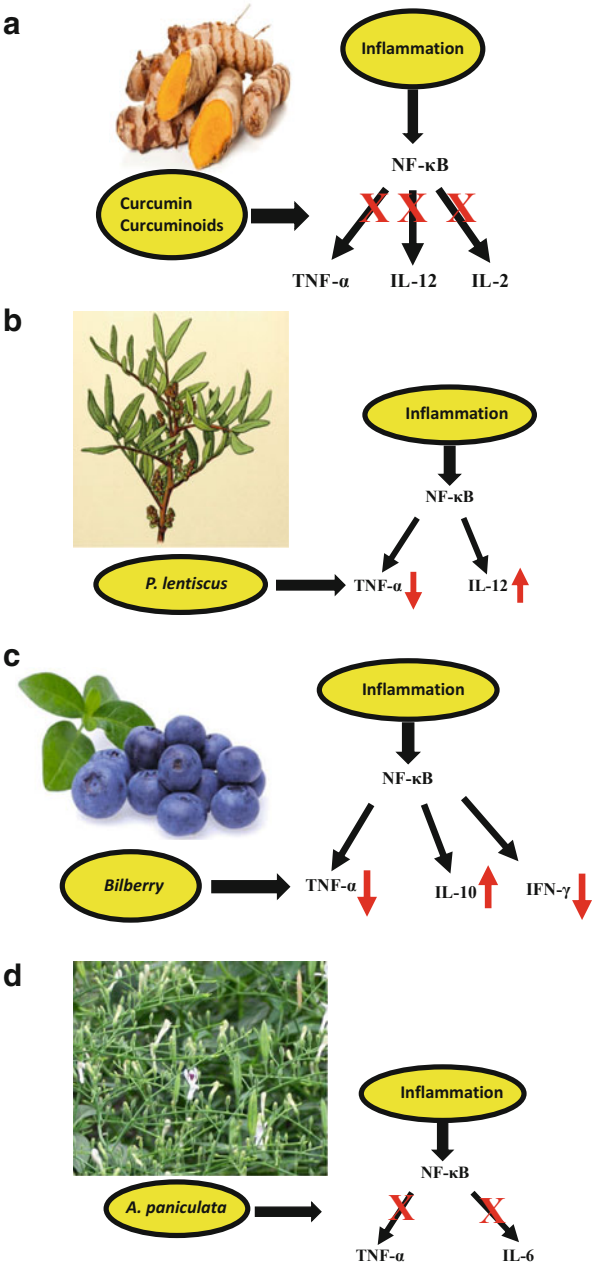


Fig. 2 (**a**–**d**) Phytochemicals and their interactions with inflammatory pathways. *IL* interleukin, *NF-kB* nuclear factor kappa-light-chain-enhancer of activated B cells, *TNF-α* tumor necrosis factor-alpha

Capsaicin is present in hot chilies, and it requires calcium to inhibit SNAT2-mediated methionine transport and causes apoptosis and DNA damage in enterocytes (Talukder et al. 2018).

Ginger (rhizome of *Zingiber officinale*) has been widely used for centuries in GI disorders such as dyspepsia. It has a protective role in gastric ailments and irritations such as ulcers, vomiting, nausea, stomach ache, spasm, and GI cancer. It increases GI motility (Micklefield et al. 1989), and it also reduces blood pressure (Ghayur and Gilani 2005). Ginger contains gingerols, zingerone, shogaols, paradols, and related compounds (Connell and McLachlan 1972). The prokinetic activity of ginger extract revealed that it enhances the intestinal travel of charcoal meal in mice. This propulsive effect of the extract, similar to that of carbachol, was blocked in atropine-pretreated mice, a standard cholinergic antagonist. In addition, prokinetic activity showed an atropine-sensitive dose-dependent spasmogenic effect in vitro as well as in isolated rat and mouse stomach fundus tissues. In atropinized tissue, it showed spasmolytic activity via the inhibition of 5-HT- and K^+-induced contractions. A spasmolytic effect was also observed in other gut preparations either as noncompetitive inhibition of agonist dose-response curves, inhibition of high K^+ (80 mM)-induced contractions, or displacement of Ca^{2+} dose-response curves to the right, indicating a calcium antagonist effect. Phytochemical analysis revealed the presence of saponins, flavonoids, and alkaloids in the crude extract. In addition, the presence of a spasmolytic constituent(s) of the calcium antagonist type may explain its use in hyperactive states of the gut such as colic and diarrhea (Muhammad and Anwarul et al. 2005). Therefore, ginger has a unique combination of spasmogenic and spasmolytic activities mediated through cholinergic and calcium antagonist mechanisms, respectively.

Fruits and vegetables contain many flavonoids, which belong to the polyphenols class, and show promising health benefits. Therapeutic usages of flavonoids have been investigated along with their mode of actions in different GI disease conditions (Hoensch and Oertel 2015). Flavonoids from myrrh and chamomile demonstrate nutritional, anti-inflammatory, and immunomodulatory properties in chronic enteritis (Langhorst et al. 2013) with diarrhea. Furthermore, *Boswellia serrata* is a well-known herb for its benefits in enteritis. Recent studies demonstrate significant improvement in intestinal epithelial barrier preservation and oxidative and inflammatory damage attenuation in ulcerative colitis (Holtmeier et al. 2011; Gupta et al. 1997).

Anthocyanin, also a flavonoid, is responsible for the blue, purple, and red colors of flowers and fruits. In addition, anthocyanins act as antioxidants that directly restrict both gene expression and receptor-mediated inflammatory signaling pathways (Biedermann et al. 2013). The supplementation of mouse diet with blueberries or black currants provided a significant change in gut microbiota by promoting the anaerobic bacteria *Bacteroidetes* and *Actinobacteria*, probably through their antioxidative effect (Overall et al. 2017). Furthermore, bilberry contains anthocyanin, and very recently it has been revealed that it reduces pro-inflammatory cytokines IFN-γ and TNF-α along with enhanced levels of the immunoregulatory cytokine IL-10 in UC (Roth et al. 2016).

A subclass of flavonoids is called "isoflavonoids," of which substantial amount is found in soy, soy protein, and miso. The isoflavones are conjugated to glucose and not

active in their primary form. Isoflavones are converted into different compounds by the gut microbiota as well as enterocytes, and they are well absorbed by the microbes and by the gut and go through enterohepatic circulation (Vitale et al. 2013; Catinean et al. 2018). Additionally, these metabolites have an estrogen-like activity (Laparra and Sanz 2010). Isoflavonoids contain many glycosides, such as genistein, daidzein, and glycitein. Daidzein is metabolized by gut bacteria to equol (Franke et al. 2014; Catinean et al. 2018), which plays an important role in the health benefits of soy. It also has strong estrogenic activity and antioxidant capacity. The main bacteria that contribute to the conversion of the isoflavonoids into equol inhabit the distal portion of the gut and belong to the family *Coriobacteriaceae* (Guadamuro et al. 2017; Catinean et al. 2018). The isoflavones also inhibit tyrosine kinases, have antioxidant activity, bind to and activate peroxisome proliferator regulators α and γ, inhibit enzymes in steroid biosynthesis, strongly influence natural killer cell function and the activation of specific T-cell subsets, and inhibit metastasis (Stephen 2010). Along with the isoflavonoids, ellagitannins and lignans are metabolized by gut bacteria into equol, urolithins, and enterolignans, which have high bioavailability and estrogenic/antiestrogenic effects, antioxidant, anti-inflammatory, and antiproliferative effects (Gaya et al. 2016; Catinean et al. 2018).

A group of polyphenols is known as lignans, its precursors are found in a wide variety of plant-based foods, including seeds such as flaxseeds, soybeans, strawberries, carrots, cabbage, onion, garlic, and cucumber (Blanck et al. 2003; Peterson et al. 2011). They are rich in the omega-3 fatty acid, alpha-linolenic acid, aminolevulinic acid, etc. All of which have been shown to have many potential health benefits. They can be used to improve digestive health, lower blood pressure, and bad cholesterol, reduce the risk of cancer, and may benefit people with diabetes. Polyphenols are also abundant in cocoa powder, dark chocolate, berries, beans, nuts, vegetables (red onion, spinach), soy, tea (black and green), and red wine (Manach et al. 2004; Catinean et al. 2018). There is a bidirectional relationship between polyphenols and gut microbiota. The polyphenols' bioavailability is increased by the microbiota, while unabsorbed polyphenols are involved in maintaining the equilibrium of microbiota in the gut. Suggested mechanisms imply protection against GI disorders and pathogens, strengthening intestinal epithelial tight cell junctions, increasing mucus secretion, stimulating cytokines, and modulating the immune response (Ozdal et al. 2016; Catinean et al. 2018).

Quercetin, a specific polyphenols, is one of the most studied compounds in relationship with gut microbiota. Quercetin is derived from plants that belongs to the flavonols, a subclass of flavonoid compounds. It can be found in apples, berries, grapes, onions, tea, tomatoes, seed, and nuts but also in medicinal botanicals like *Hypericum perforatum*, *Ginkgo biloba*, and *Sambucus canadensis* (Li et al. 2016; Catinean et al. 2018). It was demonstrated that quercetin has a modulatory role in gut microbiota when overweight animals were fed a high-fat sucrose diet. Furthermore, quercetin ameliorates the *Firmicutes/Bacteroidetes* ratio in high-fat sucrose diet-fed rats by increasing the titer of *Bacteroides vulgatus* and *Akkermansia muciniphila*, which have been inversely correlated to obesity. Moreover, it can decrease the titer of *Eubacterium cylindroides* and *Bilophila wadsworthia*, bacteria associated with diet-induced obesity (Etxeberria et al. 2015; Catinean et al. 2018).

Pistacia lentiscus is an evergreen shrub found in the Mediterranean region. It contains oleo-gum-resin which acts as an immunomodulatory agent on peripheral blood mononuclear cells. It inhibits TNF-α and stimulates the macrophage migration inhibitory factor (MIF). It has been observed that oleo-gum-resin reduces TNF-α secretion and increases MIF in Crohn's disease (CD). Subsequently, it also inhibits migration of monocytes and chemotaxis in chronic GI disease conditions in CD (Kaliora et al. 2007).

Andrographis paniculata extract is used to treat chronic inflammatory diseases. The herbal extract HMPL-004 has been reported to significantly reduce the transcriptional activity of NF-κB and decrease secretions of pro-inflammatory cytokines such as TNF-α and IL-6. Its benefits in active UC were addressed by two randomized, double-blind, 8-week trials, showing a decrease in the total Mayo score of 3 points as well as a 30% reduction in rectal bleeding (Tang et al. 2011; Sandborn et al. 2013).

One of the most commonly consumed beverages is tea (*Camellia sinensis*) either green or black. These contain high concentrations of flavanols (epicatechin and catechin) and their esters. High levels of unabsorbed compounds of tea remain in the gut and play an important role in the intestine's health. Caffeic acid, for example, inhibits the growth of many intestinal pathogenic bacteria, such as *E. coli*, *Salmonella*, *Pseudomonas*, *Clostridium*, and *Bacteroides* (Lee et al. 2006). The main active compounds in green tea are polyphenols. They have important antioxidant and anti-inflammatory effects and influence the activity of NF-kB (nuclear factor kappa B), COX-2 (cyclooxygenase-2), and the level of IL-2 (interleukin-2) (Oz et al. 2013). Black tea also contains several metabolites such as benzoic, phenylacetic, and phenylpropionic acids with antimicrobial properties (Van Duynhoven et al. 2013).

The aromatic plant *Oregano vulgare* is being used for phyto-therapeutic purposes. Carvacrol and thymol are two main phenols from oregano, and they have antimicrobial activity in particularly on *E. coli* (Fournomiti et al. 2015; Lopez-Romero et al. 2015). In an experimental study on pigs, it was demonstrated that oregano oil has protective effects against villous atrophy and epithelial cell necrosis and at

the same time it decreases the seric endotoxin levels (Zou et al. 2016).

Alkaloids with a quaternary ammonium structure such as berberine modulate the intestinal microbiota and show antimicrobial activity on *Firmicutes* and *Bacteroidetes*. Berberine exerts cytostatic, antiproliferative, and antioxidant properties. Therefore, berberine may contribute to the increase of intestinal gene expression of fasting-induced adipose factor (Fiaf) in mice, which acts as a lipoprotein lipase inhibitor (Zhang et al. 2015a, b; Xu et al. 2017). The alkaloid is considered a broad spectrum antibiotic which may increase *Bacteroides* and decrease *Ruminococcus* in the terminal ileum and colon (Guo et al. 2016).

Blue-green algae, a kind of cyanobacteria, e.g., Spirulina (*Arthrospira platensis*), mostly used as a food supplement, has the capacity to inhibit the growth of Gram-positive and Gram-negative bacteria (*Staphylococcus aureus*, *Bacillus subtilis*, *E. coli*, *Pseudomonas aeruginosa*, etc.). This antibacterial effect is due to an extracellular metabolite produced by Spirulina. In addition, the microbiota's modulatory effect of spirulina was attributed to the active compounds found in this plant (glutamate, aspartate, carbohydrates, or phenolic compounds). These substances have well-known antimicrobial and bacteriostatic effects as well as the capacity to stimulate the growth of probiotics (Beheshtipour et al. 2013; Finamore et al. 2017). Due to this effect, the Spirulina biomass can become a natural product which could be added to fermented milk to increase the production of *Lactobacillus* and also the number of viable cells which reach the intestine (Bhowmik et al. 2009).

L-Glutamine (GLN) is a well-known amino acid which plays an important role in the gut and has an important contribution to generating energy. Talukder et al. (2008a, b) discovered the presence of two different types of GLN co-transporters in the intestine. GLN is degraded by enterocytes and intestinal luminal bacteria and oxidized by the Krebs cycle, forming ATP (Wang et al. 2014). GLN causes a change in the intestinal bacteria community and increases the intestinal immunity by affecting the NF-kB, MAPK (mitogen-activated protein kinase), and PI3K-Akt (phosphatidylinositol-3-kinases-protein kinase B) signaling pathways (Ren et al. 2014). Figure 2 summarizes the interactions between some phytochemical agents and the inflammatory network (adopted from Larussa et al. 2017).

3 Dietary Lipids and Fat-Soluble Vitamins for Gastrointestinal Conditions

Lipids, also known as fats, play many important roles in the animal body, from providing energy to producing hormones. Dietary lipids are one of the most active nutritional substrates modulating the immune response, in particular, the gut mucosal immune system. Polyunsaturated fatty acids (PUFA) are also called "essential fatty acids" as these are crucial to the body's function. These are introduced externally through the diet (Escott-Stump and Mahan 2000). PUFAs have been classified into two categories: omega-3 (n-3) fatty acids and omega-6 (n-6) fatty acids. The major omega-3 fatty acids are α-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA). ALA is the precursor of EPA and DHA. EPA and DHA are found mainly in fatty fishes such as mackerel, salmon, herring, trout, and blue fin tuna and in fish oils. The principal sources of ALA are mainly flaxseed, soybeans, canola, some nuts (e.g., walnuts), and red/black currant seeds (Trumbo et al. 2002). Omega-6 PUFAs mainly consist of linoleic acid (LA), γ-linolenic acid (GLA), and arachidonic acid (ARA). LA occurs mainly in vegetable oils, e.g., corn, safflower, soybean, and sunflower. ARA is found in animal products such as meat, poultry, and eggs. The roles of PUFAs have been widely investigated during inflammatory processes in chronic enteritis (Larussa et al. 2017). The most interesting fatty acids are the n-6 PUFA AA, which is the precursor of inflammatory eicosanoids like prostaglandin E_2 (PGE_2) and leukotriene B_4, and the n-3 PUFAs EPA and DHA, which are abundant in fish oils.

The modifying action of the lipid mediator profile is exerted by n-3 PUFAs mainly acting as a competitive substrate, which decreases the production of the eicosanoids from AA, but also reducing leukocyte chemotaxis and inflammatory cytokine production (Calder 2008). A rising incidence of enteritis has been reported in recent years particularly with dietary changes including a higher intake of n-6 PUFAs and a reduced consumption of n-3 PUFAs (Marion-Letellier et al. 2013). A correlation between a higher intake of LA, an n-6 PUFA, and an increased risk of UC has been reported (Tjonneland et al. 2009; Larussa et al. 2017), suggesting a possible role for dietary linoleic acid in the etiology of the disease. On the other hand, oleic acid, which is the predominant ingredient of olive oil, was found to be inversely associated with UC development (de Silva et al. 2014). In agreement with this finding, a prospective large study showed an inverse association between greater long-term intake of long-chain n-3 PUFAs and the risk of UC, confirming the protective effect of n-3 PUFA intake, while no specific fatty acids appeared to be associated with the risk of CD (Uchiyama et al. 2010). Another study showed that a dietary intervention focused on lowering n-6/n-3 PUFA ratio was effective in maintaining disease remission in IBD, possibly through the increasing of n-3 PUFA intake (Ananthakrishnan et al. 2014; Larussa et al. 2017). Fish oil supplementation in IBD resulted in n-3 PUFA incorporation into gut mucosal tissue and modification of inflammatory mediator profiles, showing how readily colonic lipids, prostaglandins, and

thromboxane synthesis can be altered by dietary changes (Hillier et al. 1991; Larussa et al. 2017).

A trial of dietary supplementation with fish oil in UC showed a 56% decrease of the mean disease activity index and a 4% decrease in placebo. Moreover, fish oil ingestion revealed a well-tolerated profile in all groups without any alteration in routine blood exams (Aslan and Triadafilopoulos 1992; Larussa et al. 2017). A recent study has identified an endogenous lipid mediator, resolvin E1, which is generated from eicosapentaenoic acid in vivo and demonstrates potent anti-inflammatory activity, suggesting a mechanism by which omega-3 fatty acids can modulate inflammatory pathways (Arita et al. 2005).

An interesting role in the nutraceutical scenario used with chronic enteritis has been proposed for the fat-soluble vitamins, such as A, D, E, and K. The deficiency of vitamin D, whose main source is endogenous production in the skin upon exposure to sunlight, was found to be significantly associated with IBD (Del Pinto et al. 2015; Larussa et al. 2017). Dogs with a chronic enteropathy (CE) have a lower vitamin D status than do healthy dogs. Vitamin D status has been associated with a negative clinical outcome with IBD in humans. Gow et al. (2011) compared serum vitamin D metabolites and plasma parathyroid hormone concentrations in dogs with IBD and normal albumin concentration, dogs with IBD and hypoalbuminemia, healthy dogs, and hospitalized ill dogs with non-gastrointestinal illness. They observed that concentrations of serum 25-hydroxyvitamin D were lower in hypoalbuminemic dogs with IBD than in the healthy dogs, hospitalized ill dogs, and normoalbuminemic dogs with IBS. Dogs with IBD and hypoalbuminemia had a higher plasma concentration of parathyroid hormone and a lower plasma concentration of ionized calcium than hospitalized ill dogs. Dogs with IBD had a positive correlation between serum 25-hydroxyvitamin D concentrations and serum albumin, serum calcium, and plasma ionized calcium concentrations. Furthermore, evidence supports an immunological role of vitamin D in chronic enteritis both promoting tissue barrier formation through the expression of cell adhesion proteins and stabilization of tight junctions between epithelial cells and inhibiting the production of pro-inflammatory cytokines through the activation of vitamin D receptor (Mouli and Ananthakrishnan 2014). Results from a randomized controlled trial support the benefits of vitamin D supplementation in UC patients, since a decrease in erythrocyte sedimentation rate (ESR) and c-reactive protein (CRP) levels was found in the treatment group (Jørgensen et al. 2010). It has been suggested that vitamin K may be involved in the modulation of disease activity and maintenance of bone health in IBD patients. Nakajima et al. (2011) evaluated vitamin K levels of patients with IBD by measuring serum undercarboxylated osteocalcin and found a significant correlation with the clinical activity index of CD. The complexity of these interactions has been highlighted by Kuwabara et al. (2009), who found that a low plasma concentration of vitamin K was an independent risk factor for low bone mineral density in IBD patients, but these low levels were associated with the patients' fat intake, and not with the intake of vitamin K. This suggested a malabsorption rather than a poor dietary intake.

4 Dietary Peptides and Amino Acids for Gastrointestinal Conditions

Amino acids (AA) are the building blocks of protein that includes essential and nonessential AA. Dietary peptides and amino acids have been shown to modulate intestinal immune functions and influence inflammatory responses, being involved in reducing inflammation, oxidative stress, and apoptosis in the gut (Zhang et al. 2015a, b; Larussa et al. 2017). Processes that lead to bioactive peptide release include in vivo enzymatic digestion in the GI tract both by human and microbiota enzymes or in vitro food processing. Any protein source can produce bioactive peptides, with milk being the most studied. However, bioactive peptides from egg, fish, meat, algae, or soy have also been reported (Martínez-Augustin et al. 2014). Milk-derived products are already in clinical use for the treatment of chronic enteritis, such as casein-based enteral feeds which are used for the treatment of CD and whose efficacy might be due, in part, to the presence of the anti-inflammatory cytokine transforming growth factor-β (Richman and Rhodes 2013; Larussa et al. 2017).

Colostrum is a form of milk produced by the mammary glands of mammals just prior to birthing. Bovine colostrum is a rich source of nutrients, antibodies, antimicrobial peptides (e.g., lactoferrin, lactoperoxidase), and growth factors. Talukder et al. (2002, 2003) discovered the transport of colostral macromolecules into the cerebrospinal fluid (CSF) via plasma in newborn and young calves. They also found the characteristics of the lactoferrin receptor in bovine intestine: higher binding activity to the epithelium overlying Peyer's patches (Talukder et al. 2003). Talukder and Harada (2007) also observed that bovine lactoferrin protects lipopolysaccharide (LPS)-induced diarrhea modulating nitric oxide (NO) and PE_2 in mice. In addition, lactoferrin ameliorates PE_2-mediated inhibition of Na^+-glucose cotransport in enterocytes (Talukder et al. 2014). Furthermore, beneficial properties of colostrum have been demonstrated during an initial study with UC patients treated by enema administration (Khan et al. 2002). An amelioration in body composition, with a decrease in fat percentage, has been obtained in CD patients after whey and soy protein dietary supplementation (Machado et al. 2015). Considering that a reduction of

body fat contributes to the control of inflammation, these nutritional strategies may be useful as alternative or ancillary treatments in IBD. The current available information indicates a potential for food-derived peptides to counteract acute and chronic intestinal inflammation. Experimental studies in animal models evaluating isolated AAs such as tryptophan, glutamine, cysteine, and arginine offer promising in CD (Coëffier et al. 2010).

5 Probiotics, Prebiotics, and Synbiotics

Probiotics are "live microorganisms" that benefit the host by modulating mucosal and systemic immunity and provide nutritional and microbial balance in the intestinal tract. In fact, coevolution led to a symbiotic relationship between the host and microorganisms and thereby developed a bidirectional signaling system through the intestinal lining that contains immune cells such as lymphocytes and dendritic cells. More than 400 species of bacteria have been identified from GI tract including beneficial (e.g., *Bifidobacterium* and *Lactobacillus*) and pathogenic (e.g., *Enterobacteriaceae* and *Clostridium* spp.) Beneficial bacteria use fibers (prebiotic) for fermentation and provide indispensable nutrients to the intestinal cells and the host as well. These nutrients include amino acids such as Arg, Cys, and Gln and short-chain fatty acids (SCFA) such as acetate, propionate, and butyrate (O'Sullivan et al. 2005). These SCFAs are being used as a source of basal metabolic energy (10–30%) by different cells of the host including colonocytes and hepatocytes; 5% of SCFAs is excreted in the feces (O'Sullivan et al. 2005). The SCFAs are important for the liver to regulate glucose homeostasis and lipogenesis. Colonocytes require butyrate, the most important and integral part of energetic metabolism, whereas acetate and propionate are used as substrates for lipogenesis, gluconeogenesis, and protein synthesis (Catinean et al. 2018). Furthermore, beneficial bacteria make vitamins K and B complex, bile acid, and neutralize dietary carcinogens such as nitrosamines. They also convert prodrugs into active metabolites. The indigenous intestinal microbiota is known as autochthonous. They compete with pathogenic bacteria (allochthonous) for nutrients and adherence to mucosal lining and produce different types of antigens, known as bacteriocins. Bacteriocins are potential to inhibit the growth and replication of allochthonous or kill them. In contrast, allochthonous can be detrimental by making toxins or other harmful metabolites. Accordingly, both autochthonous and allochthonous may affect the GI homeostasis as well as systemic physiology of the host. However, autochthonous are essential for the host to protect GI mucosa and its immune system. Different factors such as diet, drugs, and toxins can affect the usual abundance and diversity of gut microbiota (microbiome), known as dysbiosis. The diet includes "prebiotic." The latter is a nondigestible, dietary carbohydrate metabolized by gut microorganisms for their growth and development. Thus, dysbiosis directly alters the production of SCFAs. The proper development and maintenance of GI homeostasis including secretion of antibodies in the lumen against harmful antigens or allochthonous require certain types and loads of microflora for interaction with the mucosal immune system (Catinean et al. 2018). That is why they are known as the most adaptable and renewable metabolic organ of the body and their biological effects are summarized in Table 1.

Table 1 Biological effects of probiotic bacteria

Modulation of host immune response
Enhanced antibody production
Enhanced natural killer cell activity
Modulation of dendritic cell phenotype and function
Modulation of NF-kB and AP-1 pathway
Altered cytokine release
Induction of regulatory T cells
Induction of PPAR-g
Modulation of apoptosis
Inhibition of proteasome activity
Enhanced epithelial barrier function
Enhanced tight junction protein phosphorylation
Upregulation of mucous production
Enhanced epithelial cell glycosylation
Increased sIgA production
Antimicrobial effects
Decreased luminal pH
Stimulation of defensin secretion
Secretion of antimicrobial peptides
Inhibition of pathogenic bacterial invasion
Blockade of bacterial adhesion to epithelial cells
Release of nitric oxide

Nowadays, a variety of probiotics including their strains are being used to support GI health such as "probiotic chewable tablets" produced and marketed by Chr. Hansen A/S, Denmark, containing *Bifidobacterium animalis* subsp. *lactis*. In addition, probiotic bacteria, e.g., *Enterococcus faecium SF68*, and yeast, e.g., *Saccharomyces boulardii*, are also directly used in foods, particularly fermented milk products. Investigations on probiotics with regard to their medicinal use leading to determine new genera and strains with many health benefits such as *Lactobacillus plantarum* isolate (PCS20, PCS22, PCS25, and PCS26) from Slovenian cheese with high antimicrobial (Maragkoudakis et al. 2010) and immunomodulatory (Bengmark 1998) competences. Utilization of beneficial microorganisms was considered to benefit health through alterations in gut microbiota, and thereby single or mixed microbial culture preparations are commonly considered to use. Thus, it has been claimed that benefits of probiotics depend on types of probiotic strains (de Vrese and Schrezenmeir 2008; Bengmark 1998; Nissen et al. 2009). Although benefits of probiotics on gut health and in diarrhea

are documented, unlikely the dose of probiotics or the duration of such treatments are not clearly defined (Minelli and Benini 2008), while the dose for treatment of an acute illness by a specific probiotic may be lower or higher in the order of tenfold or hundredfold or more in terms of colony-forming units (CFU). In addition, clinical studies did not show conclusive results of the relationship among strains of probiotics and type of infections such as acute or chronic GI infections and immunological or inflammatory disease (Minelli and Benini 2008). The concentration of probiotics needed to obtain a clinical effect is often quoted as $\geq$106 colony-forming units (CFU) per mL in the small bowel and $\geq$ 108 CFU per mL in the colon. In acute infectious diarrhea, it is postulated that higher doses of probiotics for short courses are more effective than lower doses. It has been also suggested that the advantages of probiotics for health can only be achieved if proper strain or product selection and dose guidelines are followed in either food or medicine (Kalliomaki et al. 2010; Douglas and Sanders 2008). In addition to the importance and application of probiotics for GI, it has been shown the benefits in alleviating symptoms of allergies (Yao et al. 2010; Kalliomaki et al. 2010), respiratory and urinary tract infections (Kaur et al. 2009), AIDS (Trois et al. 2008), and cancer (Kumar et al. 2010). It has been demonstrated that the effects of probiotics depend also on the interactions between the respective microorganisms and the gut immune system and the duration of treatment in chronic diseases such as allergic, inflammatory, and/or immune diseases. Moreover, it has been revealed that probiotics improve in alleviating symptoms associated with osteoporosis, obesity, fatigue, autism, and aging and reducing the risks of type 2 diabetes (Douglas and Sanders 2008).

In order to evaluate the usefulness of probiotics, it is essential to identify the specific target groups of individuals with more specific higher susceptibilities to the potential effects of probiotics. Furthermore, depending on functional evidence of different probiotics, the International Life Sciences Institute (ILSI) categorized probiotics in four areas of application. Areas include (1) metabolism, (2) chronic intestinal inflammatory and functional disorders, (3) infections, and (4) allergy (Rijkers et al. 2010). The main goals of ILSI were to substantiate the current body of information on probiotic benefits. The ILSI also provided the pros and cons including recommendations to design the next generation of probiotic research.

The main probiotic preparations currently on the market belong to a large group of bacteria designated as lactic acid bacteria (e.g., *Lactobacillus*, *Streptococcus*, *Bifidobacterium*), which are important and normal constituents of the GI microflora. These are presented in Table 2. However, studies are also investigating potential probiotic roles of other microbes such as yeast (*Saccharomyces boulardii*), which are not normally found in the gastrointestinal tract. Probiotic strains exert their beneficial effects through a variety of mechanisms (Cong et al. 2003) that are unique to each strain (Table 2). Whether living microorganisms are required, or even whether oral administration is necessary for clinical benefit, is still uncertain and might depend on the particular bacterial strain. For instance, several recent studies in animal models have demonstrated that nonviable bacterial components may have beneficial effects.

The mechanisms of probiotic action are poorly understood (Oelschlaeger 2010). Probiotics may act by modulating the host's immune system, affecting other microorganisms directly, or acting on microbial products, host products, or food components (Oelschlaeger 2010). The effectiveness of a probiotic depends on its metabolic properties, the set of molecules presented at its surface, the components it secretes, and the integral parts of the microorganism such as its DNA or peptidoglycan (Oelschlaeger 2010). DNA isolated from the VSL3 probiotic compound attenuated colitis, an effect

Table 2 Probiotics used in experimental studies

Lactobacilli	Bifidobacteria	Others	Fungi
L. acidophilus	*B. bifidum*	*Streptococcus thermophilus*	*Saccharomyces cerevisiae*
L. casei	*B. infantis*	*Enterococcus faecium*	*Saccharomyces boulardii*
L. delbrueckii subsp. *bulgaricus*	*B. longum*	*Lactococcus lactis*	
L. reuteri	*B. thermophilum*	*Propionibacterium freudenreichii*	
L. brevis	*B. adolescents*	*Escherichia coli* Nissle 1917	
L. cellobiosus	*B. lactis*	*Bacillus clausii*	
L. curvatus	*B. animalis*	*Bacillus oligonitrophilis*	
L. fermentum	*B. breve*		
L. plantarum			
L. rhamnosus (GG)			
L. salivarius			
L. gasseri			
L. johnsonii			
L. helveticus			
L. farciminis			

that was dependent upon Toll-like receptor (TLR)-9 (Rachmilewitz et al. 2004). TLRs comprise a family of pattern recognition receptors that function in the maintenance of intestinal homeostasis by recognizing and responding to conserved molecular products of microorganisms (Abreu et al. 2005). Another study demonstrated that isolated DNA from VSL3, but not from *E. coli*, inhibited nuclear factor-kB (NF-kB) activation and pro-inflammatory cytokine secretion (Jijon et al. 2004). Furthermore, probiotic compounds might not even have to be taken orally to have benefit. Sheil et al. (2004) showed that subcutaneous administration of *Lactobacillus salivarius* attenuated colitis and pro-inflammatory cytokine production in a mouse model. This suggests that in the future it may be possible to use nonviable and purified probiotic bacterial components for the treatment of GI diseases.

The term prebiotic refers to dietary carbohydrates that stimulate the growth of gut bacteria or probiotics when these are administered externally. A prebiotic is a nondigestible compound that through its metabolization by microorganisms in the gut modulates the composition and/or activity of the gut microbiota, thus conferring a beneficial physiological effect on the host. Prebiotic is defined as "a selectively fermented ingredient, or a fiber that allows specific changes, both in the composition and/or activity of the gastrointestinal microflora, resultantly conferring benefits on the wellbeing and health of the host" (de Vrese and Schrezenmeir 2008; Douglas and Sanders 2008).

A prebiotic agent must fulfill certain criteria, such as not being hydrolyzed and absorbed in the first part of the gastrointestinal tract and is fermented by a limited number of beneficial bacteria in the colon (e.g., *Lactobacillus*). Moreover, the prebiotic must be able to stimulate or metabolically activate the growth of these beneficial bacteria, in order to change the microflora into those that are healthier. The main effects of prebiotics include modulation of gut microbiota composition, production of energy metabolism, increasing mineral absorption, regulation of immune function, and improvement of the intestinal barrier functions. Other more specific effects of prebiotics on health are indirect, namely, prevention of diarrhea or obstipation, modulation of the metabolism of the intestinal flora, cancer prevention, effects on lipid metabolism, stimulation of mineral adsorption, and immunomodulatory properties.

Prebiotics form a group of diverse carbohydrate ingredients that is poorly understood regarding their origin, fermentation profiles, and dosages required for health effects, although they do provide nutraceutical and nutritional value (Douglas and Sanders 2008). Fructooligosaccharides (FOS), galactooligosaccharides (GOS), xylooligosaccharides (XOS), lactulose, the nondigestible carbohydrate inulin, cellulose, resistant starches, hemicelluloses, and pectins are currently the most used prebiotics. Today, only bifidogenic, nondigestible oligosaccharides (particularly inulin, its hydrolysis product oligofructose, and (trans)galactooligosaccharides) fulfill all the criteria for prebiotic classification (de Vrese and Schrezenmeir 2008). In the last few years, successful attempts have been reported to make infant formula more breast milk-like by the addition of fructo- and (primarily) galactooligosaccharides.

Soy beans and lactose from cow's milk are the main sources of GOS. These are included especially in different foods. Probiotics and prebiotics share unique roles in human nutrition, largely centered on manipulation of populations, or activities of the microbiota that colonize the human GI tract (Douglas and Sanders 2008).

Regular consumption of probiotics or prebiotics has health implications that include enhanced immune function, improved colonic integrity, decreased incidence and duration of intestinal infections, downregulated allergic response, and improved digestion and elimination (Douglas and Sanders 2008). It is noteworthy that human subjects and their enteric microbiota have evolved together to reach a state of mutual tolerance. There is mounting evidence from both animal models and human studies to suggest that IBD is a result of a malfunction of this mutual relationship (Hedin et al. 2007). Probiotics and prebiotics, however, have been investigated in clinical trials as treatments for IBD, with conflicting results (Hedin et al. 2007). While there is consensus about the value of probiotics and prebiotics, their influence on bowl health in terms of Crohn's disease is less convincing (Hedin et al. 2007). This may be attributed to variations in methodologies; differences in the range of probiotic, prebiotic, and combination (synbiotic) treatments tested; and variability in test subjects used, such as different patient groups (Hedin et al. 2007). The "synbiotics" are synergistic combinations of pro- and prebiotics (de Vrese and Schrezenmeir 2008). Figure 3 depicts the interactions between nutraceuticals and gut microbiome and other associated disease conditions (adopted from Larussa et al. 2017).

6 Concluding Remarks and Future Directions

Physiology of the digestive system and its microbiome greatly depends on the types of foods ingested. Pharmaceutical drugs, especially antibiotics, significantly alter the gut microbiome which is very critical for normal GI functions and can be restored by using nutraceuticals including probiotics, prebiotics, and phytochemicals (phytoestrogens and polyphenols). Recent developments in nutraceutical research, including elucidation of the mechanism of action, is very promising which will greatly help understanding of new nutraceuticals to control different GI conditions. Future basic and applied nutritional research will pave the way. In

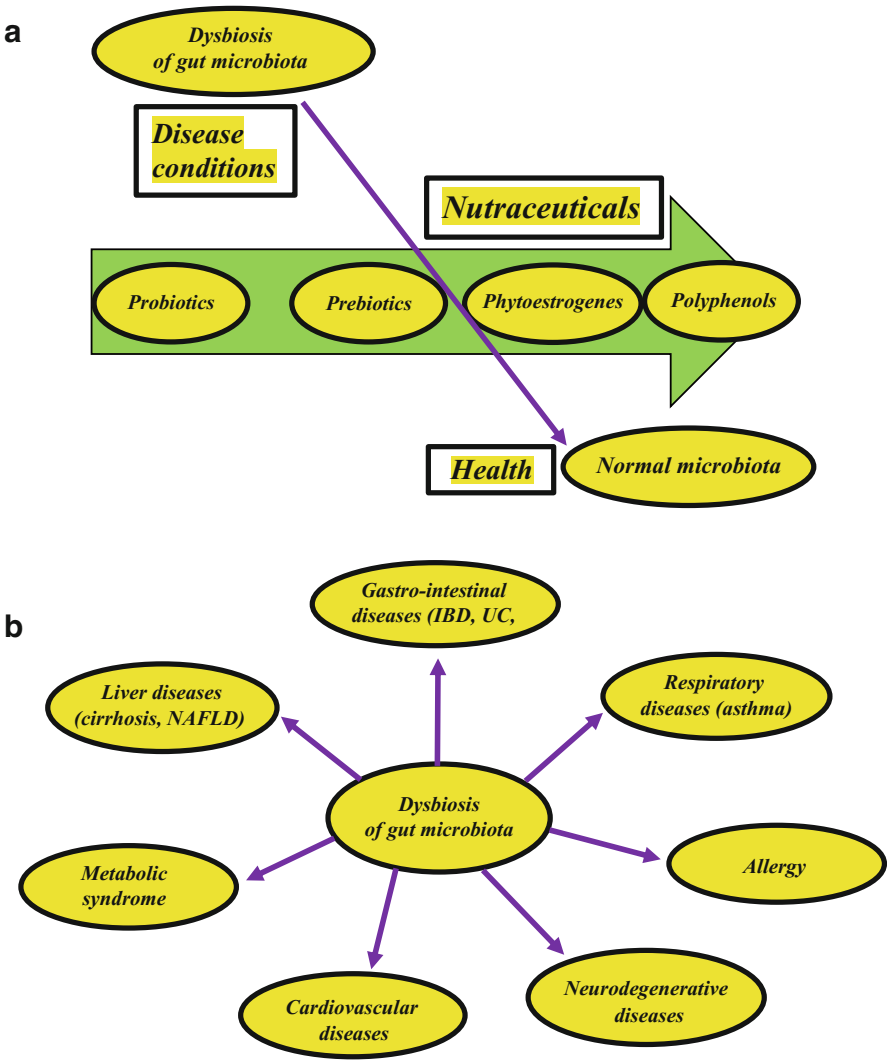


Fig. 3 (**a**, **b**) The role of nutraceuticals on intestinal microbiota. Gut microbiota and associated other disease conditions

this regard, in vitro and clinical trials are very important to elucidate the mode of actions along with appropriate doses for different animals, including humans. Therefore, scientists and healthcare professionals must work together to improve scientific understanding of nutraceuticals for different GI conditions to improve the quality of life. The nutraceutical revolution will lead us into a new era of medicine and health for animals and humans.

References

Abreu MT, Fukata M, Arditi M (2005) TLR signaling in the gut in health and disease. J Immunol 174:4453–4460

Adelaja AO, Schilling BJ (1999) Nutraceutical: blurring the line between food and drugs in the twenty-first century. Mag Food Farm Resour Issues 14:35–40

Ananthakrishnan AN, Khalili H, Konijeti GG et al (2014) Long-term intake of dietary fat and risk of ulcerative colitis and Crohn's disease. Gut 63:776–784

Arita M, Yoshida M, Hong S et al (2005) Resolvin E1, an endogenous lipid mediator derived from omega-3 eicosapentaenoic acid, protects against 2, 4,6-trinitrobenzene sulfonic acid-induced colitis. Proc Natl Acad Sci USA 102:7671–7676

Aslan A, Triadafilopoulos G (1992) Fish oil fatty acid supplementation in active ulcerative colitis: a double-blind, placebo-controlled, cross-over study. Am J Gastroenterol 87:432–437

Beheshtipour H, Mortazavian AM, Mohammadi R et al (2013) Supplementation of *Spirulina platensis* and *Chlorella vulgaris* algae into probiotic fermented milks. Compr Rev Food Sci Food Saf 12:144–154

Bengmark S (1998) Ecological control of the gastrointestinal tract. The role of probiotic flora. Gut 42:2–7

Bhowmik D, Dubey J, Mehra S (2009) Probiotic efficiency of spirulina platensis – stimulating growth of lactic acid bacteria. World J Dairy Food Sci 4:160163

Biedermann L, Mwinyi J, Scharl M et al (2013) Bilberry ingestion improves disease activity in mild to moderate ulcerative colitis – an open pilot study. J Crohns Colitis 7:271–279

Blanck HM, Bowman BA, Cooper GR et al (2003) Biomarkers of nutritional exposure and nutritional status laboratory issues: use of nutritional biomarkers 1. Environ Health 133:888S–894S

Bilal I, Chowdhury A, Davidson J, Whitehead S (2014) Phytoestrogens and prevention of breast cancer: the contentious debate. World J Clin Oncol 5:705–712. https://doi.org/10.5306/wjco.v5.i4.705

Buchanan RL, Baker RC, Charlton AJ et al (2011) Pet food safety: a shared concern. Br J Nutr 106(Suppl. 1):S78–S84

Bundy R, Walker AF, Middleton RW et al (2004) Turmeric extract may improve irritable bowel syndrome symptomology in otherwise healthy adults: a pilot study. J Altern Complement 10:1015–1018
Calder PC (2008) Polyunsaturated fatty acids, inflammatory processes and inflammatory bowel diseases. Mol Nutr Food Res 52:885–897
Carter RA, Bauer JE, Kersey JH et al (2014) Awareness and evaluation of natural pet food products in the United States. J Am Vet Med Assoc 245:1241–1248
Cassidy A, Minihane AM (2017) The role of metabolism (and the microbiome) in defining the clinical efficacy of dietary flavonoids. Am J Clin Nutr 05:10–22. https://doi.org/10.3945/ajcn.116.136051
Catinean A, Neag MA, Muntean DM, Bocsan IC, Buzoianu AD (2018) An overview on the interplay between nutraceuticals and gut microbiota. PeerJ 6:e4465. https://doi.org/10.7717/peerj.4465
Coëffier M, Marion-Letellier R, Déchelotte P (2010) Potential for amino acids supplementation during inflammatory bowel diseases. Inflamm Bowel Dis 16:518–524
Cong Y, Konrad A, Iqbal N et al (2003) Probiotics and immune regulation of inflammatory bowel diseases. Curr Drug Targets Inflamm Allergy 2:145–154
Connell DW, McLachlan R (1972) Natural pungent compounds: examination of gingerols, shogaols, paradols and related compounds by thin-layer and gas chromatography. J Chromatogr 67:29–35
de Silva PS, Luben R, Shrestha SS et al (2014) Dietary arachidonic and oleic acid intake in ulcerative colitis etiology: a prospective cohort study using 7-day food diaries. Eur J Gastroenterol Hepatol 26:11–18
de Vrese M, Schrezenmeir J (2008) Probiotics, prebiotics, and synbiotics. Adv Biochem Eng Biotechnol 111:1–66
Del Pinto R, Pietropaoli D, Chandar AK et al (2015) Association between inflammatory bowel disease and vitamin D deficiency: a systematic review and meta-analysis. Inflamm Bowel Dis 21:2708–2717
Douglas LC, Sanders ME (2008) Probiotics and prebiotics in dietetics practice. J Am Diet Assoc 108:510–521
Eckburg PB, Bik EM, Bernstein CN et al (2005) Diversity of the human intestinal microbial flora. Science 308:1635–1638
Escott-Stump E, Mahan LK (2000) Krause's food, nutrition and diet therapy, 10th edn. WB Saunders Company, Philadelphia, pp 553–559
Etxeberria U, Arias N, Boqué N et al (2015) Reshaping faecal gut microbiota composition by the intake of trans-resveratrol and quercetin in high-fat sucrose diet-fed rats. J Nutr Biochem 26:651–660
Finamore A, Palmery M, Bensehaila S et al (2017) Antioxidant, immunomodulating, and microbial-modulating activities of the sustainable and ecofriendly spirulina. Oxidative Med Cell Longev 2017:3247528. https://doi.org/10.1155/2017/3247528
Foster BC, Arnason JT, Briggs CJ (2005) Natural health products and drug disposition. Annu Rev Pharmacol Toxicol 45:203–226
Fournomiti M, Kimbaris A, Mantzourani I et al (2015) Antimicrobial activity of essential oils of cultivated oregano (*Origanum vulgare*), sage (*Salvia officinalis*), and thyme (*Thymus vulgaris*) against clinical isolates of *Escherichia coli*, *Klebsiella oxytoca*, and *Klebsiella pneumoniae*. Microb Ecol Health Dis 1:1–7
Franke AA, Lai JF, Halm BM et al (2014) Absorption, distribution, metabolism, and excretion of isoflavonoids after soy intake. Arch Biochem Biophys 559:24–28
Gaya P, Medina M, Sánchez-Jiménez A, Landete J (2016) Phytoestrogen metabolism by adult human gut microbiota. Molecules 21:1034. https://doi.org/10.3390/molecules21081034
Ghayur MN, Gilani AH (2005) Ginger lowers blood pressure through blockade of voltage-dependent calcium channels. J Cardiovasc Pharmacol 45:74–80
Gow AG, Else R, Evans H et al (2011) Hypovitaminosis D in dogs with inflammatory bowel disease and hypoalbuminaemia. J Small Anim Pract 52(8):411–418
Guadamuro L, Dohrmann AB, Tebbe CC et al (2017) Bacterial com-munities and metabolic activity of faecal cultures from equol producer and non- producer menopausal women under treatment with soy isoflavones. BMC Microbiol 17:93. https://doi.org/10.1186/s12866-017-1001-y
Guo Y, Zhang Y, Huang W, Selwyn FP, Klaassen CD (2016) Dose response effect of berberine on bile acid profile and gut microbiota in mice. BMC Complement Altern Med 16:394. https://doi.org/10.1186/s12906-016-1367-7
Gupta I, Parihar A, Malhotra P et al (1997) Effects of *Boswellia serrata* gum resin in patients with ulcerative colitis. Eur J Med Res 2:37–43
Hanai H, Iida T, Takeuchi K et al (2006) Curcumin maintenance therapy for ulcerative colitis: randomized, multicenter, double-blind, placebo-controlled trial. Clin Gastroenterol Hepatol 4(12):1502–1506
Hasler CM (2000) The changing face of functional foods. J Am Coll Nutr 19:499S–506S
Hedin C, Whelan K, Lindsay JO (2007) Evidence for the use of probiotics and prebiotics in inflammatory bowel disease: a review of clinical trials. Proc Nutr Soc 66:307–315
Hillier K, Jewell R, Dorrell L et al (1991) Incorporation of fatty acids from fish oil and olive oil into colonic mucosal lipids and effects upon eicosanoid synthesis in inflammatory bowel disease. Gut 32:1151–1155
Hoensch HP, Oertel R (2015) The value of flavonoids for the human nutrition: short review and perspectives. Clin Nutr Exp 3:8–14
Holt PR, Katz S, Kirshoff R (2005) Curcumin therapy in inflammatory bowel disease: a pilot study. Dig Dis Sci 50:2191–2193
Holtmeier W, Zeuzem S, Preiss J et al (2011) Randomized, placebo-controlled, double-blind trial of *Boswellia serrata* in maintaining remission of Crohn's disease: good safety profile but lack of efficacy. Inflamm Bowel Dis 17:573–582
Hsu CH, Cheng AL (2007) Clinical studies with curcumin. Adv Exp Med Biol 595:471–480
Iranshahi M, Rezaee R, Parhiz H, Roohbakhsh A, Soltani F (2015) Protective effects of flavonoids against microbes and toxins: the cases of hesperidin and hesperetin. Life Sci 137:125–132. https://doi.org/10.1016/j.lfs.2015.07.014
Jarosová B, Javrek J, Adamovský O, Hilscherová K (2015) Phytoestrogens and mycoestrogens in surface waters—their sources, occurrence, and potential contribution to estrogenic activity. Environ Int 81:26–44. https://doi.org/10.1016/j.envint.2015.03.019
Jijon H, Backer J, Diaz H et al (2004) DNA from probiotic bacteria modulates murine and human epithelial and immune function. Gastroenterology 126:1358–1373
Jørgensen SP, Agnholt J, Glerup H, Lyhne S, Villadsen GE, Hvas CL, Bartels LE, Kelsen J, Christensen LA, Dahlerup JF (2010) Clinical trial: vitamin D3 treatment in Crohn's disease – a randomized double-blind placebo-controlled study. Aliment Pharmacol Ther 32:377–383. https://doi.org/10.1111/j.1365-2036.2010.04355.x
Kaliora AC, Stathopoulou MG, Triantafillidis JK et al (2007) Alterations in the function of circulating mononuclear cells derived from patients with Crohn's disease treated with mastic. World J Gastroenterol 13:6031–6036
Kalliomaki M, Antoine JM, Herz U et al (2010) Guidance for substantiating the evidence for beneficial effects of probiotics: prevention and management of allergic diseases by probiotics. J Nutr 140:713S–721S
Kaur C, Kapoor HC (2001) Antioxidants in fruits and vegetables-the millennium's health. Int J Food Sci Technol 36:703–725
Kaur IP, Kuhad A, Garg A et al (2009) Probiotics: delineation of prophylactic and therapeutic benefits. J Med Food 12:219–235
Khan Z, Macdonald C, Wicks AC et al (2002) Use of the 'nutriceutical', bovine colostrum, for the treatment of distal colitis: results from an initial study. Aliment Pharmacol Ther 16:1917–1922
Kumar M, Kumar A, Nagpal R et al (2010) Cancer-preventing attributes of probiotics: an update. Int J Food Sci Nutr 61(5):473–496
Kuwabara A, Tanaka K, Tsugawa N et al (2009) High prevalence of vitamin K and D deficiency and decreased BMD in inflammatory bowel disease. Osteoporos Int 20:935–942

Lacy BE, Lee RD (2005) Irritable bowel syndrome: a syndrome in evolution. J Clin Gastroenterol 39:S230–S242

Lang A, Salomon N, Wu JC et al (2015) Curcumin in combination with mesalamine induces remission in patients with mild-to- moderate ulcerative colitis in a randomized controlled trial. Clin Gastroenterol Hepatol 13:1444–9.e1. PMID: 25724700. https://doi.org/10.1016/j.cgh.2015.02.019

Langhorst J, Varnhagen I, Schneider SB et al (2013) Randomised clinical trial: a herbal preparation of myrrh, chamomile and coffee charcoal compared with mesalazine in maintaining remission in ulcerative colitis--a double-blind, double-dummy study. Aliment Pharmacol Ther 38:490–500

Langmead L, Feakins RM, Goldthorpe S et al (2004) Randomized, double-blind, placebo-controlled trial of oral *aloe vera* gel for active ulcerative colitis. Aliment Pharmacol Ther 19:739–747

Laparra JM, Sanz Y (2010) Interactions of gut microbiota with functional food components and nutraceuticals. Pharmacol Res 61:219–225

Larussa T, Imeneo M, Luzza F (2017) Potential role of nutraceutical compounds in inflammatory bowel disease. World J Gastroenterol 23(14):2483–2492. https://doi.org/10.3748/wjg.v23.i14.2483

Lee HC, Jenner AM, Low CS et al (2006) Effect of tea phenolics and their aromatic fecal bacterial metabolites on intestinal microbiota. Res Microbiol 157:876–884

Li Y, Yao J, Han C et al (2016) Quercetin, inflammation and immunity. Nutrients 8:1–14

Lopez-Romero JC, González-Ríos H, Borges A et al (2015) Antibacterial effects and mode of action of selected essential oils components against *Escherichia coli* and *Staphylococcus aureus*. Evid-Based Complement Alternat Med 2015:795435. https://doi.org/10.1155/2015/795435

Machado JF, Oya V, Coy CS et al (2015) Whey and soy protein supplements changes body composition in patients with Crohn's disease undergoing azathioprine and anti-TNF-alpha therapy. Nutr Hosp 31:1603–1610

Manach C, Scalbert A, Morand C et al (2004) Polyphenols: food sources and bioavailability. Am J Clin Nutr 79:727–747

Maragkoudakis PA, Chingwaru W, Gradisnik L et al (2010) Lactic acid bacteria efficiently protect human and animal intestinal epithelial and immune cells from enteric virus infection. Int J Food Microbiol 141 (1):S91–S97

Marion-Letellier R, Savoye G, Beck PL et al (2013) Polyunsaturated fatty acids in inflammatory bowel diseases: a reappraisal of effects and therapeutic approaches. Inflamm Bowel Dis 19:650–661

Martínez-Augustin O, Rivero-Gutiérrez B, Mascaraque C et al (2014) Food derived bioactive peptides and intestinal barrier function. Int J Mol Sci 15:22857–22873

Micklefield GH, Redeker Y, Meister V et al (1989) Effects of ginger on gastroduodenal motility. Int J Clin Pharmacol Ther 37:341–346

Minelli EB, Benini A (2008) Relationship between number of bacteria and their probiotic effects. Microb Ecol Health Dis 20:1651–2235

Mosele JI, Macià A, Motilva MJ (2015) Metabolic and microbial modulation of the large intestine ecosystem by non-absorbed diet phenolic compounds: a review. Molecules 20:17429–17468

Mouli VP, Ananthakrishnan AN (2014) Review article: vitamin D and inflammatory bowel diseases. Aliment Pharmacol Ther 39:125–136

Muhammad NG, Anwarul HG (2005) Pharmacological basis for the medicinal use of ginger in gastrointestinal disorders. Dig Dis Sci 50 (10):1889–1897

Nakajima S, Iijima H, Egawa S et al (2011) Association of vitamin K deficiency with bone metabolism and clinical disease activity in inflammatory bowel disease. Nutrition 27:1023–1028

Nicoli MC, Anese M, Parpinel M (1999) Influence of processing on the antioxidant properties of fruits and vegetables. Trends Food Sci Technol 10:94–100

Nissen L, Chingwaru W, Sgorbati B et al (2009) Gut health promoting activity of new putative probiotic/protective *Lactobacillus* spp. strains: a functional study in the small intestinal cell model. Int J Food Microbiol 135:288–294

O'Sullivan GC, Kelly P, O'Halloran S et al (2005) An emerging therapy. Curr Pharm Design 11:3–10

Oelschlaeger TA (2010) Mechanisms of probiotic actions – a review. Int J Med Microbiol 300:57–62

Overall J, Bonney SA, Wilson M et al (2017) Metabolic effects of berries with structurally diverse anthocyanins. Int J Mol Sci 18:422. https://doi.org/10.3390/ijms18020422

Oz HS, Chen T, De Villiers WJS (2013) Green tea polyphenols and sulfasalazine have parallel anti-inflammatory properties in colitis models. Front Immunol 4:132. https://doi.org/10.3389/fimmu.2013.00132

Ozdal T, Sela DA, Xiao J et al (2016) The reciprocal interactions between polyphenols and gut microbiota and effects on bioaccessibility. Nutrients 8:78. https://doi.org/10.3390/nu8020078

Peterson J, Dwyer J, Adlercreutz H et al (2011) Dietary lignans: physiology and potential for cardiovascular disease risk reduction. Nutr Rev 68:571–603

Rachmilewitz D, Katakura K, Karmeli F et al (2004) Toll-like receptor 9 signaling mediates the anti-inflammatory effects of probiotics in murine experimental colitis. Gastroenterology 126:520–528

Ren W, Duan J, Yin J et al (2014) Dietary l-glutamine supplementation modulates microbial community and activates innate immunity in the mouse intestine. Amino Acids 46:2403–2413

Richman E, Rhodes JM (2013) Review article: evidence-based dietary advice for patients with inflammatory bowel disease. Aliment Pharmacol Ther 38:1156–1171

Rijkers GT, Bengmark S, Enck P et al (2010) Guidance for substantiating the evidence for beneficial effects of probiotics: current status and recommendations for future research. J Nutr 140:671S–676S

Roth S, Spalinger MR, Gottier C et al (2016) Bilberry-derived anthocyanins modulate cytokine expression in the intestine of patients with ulcerative colitis. PLoS One 11:e0154817

Sandborn WJ, Targan SR, Byers VS et al (2013) *Andrographis paniculata* extract (HMPL-004) for active ulcerative colitis. Am J Gastroenterol 108:90–98

Saxena A, Kaur K, Hegde S et al (2014) Dietary agents and phytochemicals in the prevention and treatment of experimental ulcerative colitis. J Tradit Complement Med 4:203–217

Sheil B, McCarthy J, O'Mahony L et al (2004) Is the mucosal route of administration essential for probiotic function? Subcutaneous administration is associated with attenuation of murine colitis and arthritis. Gut 53:694–700

Singla V, Pratap Mouli V et al (2014) Induction with NCB-02 (curcumin) enema for mild-to-moderate distal ulcerative colitis - a randomized, placebo-controlled, pilot study. J Crohns Colitis 8:208–214

Sirotkin AV, Harrath AH (2014) Phytoestrogens and their effects. Eur J Pharmacol 741:230–236

Stephen B (2010) The biochemistry, chemistry and physiology of the isoflavones in soybeans and their food products. Lymphat Res Biol 8 (1):89–98. https://doi.org/10.1089/lrb.2009.0030

Suskind DL, Wahbeh G, Burpee T et al (2013) Tolerability of curcumin in pediatric inflammatory bowel disease: a forced-dose titration study. J Pediatr Gastroenterol Nutr 56:277–279

Talukder MJR, Harada E (2007) Bovine lactoferrin protects lipopolysaccharide-induced diarrhea modulating nitric oxide and prostaglandin E_2 in mice. Can J Physiol Pharmacol 85(2):200–208

Talukder MJ, Takeuchi T, Harada E (2002) Transport of colostral macromolecules into the cerebrospinal fluid via plasma in newborn calves. J Dairy Sci 85(3):514–524

Talukder MJ, Takeuchi T, Harada E (2003) Characteristics of lactoferrin receptor in bovine intestine: higher binding activity to the epithelium overlying Peyer's patches. J Vet Med A 50(3):123–131

Talukder JR, Kekuda R, Saha P et al (2008a) Functional characterization, localization and molecular identification of rabbit intestinal N-amino acid transporter. Am J Phys 294(6):G1301–G1310

Talukder JR, Kekuda R, Saha P et al (2008b) Identification and characterization of rabbit small intestinal villus cell brush-border membrane Na-glutamine co-transporter. Am J Phys 295(1):G7–G15

Talukder JR, Griffin A, Jaima A et al (2014) Lactoferrin ameliorates prostaglandin E_2-mediated inhibition of Na+ −glucose cotransport in enterocytes. Can J Physiol Pharmacol 92(1):9–20

Talukder JR, Antara Jaima, Cameron Hill, et al. (2018) Capsaicin requires calcium to inhibit SNAT2 mediated methionine transport, causes apoptosis and DNA damage in enterocytes. J Funct Foods. Under Review. MS# JFF-D-17-01076

Tang T, Targan SR, Li ZS et al (2011) Randomised clinical trial: herbal extract HMPL-004 in active ulcerative colitis – a double-blind comparison with sustained release mesalazine. Aliment Pharmacol Ther 33:194–202

Tjonneland A, Overvad K, Bergmann MM et al (2009) Linoleic acid, a dietary n-6 polyunsaturated fatty acid, and the etiology of ulcerative colitis: a nested case-control study within a European prospective cohort study. Gut 58:1606–1611

Trois L, Cardoso EM, Miura E (2008) Use of probiotics in HIV-infected children: a randomized double-blind controlled study. J Trop Pediatr 54:19–24

Trumbo P, Schlicker S, Yates AA et al (2002) Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein and amino acids. J Am Diet Assoc 102(11):1621–1630

Uchiyama K, Nakamura M, Odahara S et al (2010) N-3 polyunsaturated fatty acid diet therapy for patients with inflammatory bowel disease. Inflamm Bowel Dis 16:1696–1707

Van Duynhoven J, Vaughan EE, Van Dorsten F et al (2013) Interactions of black tea polyphenols with human gut microbiota: implications for gut and cardiovascular health. Am J Clin Nutr 98(6):1631S–1641S

Vecchi Brumatti L, Marcuzzi A, Tricarico PM et al (2014) Curcumin and inflammatory bowel disease: potential and limits of innovative treatments. Molecules 19:21127–21153

Vitale DC, Piazza C, Melilli B et al (2013) Isoflavones: estrogenic activity, biological effect and bioavailability. Eur J Drug Metab Pharmacokinet 38:15–25

Wang B, Wu G, Zhou Z et al (2014) Glutamine and intestinal barrier function. Amino Acids 47:2143–2154

Wood PJ, Braaten JT, Scott FW et al (1994) Effect of dose and modification of viscous properties of oat gum on plasma glucose and insulin following an oral glucose load. Br J Nutr 72:731–743

Xu J, Liu X, Pan W et al (2017) Berberine protects against diet-induced obesity through regulating metabolic endotoxemia and gut hormone levels. Mol Med Rep 15:2765–2787

Yao TC, Chang CJ, Hsu YH et al (2010) Probiotics for allergic diseases: realities and myths. Pediatr. Allerg Immunol 21(6):900–919

Zhang H, Hu CA, Kovacs-Nolan J et al (2015a) Bioactive dietary peptides and amino acids in inflammatory bowel disease. Amino Acids 47:2127–2141

Zhang X, Zhao Y, Xu J et al (2015b) Modulation of gut microbiota by berberine and metformin during the treatment of high-fat diet-induced obesity in rats. Sci Rep 5:14405. https://doi.org/10.1038/srep14405

Zoran D (2003) Nutritional management of gastrointestinal disease. Clin Tech Small Anim Pract 18(4):211–217

Zou Y, Xiang Q, Wang J et al (2016) Oregano essential oil improves intestinal morphology and expression of tight junction proteins associated with modulation of selected intestinal bacteria and immune status in a pig model. Bio Med Res Int:5436738. https://doi.org/10.1155/2016/5436738

Nutraceuticals in Reproductive Disorders

Moges Woldemeskel

Abstract

Reproductive disorders or abnormalities that occur in male and female reproductive tracts are induced by infectious and noninfectious agents and various other conditions. In the contemporary world, common use of synthetic chemicals in cosmetics and other items for domestic day-to-day uses undoubtedly contributes to the noninfectious causes of reproductive abnormalities. In both animals and humans, modern drugs are used to treat reproductive disorders. However, unwanted side effects that follow often pose severe problems and concerns, consequently increasing global use of natural products including nutraceuticals to treat reproductive abnormalities. In this chapter, the use of nutraceuticals in the treatment and management of female and male reproductive tract abnormalities is briefly highlighted.

Keywords

Nutraceuticals · Veterinary nutraceuticals · Reproductive disorders

1 Introduction

Since the ancient time, plant products and their crude extracts are used in traditional medicine for the treatment of various disorders until the development of synthetic drugs (Patel and Patel 2017). Despite development of modern synthetic drugs, medicinal plants still serve as mainstream therapeutics and are central in traditional medicine (Akhtar et al. 2017) in many parts of the world. More than 40% of prescription drugs in the world were mainly derived from herbal source (Patel and Patel 2017). Although modern medicine is at the forefront in treating diseases, plant-based remedies in alternative medicine are still highly used throughout the world, mainly in the developing countries (Fadus et al. 2017). Nowadays, medicinal plants play a key role in health care as more than 80% of the world's populations rely on the traditional medicine for their primary health care (Patel and Patel 2017). This is largely because natural products are effective and relatively nontoxic and have remedial doses far below their toxic levels (Fadus et al. 2017).

Nutraceuticals are natural products derived from various food sources that provide health benefits in addition to nutritional values (Kalra 2003; Larussa et al. 2017). Nutraceuticals can be substances such as vitamins, minerals, amino acids, fatty acids, herbs, and botanical products or substances derived from other sources taken as dietary supplements including pyruvate, chondroitin sulfate, steroid hormone precursors, etc. (Chauhan et al. 2013).

Natural products or nutraceuticals have been shown to elicit antiaging, anticancer, and other health-enhancing effects. A key target of the effects of natural products may be the regulation of microRNA expression which results in cell death or prevents aging, diabetes, and cardiovascular and other diseases. Nutraceuticals have also been proposed to exert their effects on cancer stem cells by interacting with the expression of microRNAs (McCubrey et al. 2017).

Various nutraceuticals have been in use for centuries to heal different types of diseases in humans and animals including diseases that inflict male and female reproductive system. This chapter gives a brief account on some nutraceuticals used in the treatment of reproductive diseases in males and females.

2 Nutraceutical in Female Reproductive Abnormalities

Disorders caused by microorganisms, physical agents, and other etiologies are known to affect female reproductive system. However, due to environmental pollution, use of

M. Woldemeskel (✉)
Tifton Veterinary Diagnostic and Investigational Laboratory, Department of Pathology, College of Veterinary Medicine, University of Georgia, Tifton, GA, USA
e-mail: mwoldem@uga.edu

R. C. Gupta et al. (eds.), *Nutraceuticals in Veterinary Medicine*, https://doi.org/10.1007/978-3-030-04624-8_32

synthetic chemicals in cosmetics and other items for domestic day-to-day uses, and other risk factors, currently neoplasm (cancer) is the major disease that affects reproductive tracts in women and also in pet animals. Various forms of cancer therapeutics are available nowadays. However, all the currently available cancer therapeutic options are expensive, and none of them are safe. On the contrary, traditional plant-derived medicines or nutraceuticals are relatively safe and are used in the treatment of diseases of female reproductive tract. Some nutraceuticals effective in the treatment and management of female reproductive diseases mainly cancerous diseases are briefly highlighted below.

2.1 Effects of Nutraceuticals on Breast Cancer

Breast cancer is the second leading cause of death in women worldwide, and more than one million women are diagnosed with breast tumor (Sayeed and Ameen 2015; Mandal et al. 2017) with approximately 458,000 deaths each year (Sayeed and Ameen 2015). It is also the second most frequent cancer in women in the USA with about 246,660 new breast cancer cases and 40,450 breast cancer-related deaths estimated in 2016 (Siegel et al. 2016). Citing several authors, Mandal et al. (2017) reported that chronic inflammation is suggested to contribute to development and progression of breast cancers. Therefore, agents that inhibit chronic inflammation may be effective in the prevention and treatment of breast cancer. Several natural products, phytochemicals, and dietary agents with anti-inflammatory properties have shown promise in the prevention and treatment of breast cancer (Mandal et al. 2017).

Distinct subtypes of breast tumor would response differently to treatment, which made breast cancer extremely intractable. Currently, surgical resection, adjuvant chemotherapy, radiotherapy, and hormone therapy represent the main treatment options for early-stage breast cancer. However, the development of drug resistance and major side effects have weakened the efficacy of these therapies. Besides, triple-negative breast cancer does not respond to hormone therapy. Due to poor efficacy of modern therapies, prevention of the occurrence of breast cancer appears to be the best strategy for which diet- and nutrition-based approach has been considered as an effective method. A group of dietary natural products have shown a potential role in prevention and treatment of cancers (Li et al. 2017).

A recent review summarized the potential role of dietary natural products and their major bioactive components in prevention and treatment of breast cancer. Several epidemiological and experimental studies suggested the inverse correlation between intake of vegetables and fruits and incidence of breast cancer. Many dietary natural products such as soy, pomegranate, mangosteen, citrus fruits, apple, grape, mango, cruciferous vegetables, ginger, garlic, black cumin, edible macro-fungi, and cereals could affect the development and progression of breast cancer. Experimental studies indicated that their anti-breast cancer effects involve various mechanisms of action such as downregulating estrogen receptor α expression and activity; inhibiting proliferation, migration, metastasis, and angiogenesis of breast tumor cells; inducing apoptosis and cell cycle arrest; and sensitizing breast tumor cells to radiotherapy and chemotherapy. Epidemiological studies suggested that high consumption of some dietary natural products might reduce the recurrence and increase the survival rate of breast cancer patients. Therefore, use of natural dietary substances could be a practical approach for the prevention and treatment of breast cancer (Li et al. 2017). One widely known such compound is beta-sitosterol, a plant-derived nutrient with anticancer properties against breast cancer, prostate cancer, colon cancer, lung cancer, stomach cancer, ovarian cancer, and leukemia (Sayeed and Ameen 2015). Other natural products and plant extracts with similar effects on breast cancer are given below.

Berberine is a natural isoquinoline alkaloid extracted from plants such as *Berberis (B) aquifolium*, *B. vulgaris*, *B. aristata*, and *Tinospora cordifolia* (Imanshahidi and Hosseinzadeh 2008). The antitumor effects of berberine in various human cancer cells have been reported. Its effect was evaluated on triple-negative breast cancer (TNBC), a cancer defined by negative expression of estrogen receptor, progesterone receptor, and human epidermal growth factor receptor 2. Because of lack of standard molecular targets, the current viable targeted therapies are not useful for TNBC patients. TNBC is insensitive to the antihormone receptor (HR) and HER2-targeted drugs and has higher incidence than HR- and HER2-positive patients. Berberine inhibited tumor cell proliferation and migration and induced cellular apoptosis. It activates caspase-9/cytochrome c-mediated apoptosis to inhibit the growth of TNBC breast cancer cells in vitro and in vivo. Therefore, berberine is a potential treatment agent for TNBC (Zhao et al. 2017). Furthermore, it was reported that berberine can bind to the vasodilator-stimulated protein overexpressed in breast cancer cells and suppress tumor proliferation and growth (McCubrey et al. 2017).

Resveratrol, a polyphenol found in various plants, is also reported as one of the agents effective against breast cancer. Experimentally, it inhibited the proliferation of cancer stem cells isolated from breast cancer cell lines. Resveratrol reduced the presence of mammospheres in breast cancer cell lines, inhibited tumor formation of the mammospheres in mice, and also induced autophagy in the breast mammospheres (Fu et al. 2014).

Soy isoflavones have also been reported by many studies to have a protective role against breast cancer, though some adverse effects have also been reported. Whether soy isoflavones promote or inhibit the growth of breast cancer

seemed to be dose dependent (Xie et al. 2013); thus the dosage and long-term safety of soy isoflavones need to be further investigated before they are recommended as supplement for breast cancer patients (Li et al. 2017).

Curcumin, a substance in turmeric, has been reported to exert antitumor development effects on breast cancer. It is shown to have effects on drug transporter expression in breast cancer cells with cancer stem cell properties. Curcumin could increase the tumoricidal effects of mitomycin-C, and it could also increase the sensitivity of breast cancer cell lines to various chemotherapeutic drugs such as cisplatin, doxorubicin, and paclitaxel. The combination of curcumin and mitomycin-C together prevented the sphere-forming capacity of breast cancer cell lines (Zhou et al. 2015).

Li et al. (2017) citing various studies indicated that fruits that normally contain high content of polyphenols have great antioxidant activity and may help reduce risk of cancer. Fruits, such as pomegranate, mangosteen, and citrus fruits, have shown inhibitory activities on breast cancer cells. For example, pomegranate inhibited breast cancer growth and metastasis through various mechanisms such as inhibiting breast cancer cell growth by inducing cell cycle arrest, inducing apoptosis, exerting antiproliferative effects on rat mammary tumorigenesis, and significantly decreasing cell viability of breast cancer cell lines. Pomegranate juice and its components blocked metastatic processes of breast cancer cells and proliferation of breast cancer cell lines in vitro indicating a potential role of pomegranate for the prevention of estrogen-responsive breast cancers. Whole pomegranate seed oil and fermented pomegranate juice polyphenols inhibited the tumor lesion formation in a murine mammary gland organ culture suggesting a chemopreventive property and adjuvant therapeutic potential of pomegranate. Pomegranate seed oil and fermented juice concentrate suppressed preneoplastic mammary gland lesions ex vivo in a mouse mammary organ culture model, and oral administration of pomegranate juice concentrate reduced the volume and weight of xenografted tumors in female athymic nude mice. Pomegranate emulsion containing most bioactive phytochemicals present in the whole fruit exerted a significant chemopreventive activity against DMBA-initiated mammary tumorigenesis in female rats. It reduced the incidence, total burden, and average weight of mammary tumors with a concomitant inhibition of intratumor cell proliferation and induction of apoptosis (Mehta and Lansky 2004; Banerjee et al. 2012; Li et al. 2017; Mandal et al. 2017).

Several in vitro and in vivo studies reported anti-breast cancer activities of mangosteen (*Garcinia mangostana*). Crude methanolic extract of mangosteen pericarp inhibits proliferation and induces apoptosis on breast cancer cell line; phenolics from the fruit pericarp produced cytotoxicities against human breast cancer cell lines; and alpha-mangostin treatment significantly increased survival rate of mice with mammary tumors and greatly suppressed tumor volume and lymph node metastases. Furthermore, studies report that citrus fruits such as orange, lemon, grapefruit, pomelo, and lime have an impact on overall health of a patient including reducing the risk of developing various cancers. A number of studies pointed out an inverse association between citrus fruits intake and the risk of breast cancer. For example, polysaccharides from Korean citrus hallabong peels inhibited angiogenesis, and extracts from a citrus fruit Citrus hassaku Hort. ex Tanaka, also known as phalsak, and lemon citrus extract induced apoptosis in breast cancer stem cell lines. Furthermore, extracts of apples, grape, mango, strawberries, peach, and other citrus fruits are reported to have effect on breast cancer cells in vivo and in vitro through inducing apoptosis, inhibiting cell proliferation, prevention of breast cancer cell growth, decreasing tumor volume, and activation of oxidative stress in cancer cells. Generally, intake of fruits is beneficial for the prevention and treatment of breast cancer, pomegranate, mangosteen, apple, citrus fruits, grape, and mango showing the most promising effects. The anti-breast cancer action of these fruits might be attributed to the presence of bioactive components such as ellagitannins in pomegranate and mangostin in mangosteen (Li et al. 2017).

Vegetables also are reported to have effect on breast cancers. In experimental studies, several vegetables, especially cruciferous vegetables, have shown inhibitory effect on breast cancer cells. For example, intake of broccoli, cauliflower, watercress, and Brussels sprouts was inversely associated with risk of breast cancer (Bradlow 2008; Thomson et al. 2016). Indole-3-carbinol found in *Brassica* vegetables showed anti-breast cancer action since it could interact directly with the estrogen receptor and inhibit its activity or through estrogen-independent actions, such as blocking cell cycle progression, metastasis, and inducing apoptosis (Li et al. 2017). Daily intake of fresh carrot juice might also benefit patients with breast cancer (Butalla et al. 2012). Generally, isothiocyanates and indole-3-carbinol and its metabolite 3,30-diindolylmethane found in cruciferous vegetables have shown a potential role in the prevention and treatment of breast cancer. The underlying mechanisms mainly include downregulating estrogen receptor alpha and repressing estrogen receptor signaling, inducing apoptosis and cell cycle arrest, and inhibiting metastasis of breast cancer cells (Li et al. 2017).

Consumption of certain spices is also known to have anti-breast cancer effect. Several studies suggested that ginger, garlic, and black cumin have shown promising anti-breast cancer effects among various spices. Bioactive components in spices, such as gingerols and shogaols in ginger, and organosulfur substances including diallyl disulfide, diallyl trisulfide, *S*-allyl mercaptocysteine, and allicin in garlic have anti-breast cancer activities and help in reducing risks of developing breast cancer. Methanolic extract of ginger

(*Zingiber officinale*) exhibited inhibitory effect on cancer cell proliferation in breast cancer cell lines. The anti-breast cancer property of ginger might be attributed to its bioactive constituents such as gingerols and shogaols (Lee et al. 2008; Joo et al. 2016). A supercritical CO_2 extract of black cumin (*Nigella sativa*) exhibited pro-apoptotic and anti-metastatic effect on breast cancer cell lines in vitro. Thymoquinone, a major bioactive component isolated from the seeds of *Nigella sativa*, has shown potent chemopreventive and chemotherapeutic activities and antiproliferative effect on breast cancer (Schneider-Stock et al. 2014; Li et al. 2017).

Other spices such as capsaicin, piperine isolated from black pepper (*Piper nigrum*), saffron (*Crocus sativus*), clove (*Syzygium aromaticum*), wasabi (*Wasabia japonica*), rosemary (*Rosmarinus officinalis*), and coriander are reported to have anti-breast cancer activities in vitro and in vivo experimental models. However, it should be noted that the role of capsaicin in cancer is controversial. Some studies have indicated that capsaicin itself was mutagenic and promote tumor formation and might increase the cancer risk in humans (Li et al. 2017), warranting more detailed studies to determine its real impact and benefit in breast cancer.

Several kinds of edible macro-fungi have shown inhibitory effect on breast cancer. Mushroom intake might be inversely associated with risk of breast cancer. A significant inverse association between mushroom consumption and breast cancer incidence was found in postmenopausal women in Korea. Several mechanisms such as inhibiting proliferation, inducing apoptosis, and suppressing angiogenesis have been found to explain the anti-breast cancer effects of edible macro-fungi. The anti-breast cancer effects of edible macro-fungi are mainly attributed to the polysaccharides with different molecular weights present in the fungi. A number of studies also indicated an inverse association between cereal fiber intake and breast cancer risk. For example, *sorghum* suppressed tumor growth, induced cell cycle arrest, and inhibited metastasis in nude mice bearing breast cancer xenografts, young barley (the grass of the barley plant) exhibited significant antiproliferative and pro-apoptotic activities in rat breast tumor model and in human breast cancer cells in vitro, and wheat (*Triticum aestivum*) flour inhibited growth of breast cancer cells and induced apoptosis in vitro. Sorghum, barley, and wheat have shown the potential to inhibit growth of breast cancer cells, mainly through inducing apoptosis and cell cycle arrest and inhibiting metastasis (Hong et al. 2008; Li et al. 2014, 2017).

In addition to their direct effect on cancers, dietary natural products have synergistic effect with anticancer therapies. This is very important because chemotherapy and radiotherapy frequently used nowadays in cancer therapy exhibit certain toxic adverse effects, and drug resistance is a common cause of chemotherapy failure and disease recurrence. Some dietary products and their bioactive components have shown synergistic effects with chemotherapy or radiotherapy by enhancing their therapeutic effect or reducing the adverse side effects (Li et al. 2017).

Certain vitamins and minerals are also beneficial in reducing risk of developing various cancers including breast cancer. For instance, experimental studies suggest a protective effect of B vitamins on breast cancer risk. A large prospective study also suggested dietary, supplemental, and total pyridoxine and thiamin intakes were associated with decreased breast cancer risk and have a potential protective effect against breast cancer in middle-aged women (Egnell et al. 2017). Moreover, Tsuji et al. (2015) citing various experimental studies in animal models indicated that selenium deficiency impairs immune response to infection, cancer, and other stimuli. These included reduction in CD4+ T-cell response in selenium-deficient mice challenged with a peptide/adjuvant and increased tumor growth and spread in a mouse model of breast cancer indicating that selenium may play an important role in the prevention of breast cancer development.

2.2 Effects of Nutraceuticals on Cervical Cancer

A number of diseases can affect the cervix in women. However, cancer is the major disease condition affecting the cervix and is globally among important causes of mortality in women. Several natural products (nutraceuticals) are reported to be effective in treating and/or reducing risk of cervical cancer in women. For example, McCubrey et al. (2017) citing several studies indicated that berberine and curcumin may be promising agents for treatment of cervical cancer. In a study on cancer cell lines, it was reported that *Aloe vera* alone or its simultaneous use with cisplatin exhibited antineoplastic effects in breast and cervical cancers by inducing apoptosis and modulation of expression of effector molecules. These results signify that *Aloe vera* may be an effective antineoplastic agent to inhibit cancer cell growth and increase the therapeutic efficacy of conventional drugs like cisplatin promoting the development of plant-derived therapeutic agents for novel cancer treatment strategies (Hussain et al. 2015). Due to the effect of various nutraceuticals on inhibiting tumor growth and reducing risk of cancer development in general, consumption of natural plant products and other nutraceuticals undoubtedly will help in minimizing the risk of developing cervical cancer.

2.3 Effect of Nutraceuticals on Diseases of Uterus

Different types of diseases including infectious diseases and neoplasms may affect uterus in females. A number of modern

medicinal therapies have been in use to treat uterine diseases. However, nutraceuticals, as was described earlier, are gaining increased preference due to side effects that follow treatment with modern drugs and well-documented efficacy of nutraceuticals in fighting various infections and diseases. Nutraceuticals that promote overall health of individuals with anti-inflammatory and anticancer effects described earlier certainly have impact on the treatment and prevention of uterine diseases. For example, berberine was reported to inhibit proliferation of human uterine leiomyoma cells (Wu et al. 2015). Some nutraceuticals have a synergetic effect, work in concert with various modern drugs, and may enhance the effect of modern medicines on cancer cells. Berberine and metformin induced lipolysis-stimulated lipoprotein receptor, which is a component of tricellular tight junctions in endometrial cancer cells and suppressed cell migration and invasion (McCubrey et al. 2017).

Endometriosis is a common gynecological disease that affects women and other primate females. Treatment options for endometriosis include surgery and medication, mainly pain medications such as nonsteroidal anti-inflammatory agents or hormone therapy including gonadotropin-releasing hormone agonists and androgens. Although such treatments can improve some symptoms, there is no cure for endometriosis. Trials have been conducted to determine effects of some natural products on endometriosis. It was reported that *Pueraria* flower extract suppressed adhesion of human endometriotic cells to human mesothelial cells and significantly inhibited the migration of endometriotic cells. It also significantly suppressed endometriotic lesion formation in a mouse model suggesting that *Pueraria* flower is an anti-endometriotic agent for the inhibition of endometriotic cell adhesion and endometriotic cell migration and establishment of endometriosis-like lesions and has a potential use in the treatment and prevention of endometriosis (Kim et al. 2017).

2.4 Effect of Nutraceuticals on Ovarian Cancer

Ovarian cancer is the most lethal gynecological malignancy in developed countries. This is due to the lack of specific symptoms that hinder early diagnosis and to the high relapse rate after treatment with radical surgery and chemotherapy (Áyen et al. 2018). A number of studies reported effects of different nutraceuticals on ovarian cancer. Berberine suppressed proliferation of ovarian cancer cells, and in concert with cisplatin, it induced death of ovarian cancer cells and augmented the effects of cisplatin in inducing cell cycle arrest in ovarian carcinoma cells (McCubrey et al. 2017). Resveratrol was also found to kill ovarian cancer stem cells. Further studies reported that bitter melon extracts contain compounds that inhibit growth of many types of cancers including growth of ovarian cancer and ameliorate resistance to common chemotherapeutic compounds (Yung et al. 2016). Bitter melon extracts inhibited ovarian cancer growth via activation of various signaling cascades. Alpha-momorcharin, a ribosome-inactivating protein present in bitter melon extracts, may in part be responsible for the anticancer effects (McCubrey et al. 2017). Dietary calcium was significantly associated with a reduced risk of ovarian cancer, and increased dietary calcium intake might be inversely correlated with the risk of ovarian cancer (Song et al. 2017). Briefly, large numbers of natural products that have significant effect on overall health of animals and humans are beneficial in minimizing risk of developing ovarian cancer and in the management of clinical disease.

2.5 Effects of Nutraceuticals on Other Disorders of Female Reproductive System

In different parts of the world, natural products, mainly of plant origin, are used in the treatment and management of various conditions and symptoms associated with female reproductive system. For example, in India, the bark of *Adenium obesum* is used as an abortifacient agent, and in Omani ethnic community, it is used for the treatment of venereal diseases, among many others (Akhtar et al. 2017).

Some nutraceuticals are also used in the treatment of a number of reproductive tract-associated ailments in females. For instance, *Agnus castus* has also been used in the treatment of many female conditions including menstrual disorders such as amenorrhea and dysmenorrhea, premenstrual dysphoric disorder, corpus luteum insufficiency, hyperprolactinemia, infertility, acne, disrupted lactation, cyclic breast pain, cyclical mastalgia, and inflammatory conditions. It was further indicated that *Agnus castus* and magnolia, combined with soy isoflavones plus lactobacilli, were effectively and safely used in the treatment of vasomotor symptoms in postmenopausal women, mainly with poor quality of sleep. Such alternative treatments are preferred to hormone replacement therapy due to adverse side effects, poor compliance, and fear of increasing the risk of cancer or weight gain associated with hormone therapy (De Franciscis et al. 2017).

Many studies indicated that combined supplementation with vitamin E and selenium, given a few weeks before calving, either by injection or orally can significantly reduce the incidence of retained placenta in cattle. It was also indicated that selenium as sodium selenite injected 3 weeks prepartum, with or without oral vitamin E, reduced incidence of metritis from 83 to 57%. Furthermore, an injection of 50 mg selenium (sodium selenite) 3 weeks before parturition

reduced the incidence of cystic ovaries from 47 to 19% (Hemingway 2003).

Nutraceuticals such as garlic that are effective against bacteria (Zardast et al. 2016) and other plant products including *Balanites aegyptiaca* and *Capparis erythrocarpos* known to be effective against fungi (Anywar et al. 2014) may help in the management and treatment of bacterial and mycotic reproductive tract infections.

3 Nutraceutical in Male Reproductive Abnormalities/Diseases

Abnormalities in male reproductive system can be induced by infectious organisms, exposure to environmental toxins, and individual's lifestyle among others. Male reproductive disorders such as sexual dysfunction, infertility, cryptorchidism, hypospadias, and testicular cancer are diseases of much concern currently (Bonde 2010). Among many abnormalities and diseases of the male reproductive system, prostate cancer is the major problem. Benign prostatic hyperplasia also affects large numbers of men above 50 years of age. It was reported that unhealthy dietary pattern was associated with increased risk of prostate cancer, while a healthy dietary pattern was associated with decreased risk of prostate cancer (Bagheri et al. 2018). This section will highlight on the use of some nutraceuticals on disorders and diseases of the prostate gland.

3.1 Effects of Nutraceuticals on Diseases of Prostate Gland

Plant-based healthy diet and lifestyles are reported to play an important role in reducing risks of developing prostate diseases and cancer in men. A number of trials have been made to look into the effect of nutritional plants and plant derivatives on prostate cancer. According to the World Health Organization, one-third of all cancer deaths are preventable through an increased consumption of natural compounds capable of modulating key molecular signaling cascades that ultimately inhibit cancer cell proliferation and induce apoptosis. A number of dietary phytochemicals including curcumin, ursolic acid, epigallocatechin-3-gallate, resveratrol, sulforaphane, and 6-shogaol have shown potential chemopreventive effects in vitro and in vivo in either animal models or in clinical studies on several cancers, including prostate cancer. Combination of ursolic acid, curcumin, and resveratrol administered to mice allograft model of prostate cancer through the diet, either alone or in combination, produced synergistic effects on tumor size and weight, indicating that synergistic combinations of natural compounds may be important for cancer therapeutic interventions (Lodi et al. 2017). Curcumin has been shown to have some effects on signaling pathways implicated in prostate cancer. It has effect on cancer-associated fibroblasts, which are important in regulation of epithelial to mesenchymal transition in tumor cells and in the induction of stem cell characteristics (McCubrey et al. 2017).

Other plant derivative compounds such as beta-sitosterol are reported to have anticancer properties against prostate cancer among many others (Sayeed and Ameen 2015). Berberine also decreased prostate cancer growth and cellular testosterone formation. It suppressed migration and invasion of prostate cancer cells and decreased expression of certain mesenchymal genes such as bone morphogenetic protein-7, nodal growth differentiation factor, and SNAIL, normally associated with shorter survival of prostate cancer patients. Moreover, berberine inhibited hypoxia-inducible factor-1 alpha and vascular endothelial growth factor expression in prostate cancer cells and increased their radiosensitivity in vitro and in vivo. Berberine also targets epidermal growth factor receptor signaling to suppress proliferation of prostate cancer cells in vitro and was shown to increase induction of apoptosis (McCubrey et al. 2017).

Other plants such as pomegranate fruit has been gaining a widespread reputation as a dietary supplement as well as a functional food due to emerging scientific evidence on potential health benefits, including prevention and treatment of erectile dysfunction in male subjects among many other effects. Pomegranate contains phytochemicals, including flavonoids (anthocyanins and catechins), flavonols (kaempferol and quercetin), flavones (apigenin and luteolin), conjugated fatty acids, hydrolyzable tannins, and related compounds, which are thought to be responsible for various biological and pharmacological activities including cancer preventive and therapeutic effects against many cancers including prostate cancer (Mandal et al. 2017).

Apart from prostate cancer, benign prostatic hyperplasia is known to commonly affect most men over the age 50. Various modern drugs are prescribed for benign prostatic hyperplasia. A cure for benign prostatic hyperplasia without side effects is not available so far. Due to adverse effects of the drugs, it is desirable to develop effective and long-term safety herbal medicines to inhibit the progress of benign prostatic hyperplasia (Chung et al. 2016). Pumpkin seed has long been used to treat micturition disorders and is found to substantially improve lower urinary tract symptoms associated with benign prostatic hyperplasia. The observed symptom relief is accompanied by a clinically significant improvement in International Prostate Symptom Score (IPSS)-related quality of life (Vahlensieck et al. 2015). Another study showed that garlic may have suppressing effects on benign prostatic hyperplasia. Its administration decreased relative prostate weight ratio and suppressed mRNA expression level of androgen receptor, dihydrotestosterone serum levels, and

growth of prostatic tissue in benign prostatic hyperplasia-induced rats. Garlic administration prevented progress of benign prostatic hyperplasia by regulating inflammatory responses through decreased levels of inflammatory proteins and apoptosis, which will be related to antioxidant properties of garlic and its components suggesting that garlic has a great potential as a treatment for benign prostatic hyperplasia (Chung et al. 2016).

Generally, many nutraceuticals with known antibacterial and antifungal properties such as garlic (Zardast et al. 2016) and potent antifungal and antibacterial nutraceutical plant species such as *Balanites aegyptiaca* and *Capparis erythrocarpos* (Anywar et al. 2014) will have potential to treat bacterial- and fungal-induced reproductive diseases in humans and animals.

4 Concluding Remarks and Future Directions

Reproductive disorders in males and females including infertility, infection-associated inflammation, cancer, and many other abnormalities are among common problems in humans and animals. Environmental pollution due to immense industrial developments, use of pesticides in profit-oriented modern agriculture, and chemicals in various household uses in humans and animals have immensely contributed to reproductive disorders mainly in industrialized and developing countries. In the present day, modern drugs are routinely prescribed to manage and treat reproductive abnormalities and other diseases. However, common occurrence of side effects of modern medical treatments has made the patients to resort to the use of traditional and alternative medicine, mainly nutraceuticals, to prevent or minimize the incidence of reproductive abnormalities and to treat clinical diseases. As a result, use of natural products including nutraceuticals to tackle with reproductive disorders has increased. Encouraging results supported by research undertakings and clinical trials are inspiring more hope in the future use of nutraceuticals for the treatment of various reproductive diseases in animals and humans.

References

Akhtar MS, Hossain MA, Said SA (2017) Isolation and characterization of antimicrobial compound from the stem-bark of the traditionally used medicinal plant, *Adenium obesum*. J Tradit Complement Med 7 (2017):296e300

Anywar G, Oryem-Origa H, Kamatenesi-Mugisha M (2014) Antibacterial and antifungal properties of some wild nutraceutical plant species from Nebbi District, Uganda. Br J Pharm Res 4 (14):1753–1761

Áyen Á, Jiménez Martínez Y, Marchal JA et al (2018) Recent progress in gene therapy for ovarian cancer. Int J Mol Sci 19(7):E1930. https://doi.org/10.3390/ijms19071930

Bagheri A, Nachvak SM, Rezaei M et al (2018) Dietary patterns and risk of prostate cancer: a factor analysis study in a sample of Iranian men. Health Promot Perspect 8(2):133–138

Banerjee N, Talcott S, Safe S et al (2012) Cytotoxicity of pomegranate polyphenolics in breast cancer cells in vitro and vivo: potential role of miRNA-27a and miRNA-155 in cell survival and inflammation. Breast Cancer Res Treat 136:21–34

Bonde JP (2010) Male reproductive organs are at risk from environmental Hazards. A review. Asian J Androl 12:152–156

Bradlow HL (2008) Indole-3-carbinol as a chemoprotective agent in breast and prostate cancer. In Vivo 22:441–445

Butalla AC, Crane TE, Patil B et al (2012) Effects of a carrot juice intervention on plasma carotenoids, oxidative stress, and inflammation in overweight breast cancer survivors. Nutr Cancer 64:331–341

Chauhan B, Kumar G, Kalam N et al (2013) Current concepts and prospects of herbal nutraceutical: a review. J Adv Pharm Technol Res 4:4–8

Chung KS, Shin SJ, Lee NY et al (2016) Anti-proliferation effects of garlic (Allium sativum L.) on the progression of benign prostatic hyperplasia. Phytother Res 30(7):1197–1203

De Franciscis P, Grauso F, Luisi A et al (2017) Adding *Agnus Castus* and Magnolia to soy isoflavones relieves sleep disturbances besides postmenopausal vasomotor symptoms long-term safety and effectiveness. Nutrients 9:129. https://doi.org/10.3390/nu9020129

Egnell M, Fassier P, Lécuyer L et al (2017) B-Vitamin intake from diet and supplements and breast cancer risk in middle-aged women: results from the prospective NutriNet-Santé cohort. Nutrients 9:488. https://doi.org/10.3390/nu9050488

Fadus MC, Lau C, Bikhchandani J et al (2017) Curcumin: an age-old anti-inflammatory and anti-neoplastic agent. J Tradit Complement Med 7(2017):339e346

Fu Y, Chang H, Peng X et al (2014) Resveratrol inhibits breast cancer stem-like cells and induces autophagy via suppressing Wnt/β-catenin signaling pathway. PLoS One 9:e102535. https://doi.org/10.1371/journal.pone.0102535

Hemingway RG (2003) The influences of dietary intakes and supplementation with selenium and vitamin-E on reproduction diseases and reproductive efficiency in cattle and sheep. Vet Res Commun 27 (2):159–174

Hong SA, Kim K, Nam SJ et al (2008) A case-control study on the dietary intake of mushrooms and breast cancer risk among Korean women. Int J Cancer 122:919–923

Hussain A, Sharma C, Khan S et al (2015) *Aloe vera* inhibits proliferation of human breast and cervical cancer cells and acts synergistically with cisplatin. Asian Pac J Cancer Prev 16(7):2939–2946

Imanshahidi M, Hosseinzadeh H (2008) Pharmacological and therapeutic effects of *Berberis vulgaris* and its active constituent, berberine. Phytother Res 22(8):999–1012

Joo JH, Hong SS, Cho YR et al (2016) 10-Gingerol inhibits proliferation and invasion of MDA-MB-231 breast cancer cells through suppression of Akt and p38(MAPK) activity. Oncol Rep 35:779–784

Kalra EK (2003) Nutraceutical-definition and introduction. AAPS PharmSci 5:E25. https://doi.org/10.1208/ps050325

Kim JH, Woo J-H, Kim HM et al (2017) Anti-endometriotic effects of Pueraria flower extract in human endometriotic cells and mice. Nutrients 9:212. https://doi.org/10.3390/nu9030212

Larussa T, Imeneo M, Luzza F (2017) Potential role of nutraceutical compounds in inflammatory bowel disease. World J Gastroenterol 23(14):2483–2492

Lee HS, Seo EY, Kang NE et al (2008) (6)-Gingerol inhibits metastasis of MDA-MB-231 human breast cancer cells. J Nutr Biochem 19:313–319

Li JY, Zou L, Chen W et al (2014) Dietary mushroom intake may reduce the risk of breast cancer: Evidence from a meta-analysis of observational studies. PLoS One 9:e93437

Li Y, Li S, Meng X et al (2017) Dietary natural products for prevention and treatment of breast cancer. Nutrients 9:728. https://doi.org/10.3390/nu9070728

Lodi A, Saha A, Lu X et al (2017) Combinatorial treatment with natural compounds in prostate cancer inhibits prostate tumor growth and leads to key modulations of cancer cell metabolism. NPJ Precis Oncol 1:18. https://doi.org/10.1038/s41698-017-0024-z

Mandal A, Bhatia D, Bishayee A (2017) Anti-inflammatory mechanism involved in pomegranate-mediated prevention of breast cancer: the role of NF-κB and Nrf2 signaling pathways. Nutrients 9:436. https://doi.org/10.3390/nu9050436

McCubrey JA, Lertpiriyapong K, Steelman LS et al (2017) Effects of resveratrol, curcumin, berberine and other nutraceuticals on aging, cancer development, cancer stem cells and microRNAs. Aging 9 (6):1477–1536

Mehta R, Lansky EP (2004) Breast cancer chemopreventive properties of pomegranate (*Punica granatum*) fruit extracts in a mouse mammary organ culture. Eur J Cancer Prev 13:345–348

Patel K, Patel DK (2017) Medicinal importance, pharmacological activities, and analytical aspects of hispidulin: a concise report. J Trad Complem Med 7(2017):360e366

Sayeed B, Ameen SS (2015) Beta-sitosterol: a promising but orphan nutraceutical to fight against cancer. Nutr Cancer 67(8):1214–1220

Schneider-Stock R, Fakhoury IH, Zaki AM et al (2014) Thymoquinone: fifty years of success in the battle against cancer models. Drug Discov Today 19:18–30

Siegel RL, Miller KD, Jemal A (2016) Cancer statistics, 2016. CA Cancer J Clin 66:7–30

Song X, Li Z, Ji X et al (2017) Review: calcium intake and the risk of ovarian cancer: a meta-analysis. Nutrients 9:679. https://doi.org/10.3390/nu9070679

Thomson CA, Ho E, Strom MB (2016) Chemopreventive properties of 3,30-diindolylmethane in breast cancer: evidence from experimental and human studies. Nutr Rev 74:432–443

Tsuji PA, Carlson BA, Anderson CB et al (2015) Dietary selenium levels affect selenoprotein expression and support the interferon- and IL-6 immune response pathways in mice. Nutrients 7:6529–6549. https://doi.org/10.3390/nu7085297

Vahlensieck W, Theurer C, Pfitzer E et al (2015) Effects of pumpkin seed in men with lower urinary tract symptoms due to benign prostatic hyperplasia in the one-year, randomized, placebo-controlled GRANU study. Urol Int 94(3):286–295

Wu HL, Chuang TY, Al-Hendy A et al (2015) Berberine inhibits the proliferation of human uterine leiomyoma cells. Fertil Steril 103:1098–1106

Xie Q, Chen ML, Qin Y et al (2013) Isoflavone consumption and risk of breast cancer: a dose-response meta-analysis of observational studies. Asia Pac J Clin Nutr 22:118–127

Yung MM, Ross FA, Hardie DG et al (2016) Bitter melon (*Momordica charantia*) extract inhibits tumorigenicity and overcomes cisplatin-resistance in ovarian cancer cells through targeting AMPK signaling cascade. Integr Cancer Ther 15:376–389

Zardast M, Namakin K, KahoJ E et al (2016) Assessment of antibacterial effect of garlic in patients infected with Helicobacter pylori: assessment of garlic's effect using urease breath test. Avicenna J Phytomed 6(5):495–501

Zhao Y, Jing Z, Lv J et al (2017) Berberine activates caspase-9/cytochrome c-mediated apoptosis to suppress triple-negative breast cancer cells *in vitro* and *in vivo*. Biomed Pharmacother 95:18–24

Zhou Q, Ye M, Lu Y et al (2015) Curcumin improves the tumoricidal effect of mitomycin C by suppressing ABCG2 expression in stem cell-like breast cancer cells. PLoS One 10:e0136694. https://doi.org/10.1371/journal.pone.0136694

Nutraceuticals in Genitourinary Maladies

Robert W. Coppock

Abstract

Nutraceuticals are used as prophylactics and remedies for genitourinary maladies in domestic animals and have been used throughout recorded history. They are also used to control and improve reproductive performance, improve the storability of semen and enhance ovum maturation, and improve in vitro fertilization. In the last decade, the effects and mechanisms of oxidative stress on reproductive performance and immune dysfunction have been elucidated. The antioxidative effects of certain nutraceuticals during periods of the reproductive cycle with high oxidative stress improve reproductive performance and reduce infections and other gentiourinary diseases. This chapter also reviews the current use of nutraceuticals to prevent and treat genitourinary diseases.

Keywords

Nutraceutical · Reproduction · Genitourinary · Bladder · Uterus · Ovum · Ovary · Testicle · Semen · Fish oil · Vegetable oil · Maca · *Lepidium* · Flax · *Linum* · Cohosh · Bulls · Stallions · Rams · Goats · Cows · Roosters · Hens · Dogs · Cats · Thyme · Fatty acids · Carnitine · Coconut · *Cocos* · Quercetin · Selenium · Vitamin E · Spirulina · *Persea* · Avocado · *Pyrus* · Pears · Goldenseal · *Poria* · Lipoic acid · Mannose · Methionine · Probiotics · Ethnoveterinary · Ferrets · Grape pomace · Algae · *Spirulina* · *Vitis* · Vitamin A · Carotene · Carotenoids · Mink · *Haematococcus* · YiShenJianPi · Arugula · *Eruca* · Cranberries · *Vaccinium* · Proanthocyanidins · Berberine · *Rubus* · Centaury · *Centaurium* · Lovage · *Levisticum* · Rosemary

R. W. Coppock (✉)
DVM, Toxicologist and Assoc. Ltd, Vegreville, AB, Canada
e-mail: r.coppock@toxicologist.ca

1 Introduction

Nutraceuticals can be defined as products isolated or purified from plants and animal products in formulations not usually associated with feed ingredients. The definition can also include functional feedstuffs and probiotics which are fed as part of an unusual diet. The functional ingredient is considered to go beyond basic nutritional needs and functions to reduce the risk of disease and have physiological benefits. Probiotics are live microorganisms that are administered orally and parenterally to improve health. There is an incorrect assumption that nutraceuticals cannot be harmful because they are a natural product produced in a plant or animal or have an antioxidative activity (Vrolijk et al. 2015). Antioxidants at pharmacological levels can cause pathophysiological interferences with the formation of reactive oxygen species (ROS) required for physiological functions. There is a common assumption that numerous nutraceuticals reduce the toxicity of in vivo free radicals. ROS are produced in the body as by-products of metabolism and for physiological functions. The ROS are reactive electron seekers and in excess can cause uncontrolled chain reactions leading to cell injury and death. Biochemically important ROS include superoxide anion ($O_2^{-\bullet}$), peroxyl ($ROO^{\bullet}$), alkoxyl ($RO^{\bullet}$), and hydroxyl radical ($OH^{\bullet}$) which is produced by the Fenton reaction (peroxide reaction with Fe^{2+}). Lipid-soluble hypochlorous acid (HOCl) is another ROS formed. Reactive nitrogen species can be formed including nitrogen dioxide ($^{\bullet}NO_2$) and nonradical peroxynitrite ($ONOO^{-}$). ROS can have important physiological functions. For example, immunocytes produce in vivo ROS (singlet oxygen, hypochlorous acid, etc.) to target and inactivate microbial pathogens. Homeostatic mechanisms provide for the destruction of ROS. When the production of ROS overwhelms the capacity for neutralization of ROS, the condition is known as oxidative stress. Oxidative stress and associated endoplasmic reticulum stress activate the nuclear factor kappa B (NF-κB) pathway. For example, there is molecular genetic evidence

R. C. Gupta et al. (eds.), *Nutraceuticals in Veterinary Medicine*, https://doi.org/10.1007/978-3-030-04624-8_33

that endoplasmic reticulum stress occurs during a negative energy balance in postpartum dairy cows (Gessner et al. 2014). Stress in the endoplasmic reticulum is linked to imbalance between incoming protein load and protein-folding capacity. The imbalance results in accumulation of unfolded or misfolded proteins in the lumen of the endoplasmic reticulum. The unfolded proteins' response triggers upregulation of endoplasmic reticulum chaperones. These include immunoglobulin heavy-chain binding protein that assists in refolding of proteins, attenuation of protein translation, and upregulation in degradation of misfolded proteins. If the stress in the endoplasmic reticulum cannot be corrected, cell death by apoptosis can occur. The endoplasmic reticulum stress transducers are activated causing a cascade of macromolecular reactions that result in pathophysiological and pathology observations, namely, oxidative stress, insulin tolerance, hepatic steatosis, and inflammation. Oxidative stress associated with oxylipid metabolism has recently been reviewed (Mavangira and Sordillo 2018). Oxylipids can contribute to oxidative stress or regulate specific sites of ROS production and can be biomarkers of oxidative stress. Detailed dietary management is important for reproductive success and controlling oxidative stresses that are associated with reproductive dysfunctions and diseases. For example, older mares with ovulation problems fed a proprietary feed that had balanced fats, carbohydrates, protein, fiber, vitamins, and minerals ovulated significantly sooner than mares fed oats (25 days vs. 39 days) (Jones 1997). Terrestrial animals hydrobionts exposed to anthropogenic pollutants have increased oxidative stress. There is substantial interest in the use of nutraceuticals to improve the genitourinary health of domestic animals.

2 Oxidative Stress in Dairy Cows

There is substantial interest in the use of nutraceuticals in the dietary management of dairy cows during the parturient and postpartum intervals. The critical period in the reproductive cycle of dairy cattle, especially in high production dairy cows, is the last 3 weeks of pregnancy (9-month gestation period) and the first 3 weeks of lactation. Dairy cows genetically selected for high milk production cannot consume enough feedstuffs to match the energy requirement for peak milk production. At calving time, the immune system of cattle also has decreased functionality. Dairy cows within a breed have a large variation in the physiological capacity to adapt to metabolic stress of lactation. Oxidative stress is linked to a decrease in immune function which increases the susceptibility of cattle, especially dairy cattle, to metritis and mastitis. A dairy cow has a dry period (cessation of milking 50–70 days before parturition) that is considered an interval of metabolic rest. Omitting the dry period increases serum or plasma levels of ceruloplasmin, cholesterol, and ROS and decreased levels of bilirubin and paraoxonase (Mayasari et al. 2017). In the last 2 weeks antepartum and the first 3 weeks postpartum, there is increased mobilization of nonesterified fatty acids from adipose tissues because of negative energy balance. This is linked to the increased risk for hepatic steatosis and ketosis. In the week before parturition, the odds of retained placenta increase by 5% with each 0.1 mMol increase in serum cholesterol or fatty acids (Quiroz-Rocha et al. 2009). There are scientific suggestions that cows with higher antioxidant activity have lower occurrences of retained placenta. Dairy cattle also have a period of physiological insulin resistance before calving and immediately after parturition (De Koster and Opsomer 2013). Prolonged insulin resistance is a pathophysiologic condition that has similarities to insulin resistance in humans. Oxidative stress is considered a causative factor for insulin resistance in cattle (Abuelo et al. 2015). The most common antioxidants used in dairy cows are vitamin E (α-tocopherol) and selenium (Se). Dry dairy cows on a diet that met or exceeded the NRC-USA nutrient recommendations received daily intramuscular injections of vitamin E and sodium selenate proprietary product[1] for approximately 15 days before parturition (Abuelo et al. 2016). Administering these antioxidants increased insulin sensitivity. Supplementing dairy cow ration with extracts[2] from grape seed and grape marc meal (1% of dry matter intake) reduced the hepatic mRNA abundance of hepatokine fibroblast growth factor 21 (Gessner et al. 2014). Hepatokine fibroblast growth factor 21 is associated with reduced hepatic steatosis and improved insulin sensitivity. Lipolysis was decreased and insulin-dependent glucose uptake was increased. The extract from grape offal did not change the antioxidative system and endoplasmic reticulum stress and did increase milk yield. Dairy cattle were supplemented with 175 mg of extracts[3] of green tea (95%) and curcuma (5%)/kg dietary dry matter from 3 weeks peripartum to week 9 postpartum (Winkler et al. 2015). Cows supplemented with the extracts had increased energy-corrected milk production and decreased hepatic cholesterol and triacylglycerols at week 1 and week 3 postpartum. There was a trend for decreased hepatic mRNA for haptoglobin and fibroblast growth factor 21.

[1] Selevit adultos, Laboratorios SYVA (Leon, Spain)

[2] AntaOx, Dr. Eckel (Niederzissen, Germany)

[3] Loxidan Dairy Cow B, Lohmann Animal Nutrition GmbH (Cuxhaven, Germany)

3 Melatonin and Seasonal Breeders

Melatonin (*N*-acetyl-5-methoxytryptamine) has applications for manipulating reproduction and use as an antioxidant. Phytomelatonin has been identified in plants, for example, *Thymus vulgaris* L., *Glycyrrhiza uralensis* Fisch., *Salvia officinalis* L., and *Hypericum perforatum* L. (Arnao and Hernandez-Ruiz 2018). Melatonin has a key role in the modulation of sexual activity in seasonal breeders (Saxena 2017). Melatonin modulates neuroendocrine functions, pregnancy endocrinology, fetal circulation, and timing of parturition. Melatonin also has a modulation role in immune functions. The four major interests in melatonin are its use to improve in vitro fertilization and to alter seasonality of breeding in domestic animals, fetal programing, and its use as an antioxidant (Kutzler 2015; Brockus et al. 2016; Mura et al. 2017; Saxena 2017; El Allali et al. 2018). Dietary melatonin at pharmacological levels increases both umbilical and fetal blood flow in sheep (Lemley and Vonnahme 2017). Ewes and rams in northern Sardinia received subcutaneous implants containing 18 mg melatonin in February to May, and fertility parameters were monitored (Mura et al. 2017). The ewes and rams treated in April and May had significantly improved fertility rate, and the ewes had a decreased time to pregnancy. Treatment of doe and buck rabbits (New Zealand White) with 3 mg melatonin orally/rabbit/day for 60 days was comparable to 14 or 16 h of light for improving reproductive parameters (Mousa-Balabel 2011). At 170 days of gestation, pregnant heifers bred with sex-sorted semen received 20 mg melatonin orally/head/day for the duration of pregnancy (Brockus et al. 2016). Treatment with melatonin during pregnancy increased postnatal growth in the F_1 heifers and likely is evidence of fetal programing. At 220 days of gestation, high-milking Holstein cows were administered melatonin implants[4] (18 mg melatonin/implant, subcutaneously) to give a total dose of 216 mg melatonin, and bred heifers (220 days of gestation) were administered 162 mg melatonin by subcutaneous implants (Garcia-Ispierto et al. 2013). The temperature humidity index during the study was 80 units. No differences between treated and control animal were observed for retained placenta and metritis. Odds ratio found that the likelihood of repeat breeders and subsequent pregnancy loss was lower in the treated animals. Treatment with melatonin significantly reduced prolactin levels but did not alter total milk production and milk somatic cell counts. Camels are seasonal breeders during short photoperiods and ovulation is mating-induced. Female dromedary camels were conditioned to artificial light of 16 hours daylight and 8 hours dark for 41 days and then moved outdoors (May in Morocco) and implanted with 18 mg melatonin subcutaneous implants[5] to give a dose of 0.64 mg melatonin/kg body weight (El Allali et al. 2018). The melatonin treatment advanced the onset of follicular growth by 3.5 months and suppressed plasma prolactin. Fertility parameters were not included in the study. The use of melatonin to suppress estrus in queens (female cats), a long-day breeder, has varying success (Kutzler 2015; Schafer-Somi 2017). Estrous suppression can last for 4 months. In male cats, melatonin subcutaneous implant (18 mg) reduced semen quality parameters.

4 Herbs

4.1 Raspberry (*Rubus idaeus* L.)

Raspberry is used in veterinary and human medicine as a nutraceutical. In humans, raspberry leaf preparations are used to prevent nausea associated with pregnancy, to increase uterine efficiency during labor and delivery, and to decrease the risk of postpartum hemorrhage (Holst et al. 2009). Simpson et al. (2001) reported that oral administration of raspberry leaf tablets to pregnant women tended to shorten the second stage of labor. Women were administered a 2.4 g raspberry leaf table twice a day or placebo starting at week 32 of pregnancy and continuing to delivery. No significant differences were observed for medical management of birthing, mode of birthing, labor pain management, birthing-associated blood loss, blood pressure (tendency to increase in treatment group), birth weight, Apgar score, meconium-stained amniotic fluid, infant special care needs, and gestation interval. The strength of uterine contractions was decreased in postpartum women after they were orally administered raspberry leaf extracts and intrauterine pressure subsequently measured (Holst et al. 2009). A toxicity study was done in rats on the safety of red raspberry leaf,[6] kaempferol,[4] and quercetin[7] (Johnson et al. 2009). The doses of red raspberry leaf, kaempferol, and quercetin were 10 mg/day/rat administered from gestation day zero to parturition. The dose was based on studies showing the pharmacology of kaempferol and quercetin. Fertility parameters were measured, and female offspring were monitored until puberty (vaginal opening). There were no differences between treatment and controls for litter size, birth weight, total litter weight, sex ratio, and survival to weaning. Treatment with red raspberry leaf significantly increased gestation period (22.4 $\pm$ 0.37 vs. 20.8 $\pm$ 0.63 days for controls), and 78% of dames treated with red raspberry leaf whelped. The time to vaginal opening of the F_1 females born to dams receiving red raspberry life was significantly decreased. The F_1 females born to dams receiving red raspberry leaf were

[4] Melovine®, CEVA Salud Animal (Barcelona, Spain)

[5] Melovin®, Ceva, Ceva Santé Animale (Libourne, France)

[6] Nature's Way, Canmore, Alberta, Canada

[7] Sigma Aldrich, Oakville, Ontario, Canada

bred, and fertility parameters were observed. There are no differences between treated and control for time to mating, mating success, pregnancy success, gestation period, live birth index, litter size, birth weight, total litter weight, sex ratio, or postnatal survival to post birth day 21. For kaempferol and quercetin, the only effect was the F_o females receiving quercetin had significantly increased weight gains during pregnancy. An ethnoveterinary study in British Columbia found that *Rubus idaeus* infusion (made with 250 mL boiling water and 150 mg leaves/kg body weight) is given orally once a day to dogs for increasing uterine tone and facilitate whelping (Lans et al. 2009). It is also used to support milk production.

4.2 Black Cohosh (*Actaea racemosa* L.) and Blue Cohosh (*Caulophyllum thalictroides*)

Black cohosh (squawroot or papooseroot) is a popular herb for women's health issues and has historical use by aboriginal peoples (Dietz et al. 2016). The primary use of the rhizomes and roots is for ameliorating the signs of menopause. In homeopathic medicine, blue and black cohosh formulations are used to establish labor (Smith 2010). There are conflicting reports in the literature on the efficacy of blue and black cohosh. There are reports for black cohosh being used as nutraceutical treatment of canine uterine infections (Lans et al. 2009).

4.3 Flax (*Linum usitatissimum* L.)

Flaxseed is used as a nutraceutical in veterinary medicine for improving semen resistance to freezing and thawing and to promote lactation in pets and cattle (Lans et al. 2009; Kumar and Bharati 2013; Kargar et al. 2017). It is also used to ameliorate the signs of menopause in women (Dietz et al. 2016). Flaxseeds are resistant to digestive actions, and thus it is recommended that seed be fractured before oral administration. They contain the secoisolariciresinol diglucoside lignans that are metabolized to estrogenic-like molecules. The secoisolariciresinol diglucosides, the primary lignans in flaxseed, are poorly absorbed and are metabolized by colonic microorganisms. The products of microbial fermentation of flaxseed meal are absorbed. There are concerns that high dietary intake of flax can result in hyperestrogenism of the fetus. A multigenerational study in rats using 20 or 40% of the diet as flaxseed or 13 or 26% of the diet as flax meal was done to investigate the effects of flaxseed on the F_1 generation (Sprando et al. 2000a, b). The females were exposed to the flaxseed diets from day 0 of pregnancy to weaning of the pups. At 21 days of age, randomly selected males were fed the same diet as the females for 70 days. For the F_1 males, no significant effects across treatment groups were observed in the testicular weights, epididymal weights, seminal vesicle weights, and testicular morphology. Also, for the F_1 males, no significant differences were observed for homogenization-resistant spermatid counts, daily sperm production rates, percentage of sperm abnormalities, levels of testosterone in the seminiferous tubular fluid, and testicular histopathology. Significant differences in endocrine parameters were observed in the F_1 males when flaxseed-treated rats were compared to control rats (no flaxseed). Serum luteinizing hormone was increased in the F_1 male rats receiving the 20 and 40% flaxseed diets, and serum testosterone and luteinizing hormone were increased in rats from the 26% flax meal group. Also, there were significant increases in caudal epididymal weights and increase caudal epididymal sperm numbers/g epididymis from the 20 and 40% flaxseed groups. In the 13% and 26% flax meal treatment groups, there were increased epididymal sperm numbers/g epididymis. Prostate weights were significantly decreased in the 20% flaxseed and 13 and 26% flax meal treatment groups. New Zealand White rabbits (8-month-old) were provided a diet supplemented with 5% extruded flaxseed for 5 weeks and semen parameters studied (Mourvaki et al. 2010). The high linolenic acid increased the dietary omega-3 (ω-3)/omega-6 (ω-6) (fatty ω-3/ω-6) fatty acid ratio by increasing the ω-3 fatty acids in spermatozoa and decreasing the ω-6 fatty acids. Spermatozoa from the treated bucks had increased membrane integrity and track speed. There was an increase in the phospholipids in the prostate granules. Flaxseed meal and ground flaxseed are considered efficacious in Uttar Pradesh, India, as a galactagogue in cattle (Kumar and Bharati 2013). The seed cake or crushed seeds are mixed with jaggery or gum of *Sterculia urens*. A study in Nili-Ravi buffalo bulls showed that supplementation of the diet with flaxseed oil significantly increased libido, semen volume, percent live sperm, spermatozoa numbers/mL of semen, mass activity, percent motility, and the integrity of spermatozoa membrane (Shah et al. 2016). The bulls were orally administered 0, 125, and 250 mL flaxseed oil for 12 weeks. Mating behavior was evaluated when semen was evaluated at day 0 and weekly starting on week 5. Flaxseed oil and vitamin E dietary supplement were found to improve the freeze-thaw semen evaluation parameters of cloned Bakhtiari goats (Kargar et al. 2017). Flaxseed oils have been shown to improve male and female fertility. Flaxseed oil has been shown to be a replacement dietary supplement for fish oil for improving the success of in vitro fertilization of ovum from dairy cows and for modifying the lipids and semen characteristics of rooster semen (Bongalhardo et al. 2009; Moallem et al. 2013). Flaxseed oil does not contain the bioconcentrated persistent organic pollutants associated with fish oil, an important issue in food safety and medicine.

4.4 Maca (*Lepidium meyenii* Walpers)

4.4.1 Background

Lepidium meyenii, also known as maca, is in the Brassicaceae family (Cruciferae, contain glucosinolates) and is cultivated in the Peruvian highlands. The hypocotyl is a fleshy root that is used internationally as a nutraceutical and locally consumed as a nutraceutical and as a root vegetable. Maca has traditionally been used to improve sexual function and fertility in domestic animals and humans (Clement et al. 2010, 2012; Lee et al. 2016; Beharry and Heinrich 2018). Maca is credited for offsetting the adverse effects of high altitude and harsh climate and thereby improving reproductive performance of domestic animals and humans (Leon 1964). A study in humans provides evidence that black and red maca decreases signs of chronic mountain sickness and improves mood and sexual desire (Gonzales-Arimborgo et al. 2016). A study in rats using a decoction of *Lepidium meyenii* showed that the decoction equivalent to 0.6 g of root/day administered for 21 days offset the effects of high altitude (4349 m) on epididymal spermatozoa numbers (Gonzales et al. 2004). Using a proprietary process, *Lepidium meyenii* roots were extracted with methanol, ethanol, or water, and the fractions were purified by chromatographic processes to concentrate macaene and macamide and other plant chemicals including campesterol, stigmasterol, and beta-sitosterol (Zheng et al. 2000). The extracts were standardized on macaene and macamide and novel polyunsaturated fatty acids and their amides. Male mice were orally administered mecca extract preparations at 40 mg/g body weight for 22 days. The number of sexual intromissions the male mice accomplished in 3 h with receptive females was recorded. For the maca extract treated mice, the intromissions increased 2.39 and 2.55 times, more than the control male mice. In castrated rats receiving oral doses of 45, 180, and 1800 mg of extract/kg body weight decreased the latent period between electrical stimulation and observed penile erection by 71 ± 12, 73 ± 12, and 41 ± 13 seconds, respectively, as compared to castrated untreated rats. Feeding rats a proprietary[8] hydrous ethanol extract of maca (*Lepidium meyenii*, different ecotypes of red, yellow, and black maca) at 2% of diet increased the testosterone production of cultured Leydig cells and alleviated decline with aging (Yoshida et al. 2018). Feeding this product also caused a transient increase in serum testosterone. There is limited data from controlled studies in humans (Lee et al. 2016). In healthy men, maca improved laboratory parameters for semen evaluation (motility, sperm counts, and morphology). In men classified with infertility, there was consistent improvement in sperm motility, and one study showed improvement in sperm numbers, motility, and vitality. Fertility was not reported. There are reports of maca being used to improve reproductive parameters in domestic animals.

[8] MACAXS, Towa Corporation KK (Tokyo, Japan)

4.4.2 Bulls

A Swiss study on *Lepidium meyenii* roots in peripubertal breeding bulls, using a crossover experimental design, showed that a 10-week supplementation increased spermatozoa numbers (Clement et al. 2010). A 1000 kg batch of hypocotyls (approximately 50% yellow color, 25% reddish color, and 25% black color) was milled to powder (0.8 mm particle size), and the powder was mixed into a basal concentration. Seventy-eight bulls, 55–84 weeks of age from five different breeds, kept at a commercial bull stud, were, at by-weekly intervals, ejaculated by established semen collection procedures. The bulls were fed no maca for 10 weeks, maca 233 mg powder/kg body weight/day for 10 weeks, or maca for the last 10 weeks of the study. Maca supplementation did not affect testes circumference, mating behavior, and semen volume. Supplementing the diet for 10 weeks increased spermatozoa numbers in the second 10 weeks when no additional maca supplement was provided. Other parameters improved in originally marginal bulls were DNA fragmentation index and motility.

4.4.3 Stallions

Yellow maca, grown in the highlands of Peru, has been studied in stallions used at a commercial stud farm (Del Prete et al. 2018). The yellow strain of *Lepidium meyenii* hypocotyls were traditionally dried and milled (0.8 mm) into flour. The treated stallions received 3 g of maca flour/kg body weight for 60 days (spermatogenic cycle is 57 days). Treatment with maca did not change the levels of serum testosterone and ejaculate volume. From day 60 (end of maca treatment) until 120 (end of study), the concentration of spermatozoa and total sperm counts were significantly increased (doubled) in semen from the maca-treated horses. Treatment with maca significantly improved acrosome integrity at study days 60, 90, and 120. At study day 120, DNA fragmentation was significantly reduced in semen from the treated stallions. Semen was evaluated after ejaculation and after processing for cooled shipping at 24, 48, and 72 h of storage. During cooling and storage, the total motility, progressive motility, and acrosomal integrity declined slower in semen from maca-treated horses.

5 Dietary Lipids

5.1 Background

Lipids are used as nutraceuticals to improve reproductive performance. Lipids in cell membranes have a temperature transitional point where they change from liquid-like

properties to crystalline-like with rigid properties. Having a transition point at a lower temperature is a factor in survivability of spermatozoa at rethawing. Increasing the *cis*-double bonds, shorter hydrocarbons, and increased cholesterol in the membrane lipids decrease the temperature at which transition point occurs. Polyunsaturated acids are divided into ω-3, ω-6, and ω-9 fatty acids. These fatty acids have the first double bond located at 3, 6, or 9 carbons, respectively, from the methyl end of the fatty acid molecule. Mammals cannot synthesize the ω-3 and ω-6 fatty acids making them essential dietary fatty acids. The ω-6 linoleic acid and α-linolenic acid (a ω-3 fatty acid) must be provided in the diet, and eicosapentaenoic acid, docosahexaenoic acid, and arachidonic acid are synthesized from these essential precursors (Gulliver et al. 2012). The ω-6 linoleic acid is most abundant in vegetable oils. The enzymatic pathways for the polyunsaturated fatty acids in eicosanoids for reproduction have been reviewed (Wathes et al. 2007). The polyunsaturated fatty acids are precursors for eicosanoids which include the prostaglandins. In mated mammalian females, the critical period for pregnancy is the interval from insemination to implantation of the embryo. Lipid catabolism by the embryo before implantation is an important source of energy. Polyunsaturated fatty acids are also important in membrane fluidity of spermatozoa. Oversupplementation with polyunsaturated fatty acids has a risk of being harmful. The testicles contain arachidonic acid, docosapentaenoic acid, and docosahexaenoic acid with the ratios varying between species. Adequate dietary polyunsaturated fatty acids are important for fertility (Gulliver et al. 2012). Resistance of spermatozoa to cold preservation is considered linked to the levels of ω-3 polyunsaturated fatty acids (e.g., linolenic, eicosapentaenoic, and docosahexaenoic acids) in their cell membranes. An Austrian study in Shetland pony stallions showed that ω-6-docosapentaenoic acid was the predominant polyunsaturated acid in equine spermatozoa (Aurich et al. 2018). This study also showed a January to June increase in the relative content of docosapentaenoic acid and an increase in ω-6-arachidonic acid. The ratio of ω-3/ω-6 polyunsaturated fatty acids in stallion sperm decreased from January to June.

5.2 Fish Meal and Oils

Boars were placed on a diet supplemented with 75 mL cod liver oil/day/boar for 12 weeks (Paulenz et al. 1995). Cod liver oil is rich in ω-3 polyunsaturated acids. The spermatozoa were analyzed for fatty acid composition. Supplementing with cod liver oil increased the levels of the ω-3 docosahexaenoic acid and decreased the levels of ω-6 docosapentaenoic acid compared to levels in spermatozoa in semen from the control group. No differences were observed between groups in the negative effects of freezing on semen evaluation parameters. A study showed fish oil supplemented diets in boars alters testicular steroid production (Castellano et al. 2011). Duroc boars were provided a diet supplemented with 62 g hydrogenated fatty acids/boar/day, 60 g of menhaden fish oil (contained 10.8 g of docosahexaenoic acid and 9.0 g of eicosapentaenoic acid)/boar/day, or 60 g tuna oil (containing 19.8 g docosahexaenoic acid and 3.9 g of eicosapentaenoic acid)/boar/day for 7 months. Body condition and testicular lipid and protein content were not different among groups. Lipid classes of phosphatidylcholine, phosphatidylethanolamine, and sphingomyelin were significantly different between groups. Supplementing with fish oils decreased ω-6/ω-3 fatty acid ratio in phosphatidylcholine with ω-6 fatty acids being the most affected. The fish oil diets increased the ω-3 fatty acids compared to the ω-6 fatty acids in phosphatidylethanolamine. Fish oil diets decreased the ω-6/ω-3 fatty acid ratio in sphingomyelin. The levels of testicular testosterone, dihydrotestosterone, and estradiol were measured. Testosterone was 46% lower in the boars receiving the diet supplemented with tuna oil, and estradiol was also lower compared to the boars receiving the menhaden fish oil supplemented diet. Three different breeds of boar pigs (Duroc, Large White, and Pietrain) were supplemented with fish oil 0.3 kg/boar/day for 26 weeks to determine the effect of supplemental ω-3 fatty acids on semen parameters by breed (Yeste et al. 2010). Semen was collected once a week and evaluated for ejaculate volume, concentration of spermatozoa, viability, acrosome and mitochondrial sheath integrity, motility, morphology, and osmotic resistance. Of these parameters, the supplemented diet positively affected both sperm morphology in Large White and Pietrain breeds and the osmotic resistance of Pietrain spermatozoa. This study shows that different breeds of pigs respond differently to ω-3 fatty acids. The addition of fish oil plus thyme (*Thymus vulgaris*) was studied in stallions to determine their potential for improving semen storability (chilled at 5 °C) (Garmsir et al. 2014). Stallions received a basal diet, a diet supplemented with fish oil at 2.5% of dry matter intake, a diet supplemented with thyme at 0.02% of dry matter intake and a diet supplemented with fish oil plus thyme. The semen parameters for the stallions fed fish oil and thyme were improved at 24 and 48 h of storage. In a 2 × 2 crossover study design using eight stallions, adding 250 g/day/stallion for 14 weeks of a nutraceutical[9] high in docosahexaenoic acid (DHA), an ω-3 fatty acid, has been shown to improve storability of stallion semen (Brinsko et al. 2005). The proprietary nutraceutical consisted of wheat, fish oil, soybean meal, DL-tocopherol acetate, dried yeast, and ascorbyl palmitate. The nutraceutical contained 25% crude fat and 30% ω-3 fatty acids. The fish oil consisted of approximately 25% DHA, ~5% eicosapentaenoic acid, and ~ 34% ω-3 fatty acids.

[9] ProSperm, Minitube of America (Verona, WI)

Ejaculates from the artificial vagina were divided and evaluated as fresh, cooled, and frozen-thawed semen. Testicular volume was not changed by treatment. The mean sperm concentration from the stallions was 1.8 times higher ($p = 0.03$) in the treated horses. The level of DHA/billion spermatozoa was three times higher ($p = 0.002$) in the treated stallions. The ratio of DHA to docosapentaenoic acid (a ω-6 fatty acid) was significantly increased. Total motility, progressive motility, and average path velocity in fresh semen were not changed by treatment. After 48 h of cold storage, there was a trend for spermatozoa from treated stallions to have greater motility ($p = 0.03$). The percent of live sperm with intact acrosomes and the percent of sperm with damaged chromatin were not reduced by treatment with the nutraceutical. The numbers of stallions in this study were small, and the effects of DL-tocopherol acetate are unknown, and fertility was not a parameter in the study. A study in rams showed that fish oil supplement was superior to palm oil and sunflower oil for improving semen parameters before and after freezing and thawing (Esmaeili et al. 2014). Fish oil also increased serum testosterone. Another study in rams showed that supplementing the diet with fish oil had positive effects on semen parameters and changed the composition of fatty acid profiles of spermatozoa and the effects continued for 2 months after cessation of supplementation (Alizadeh et al. 2014). A study in Zandi rams showed that supplementing the diet with ω-3 fatty acids improved the fertility of frozen semen (Masoudi et al. 2016). The rams were fed a diet supplemented with fish oil or palm oil at 3% of dry matter for 60 days. Semen was collected and frozen using a soybean lecithin or egg yolk extender. The semen and spermatozoa parameters were not significantly different between diets. For fertility, the fish oil diet significantly improved fertility rate, parturition rate, and lambing rate. The extender used was not significant in the fertility outcomes. Holstein bulls were fed fish oil at 1.2% of the dry matter for 13 weeks, and their semen was evaluated (Khoshvaght et al. 2016). After 7 weeks, the fish oil diet significantly increased semen volume, progressive motility, and sperm viability of fresh semen. For thawed frozen semen, the progressive motility was significantly increased and abnormal spermatozoa significantly decreased. A dietary study in dogs showed that fish oil and fish oil plus vitamin E decreased the sensitivity of canine spermatozoa to lipid peroxidation (Risso et al. 2017). The effects of supplementing ewes with salmon oil and linseed oil source of (ω-3 fatty acids) or with sunflower oil (source of ω-6 fatty acids) for 6 weeks were studied on ovarian function, oocytes, and embryo quality (Wonnacott et al. 2010). Ovarian follicle size and numbers were not different between treatment groups. The diet high in ω-3 fatty acids did increase progesterone in follicular fluid. There was selective uptake of saturated fatty acids by the oocytes, predominantly stearic acid. Blastocysts from both groups had high levels of polyunsaturated fatty acids, predominantly linoleic acid.

5.3 Vegetable Oils

The use of nutraceuticals to improve the in vitro portability of stallion semen for artificial insemination is attracting attention. Nutraceuticals are also employed to improve the storability of stallion semen. Many stallions, fertile by natural mating, produce semen that does not meet the accepted standards after it is cool or cryopreserved (Arruda et al. 2010). Consequently, there is a loss of inherent fertility. Increasing dietary sources of polyunsaturated fatty acids have been shown to increase fertility in stallions. A crossover study in eight stallions conducted over 60 days showed a proprietary reproductive nutraceutical[10] improved the laboratory parameters for fresh, cooled, and frozen semen (Freitas et al. 2016). The nutraceutical, administered by oral drench for 9 weeks, contained vitamins A, B12, B6, and E, folic acid, β-carotene, L-carnitine, amino acids, ω-3 and ω-6 fatty acids, selenium, zinc, copper, and chromium. The stallions, previously assessed to meet a national semen standard (Brazil CBRA[11]), were ejaculated once a week and on the tenth week were then ejaculated every other day for four ejaculations. Semen was evaluated at collection, cooled semen was evaluated at 24, 36, and 48 h, and frozen semen was evaluated upon thawing. Semen was evaluated according to the CBRA standard. For fresh semen, the nutraceutical treatment did not change ejaculate volume, sperm concentration, numbers of abnormal spermatozoa, color, and apparent density. In fresh semen, supplementation with the proprietary nutraceutical increased ($p < 0.05$) total motility, trajectory speed, as well as plasma and acrosomal membrane integrity. In cooled semen, nutraceutical treatment preserved acrosomal membrane integrity ($p < 0.05$), total motility (≥36 h), and velocity (≤36 h). In frozen semen, supplementation increased ($p < 0.05$) spermatozoa progressive motility and plasma membrane integrity. The addition of 100 mL of linseed oil/day/stallion and a 30 mL of a proprietary antioxidant[12]/day/stallion for 84 days (November to February in Austria) attenuated a seasonal decline in motility and membrane integrity of cooled-stored semen (Schmid-Lausigk and Aurich 2014). Vegetable oils, administered intrauterine, have been used to suppress estrus in mares. Mares receiving infusions of sterile coconut oil or sterile peanut oil on day 10 of the estrus cycle had prolonged diestrus (Wilsher and

[10] Reproductive Garanhões, JCR Nutraceuticals, Vetnil (Louveira, Spain)

[11] Brazilian College of Animal Reproduction (http://www.cbra.org.br/portal/index-ar.htm)

[12] Myostem Protect, Audevard (Clichy, France)

Allen 2011). Increased uterine fluid and vaginal discharges were not observed. The use of peanut oil to suppress estrus was studied in mares using a crossover-designed study (Campbell et al. 2017). Ten days after ovulation, each mare received 1 mL of filtered (0.22 μm) sterile peanut oil[13] intrauterine using the same sterile technique used for embryo transfer or a sham treatment without any substance injected. Peanut oil infusion did not significantly increase the duration of the luteal phase but did increased erosion of endometrial epithelium and increased eosinophilic infiltration. Fractionated coconut oil was found to be ineffective in prolonging diestrus in mares (Diel de Amorim et al. 2016). Landrace boars were fed 75 and 150 mg of algal supplement[14]/boar/day for 19 weeks (Murphy et al. 2017). The algal preparation also contained ethoxyquin (6-ethoxy-2,2,4-trimethyl-1,2-dihydroquinoline). The algal supplement increased oral intake of docosahexaenoic acid. Semen parameters and fertility indices were evaluated. The algal supplement increased semen volume and numbers of spermatozoa. There were no significant differences between control and treated boars for the fertility parameters. The supplement increased semen output by 3–4 more insemination dose packages of semen. Canine spermatozoa are very sensitive to freezing and thawing.

Considerable improvements have been made through improved laboratory procedures including semen extenders. Studies have shown that dietary supplements can improve the "ruggedness" of canine spermatozoa. Supplementing canine diet with a nutraceutical[15] containing a high content of ω-3 and ω-6 fatty acids improved semen parameters (Rodrigues et al. 2017). In a crossover study, seven mixed breed stud dogs were on a commercial dog food diet and were conditioned for 8 weeks to semen collection (digital manipulation to ejaculation). After the conditioning period, semen was collected for 5 weeks and evaluated as fresh and thawed semen for volume, motility, vigor, spermatozoa numbers, and morphology. For 8 weeks, the dogs received the nutraceutical dietary supplement [containing 39.97 mg ω-3 fatty acids (eicosapentaenoic, docosahexaenoic) and 283.40 mg ω-6 fatty acid (linoleic acids)]/10 kg body weight. The supplementation regimen increased sperm motility, and concentration, but did not improve freezeability. In another study, stud dogs were on a commercial dog food or the commercial dog food supplemented with 7.2 mg ω-3-linoleic acid/kg body weight/day, 25 mg ω-6-linolenic acid/kg body weight/day, 10.1 mg ω-9-oleic acid/kg body weight/day, and 1 IU vitamin E/kg body weight/day (da Rocha et al. 2009). The supplement was provided for 60 days. The fatty acid supplement increased semen volume, spermatozoa concentration, and motility, and there was an improvement in storage at 38 °C for 4 h.

[13] Peanut oil BP20089, Augustus Oils Ltd (Alton, UK)

[14] All-G-Rich, Alltech, Dunboyne, Co (Meath, Ireland)

[15] Vetnil Industry and Trade of Veterinary Products Ltda. (Louveira, Brazil)

6 Carnitine

6.1 Background

L-Carnitine [(–)-(*R*)-3-hydroxy-4-(trimethylammonio)butyrate] is a quaternary ammonium compound found in animal and plant products. It is essential in intermediary metabolism, and its primary function is to transfer the acetyl moiety and activated long-chain fatty acids from one cellular compartment to another including mitochondrial membranes (Rebouche 2004; Adeva-Andany et al. 2018; Ringseis et al. 2018). This process assures the availability of coenzyme A. Mitochondrial β-oxidation of saturated straight-chain fatty acids is a catabolic biochemical pathway that ultimately generates acetyl-CoA, NADH+, and FADH2 from fatty acyl-CoA esters. L-Carnitine is important in regulating the acetyl-CoA/CoA ratio, and free CoA has feedback regulatory activity on pyruvate dehydrogenase complex and influences glucose metabolism. In pregnant sows, L-carnitine increases insulin-like growth factor in blood (Eder 2009). Piglets born to L-carnitine-supplemented sows have increased numbers of primary muscle fibers. All mammals can synthesize carnitine from lysine and methionine in a process requiring methylation of protein-bound lysine in a rate-limited step by N^{ϵ}-trimethyllysine hydroxylase. The liver is the primary tissue containing the enzyme γ-butyrobetaine dioxygenase required for the final reaction to form carnitine from γ-butyrobetaine. Carnitine in blood is taken up by tissues using the organic cation/carnitine transporter 2 (OCTN2), and this mechanism is also used to conserve carnitine by uptake from the glomerular filtrate. The OCTN2 transporter is the primary mechanism for uptake of carnitine from the small intestine. Some xanthines compete for the OCTN2 transporter. Plants, especially legumes and Gramineae (Poaceae) and their seeds, are considered an important source of N^{ϵ}-trimethyllysine. The highest dietary sources of carnitine are animal products. Ruminants may obtain carnitine and its precursor from their indigenous microbiome. Intravenous infusion studies have shown that the reuptake of carnitine by the kidney tubules is saturable. Carnitine is excreted in milk. The uptake of dietary carnitine in humans is estimated to be 0.05–0.25 of the dose, and there is some evidence to support that absorption may be rate limited. There is evidence that dietary uptake of L-carnitine in young piglets is 90–95% in young pigs receiving diets containing 25–100 ppm with absorption decreasing with increasing above these dietary levels (Fischer et al. 2009). In this study, levels of L-carnitine in the liver, kidney, heart, and skeletal muscle were also increased.

Feeding hens a diet with added L-carnitine increases the levels of L-carnitine and L-acetylcarnitine in eggs (Peebles et al. 2007). Chronic inflammation likely upregulates the in vivo absorption of total carnitine and acetylcarnitine (acid soluble), and absorption is increased by inflammation-linked upregulation of the OCTN2 gene expression. This is evidence that the OCTN2 is important in transferring carnitine from maternal blood to the fetus. Pregnant sows fed a diet supplemented with 50 ppm L-carnitine had increased levels of L-carnitine in fetal hearts and livers (Xi et al. 2008). The activity of the pyruvate dehydrogenase complex was increased in the heart and the activity of carnitine palmitoyltransferase in the liver but did not alter its Michaelis constant (K_m). In women, blood levels of total carnitine, acetylcarnitine (acid soluble), and nonesterified carnitine decrease during pregnancy, especially in the second and third trimester (Cho and Cha 2005). During the first and second trimesters, urinary excretion of total carnitine, nonesterified carnitine, and acylcarnitine (acid-soluble) was significantly higher, and urinary excretion decreased as pregnancy progressed. In sows, plasma carnitine does not decrease during pregnancy (Eder 2009). L-Carnitine is important for long-chain fatty acid transport inside the mitochondria and synthesis of phosphates rich in energy that improve sperm motility and survival including preparation of semen for artificial insemination. The ability of mitochondria to metabolize lipids and balance ATP supply is critical for oocyte fertilization and subsequent developmental activities (Van Blerkom 2011). L-Carnitine is essential for mitochondrial lipid metabolism in the oocyte. There are high concentrations of L-carnitine in the epididymis and spermatozoa where it serves as a vehicle for acyl groups for oxidation (Ng et al. 2004). A study on epididymides from 10-month-old Pietrain boars showed L-carnitine levels in luminal fluid increased distally with the highest levels occurring in the cauda epididymis, and the L-carnitine level in spermatozoa was not changed (Pruneda et al. 2007). Other studies in multiple species have shown that the levels of L-carnitine increase in the caudal epididymides. In many species there is a positive correlation between L-carnitine in seminal fluid and the numbers of spermatozoa. L-Carnitine has similar functions in other energy-demanding tissues including the heart. By active processes, L-carnitine is transported into epididymal plasma. Free L-carnitine in seminal fluid is directly related to sperm counts and motility (Brooks 1979; Sheikh et al. (2007). L-Carnitine has been shown to protect the testicle. A study in rats showed that dietary supplementation with L-carnitine is protective against the oligospermia induced by busulfan, an alkylating anticancer drug (Abd-Elrazek and Ahmed-Farid 2018). Double-blind placebo studies in 60 men with asthenozoospermia showed both L-carnitine and L-acetylcarnitine, given orally, improved sperm motility and semen scavenging capacity for hydroxyl and peroxyl radicals (Balercia et al. 2005). Twelve men spontaneously impregnated their partner. Of these, 9/12 of the men were receiving L-carnitine plus L-acetylcarnitine (three men for 3 months and two men for 5 months) and two impregnations occurred by men receiving acetyl-L-carnitine treatment (one for 2 months and one for 5 months) and one pregnancy occurred after 2 months of washout (carnitine treatment not specified). Similar results have been reported for another study in men (Lenzi, et al. 2003). A recent literature review did not provide clear evidence of L-carnitine improving the fertility of women (Showell et al. 2017). Another review considers L-carnitine and L-acetylcarnitine to improve female fertility, and more research is needed to understand their use in therapeutic regimens (Agarwal et al. 2018).

6.2 Bovine

A study evaluated the therapeutic use of L-carnitine and acetyl-methionine in a proprietary formulation[16] as an addition to standard propylene glycol treatment regimen for bovine ketosis diagnosed by >1.2 mMol blood levels of β-hydroxybutyrate (Jeong et al. 2018). The study also included follow-up to determine if treatment with L-carnitine decreased the probabilities for postpartum reproductive diseases. Treatment with the proprietary product improved the chances for recovery. Milk yields were improved on days 30 and 40 postpartum by the addition of the proprietary formulation to the propylene glycol regimen. Postpartum reproductive organ complications and the interval to first insemination were not different between groups.

6.3 Equine

A study in 25 young Maremmano stallions showed that L-carnitine and L-acetylcarnitine are present in seminal plasma (Stradaioli et al. 2000). Both L-carnitine and L-acetylcarnitine are higher in the first ejaculate when compared to the second ejaculate collected 1 h later. Seminal L-acetylcarnitine was positively correlated with total motile morphologically normal spermatozoa, and both L-carnitine and L-acetylcarnitine in semen and seminal plasma were positively correlated with spermatozoa concentration. L-Carnitine has a history of being used to improve fertility of stallions (Zeyner and Harmeyer 1999). Supplementing the diet with 20 g of L-carnitine/day for 60 days improved oligoasthenospermia in stallions (Stradaioli et al. 2004). Supplementation increased the progressively motile spermatozoa at 30 and 60 days in the oligoasthenospermic stallions and increased free carnitine in seminal plasma. L-Acetylcarnitine in semen was positively correlated with total motile spermatozoa that had normal morphology. Stallions with normal semen did not have

[16] Metabolase®, Fatro (Bologna, Italy)

improvements in their semen parameters. Arruda et al. (2010) cited a study that showed supplementing stallions with good, intermediate, and bad semen with 10 g of L-carnitine/horse/day for 90 days was an effective treatment for improving semen parameters. Shetland pony stallions were, for 7 weeks, fed a proprietary[17] mixture that provided tocopherol 300 mg/day, ascorbic acid 300 mg/day, L-carnitine 4000 mg/day, and folic acid 12 mg/day (Deichsel et al. 2008). No overall improvement in semen parameters was observed in semen after it was chilled at 5 °C for 24 h. There was a small decrease in the overall observations in morphological defects. Morris and Gibb (2016) reported that 6 weeks of oral supplement with 60 g of L-carnitine/day/stallion was required to improve fertility using fresh semen for artificial insemination.

6.4 Porcine

6.4.1 Sows

Research studies show sows supplemented with L-carnitine during gestation give variable results in improvement in sow reproductive performance (Eder 2009). However, the overall finding is that supplementing sows with L-carnitine improved their reproductive performance. Feeding 100 mg L-carnitine/sow/day during gestation significantly increased total litter and individual pig birth weights and the number of live births (Musser et al. 1999). The effects of supplementation with 150 mg of L-carnitine/sow/day during gestation and 250 mg/sow/day during lactation over three reproductive cycles showed that litter and piglet weights at birth and weight gains of piglets and weaning weights were increased, but litter size, stillborn piglets, and piglets fit for rearing were not changed by L-carnitine supplementation (Ramanau et al. 2002). Sows receiving 125 mg L-carnitine/day had an increased litter size of 2.8 piglets/ litter, and sows receiving 25 and 50 ppm L-carnitine/day had an increased litter size of 0.5 and 0.6 piglets/ litter, respectively (Eder 2009). Supplementing the diet of sows with L-carnitine increased the total sucking time (1–4 days of age) and increased weight gains (Birkenfeld et al. 2006). This study had two modules. The first module used 26 sows (German Landrace crossed with Large White) bred artificially to Pietrain boars. During pregnancy the treated sows were given 125 mg L-carnitine/day and 250 mg L-carnitine/day during lactation. At birth, the number of piglets in the litter was standardized, and the piglets were suckled by their mothers for 28 days. In the second module, the litters were standardized at birth, and the piglets from the L-carnitine-treated sows were placed on control sows, and piglets from the control sows (no L-carnitine supplement) were placed on the treated sows. In module one, comparisons of treated and control sows showed that the sow body weights and the numbers and birth weight of piglets born were not significantly different. On study days 3, 6, and 9, the duration of sucking/day was significantly higher as were body weight gains for piglets born to sows administered L-carnitine. In module 2, comparisons of the body weights and number of live piglets born were not significantly different treatment and control sows. The piglets from the L-carnitine-treated sows had significantly increased sucking time on day 3 and greater weight gains in the first 14 days. These results show the effects of in utero exposure of piglets to L-carnitine and also suggest that the de novo synthesis of L-carnitine may not meet the demands of pregnancy. The effects of L-carnitine supplementation during pregnancy (125 mg L-carnitine/day) and lactation (250 mg/day) were studied in sows that were on a low protein and energy diet during lactation (Ramanau et al. 2005). Feed intake for treated and control sows and the number of piglets born alive and birth weights were not different between the L-carnitine and control sows. Litter gains for treated sows was significantly higher (20%) than control sows. The sows treated with L-carnitine produced 18% more milk than control sows, and the milk composition was not different between groups. Sows in both groups had a decrease in body weight; however, sows treated with L-carnitine retained more back fat.

6.4.2 Boars

A study in Pietrain boars showed that oral supplementation with L-carnitine improved semen quality (Jacyno et al. 2007). The boars, 1.5–2.0 years of age, were orally administered 500 mg of L-carnitine for 5 weeks. The boars were ejaculated once a week and the semen evaluated. Ejaculates from each boar collected before administration of L-carnitine were used as control values. Administration of L-carnitine significantly increased total semen volume (11%), the volume of sperm-rich fraction (10%), and total ejaculate sperm count (11.5%) and reduced the activity of asparagine aminotransferase (measure of spermatozoa membrane integrity, activity increases as membrane quality decreases). The effect was observed 1 week after administration of L-carnitine. The effects of dietary L-carnitine were studied in semen from mature (~2 years old) Hampshire × Pietrain cross boars (Cerovsky et al. 2009). The boars were given 2 g of L-carnitine/day in their diet for 8 weeks. None of the indices of semen quality were significantly improved. In a study, terminal line boars aged 285 days and 504 days were fed diets supplemented with 500 mg of L-carnitine for 16 weeks (Kozink et al. 2004). Supplementing the diet with L-carnitine did not improve the storability of chilled semen. A study on L-carnitine supplementation was done in Pietrain, Duroc, and Large White boars to determine its effects on semen parameters (Yeste et al. 2010). Boars were supplemented with 0 or 625 mg L-carnitine/day for 20 weeks and semen parameters evaluated. Photoperiod and temperature increased

[17] Stallion®, Pavo Pferdenahrung GmbH (Goch, Germany)

across the study period. Supplementing the Duroc and Large White boars did not improve ejaculate volume, sperm concentration, sperm motility, sperm viability, the osmotic resistance of spermatozoa, and sperm morphology. The authors considered increased temperature and photoperiod to be detrimental to sperm morphology (increased numbers of immature spermatozoa), and sperm morphology was improved in Pietrain boars by supplementing with L-carnitine.

6.5 Poultry

6.5.1 Roosters

Avian spermatozoa have higher concentrations of unsaturated fatty acids than mammalian spermatozoa. Lipid peroxidation products can be used to index avian semen quality. The effects of dietary L-carnitine on rooster semen have been studied. At 58 weeks of age, Leghorn roosters (mature) were fed a diet supplemented with 500 ppm L-carnitine for 5 weeks (Neuman et al. 2002). Compared to controls, L-carnitine did not change feed consumption or body weight. Circulating levels of esterified fatty acids and L-carnitine were increased. The numbers of spermatozoa in semen from birds fed L-carnitine significantly increased on experimental weeks 3 and 4, and lipid peroxidation/billion spermatozoa decreased. Semen volume, relative testicular weights (correct for body mass), and viability were not changed. This study was repeated starting with 32-week-old roosters (immature) and feeding treated birds a diet containing 500 ppm L-carnitine for 5 weeks. Significantly greater weight gains were observed in control birds, and feed consumption was not significantly different between treated and control groups. On the fourth week of the study, the treated birds had a significant increase in spermatozoa numbers. Young roosters treated with L-carnitine had significantly lower lipid peroxidation/billion sperm, a decline in percent dead spermatozoa, and significantly lower numbers of giant cells in the testicle. These finding suggest that L-carnitine reduces lipid peroxidation of spermatozoa and increases testicular efficiency for producing viable spermatozoa. Another study on L-carnitine, using three different age groups, was done in a university strain of White Leghorn roosters (Zhai et al. 2007). The first study fed a diet containing 0, 125, 250, and 500 ppm L-carnitine for 8 weeks to roosters starting at 46 weeks of age. The second study fed the same levels for 17 weeks, and the third study fed 0 or 125 ppm L-carnitine from hatch to 37 weeks of age. In the first study, feed consumption and sperm viability were not changed by dietary L-carnitine. Roosters consuming the diet containing 125 ppm L-carnitine had significantly increased sperm concentration. Semen volume among diets within sampling times was not changed. In the second study, feeding L-carnitine for 8 weeks did not affect feed consumption, body weight, semen volume, and sperm vitality. There was a trend for roosters consuming the 125 ppm L-carnitine diet to have increased seminal concentration of spermatozoa. In the third study, 18-day-old embryos were injected in ovo into the amniotic fluid with 0, 1, or 2 μMol of L-carnitine, and the percent hatching was unaffected by treatment (Zhai et al. 2008a). At hatching roosters from each of embryo treatments were placed on a diet containing 0 or 125 ppm L-carnitine for 37 weeks. At 17 weeks of age, there was no effect of the in ovo exposure to L-carnitine on semen volume and sperm concentration. Dietary L-carnitine did increase sperm concentration (10^9/mL) from 22 to 37 weeks of age and decreased body weight. There were no differences in semen volume.

6.5.2 Hens

Zhai et al. (2008a) showed that hens, exposed to 2 μMol of L-carnitine in ovo, laid heavier eggs with larger albumen and shell mass. Another study using fertile Ross x Ross cross-hatching eggs, the amniotic fluid was injected with 0.0, 2.0, or 8.0 mg L-carnitine and this treatment produced a trend for increased hatchability and increased length of incubation (Keralapurath et al. 2010). There were no differences in slaughter yields. Another study showed that hens on a diet containing 25 ppm L-carnitine for 16 weeks produced progeny with improved carcass traits (Kidd et al. 2005). Another study was done in fertile eggs from Ross 308 hens fed diets containing 0 or 25 ppm L-carnitine starting when the hens were 21 weeks of age (Peebles et al. 2007). Eggs were collected at weeks 25, 30, 32, and 38 and incubated. Administration of L-carnitine to hens did not decrease incubation mortalities or hatchability of total fertilized eggs, and there were no differences between the yolk, albumen, or shell weights of the eggs from treated and control hens. Supplementing the diet of hens with 125 ppm L-carnitine did not improve egg hatchability (Zhai et al. 2008b).

7 Nutraceuticals in Assisted Reproduction

7.1 Carnitine

7.1.1 Semen

The spermatozoa of water buffalo (*Bubalus* spp.), compared to cattle, are more susceptible to the negative effects of freezing and thawing. The addition of L-carnitine to the extender used in cryopreservation of water buffalo semen significantly improved motility and decreased capacitation-like damage of spermatozoa (Longobardi et al. 2017). Lisboa et al. (2012) found that adding L-carnitine and L-acetylcarnitine to skim milk-based extenders increased semen motility and plasma membrane integrity of chilled equine semen after 48 h of cooling (5 °C). There were no differences between the semen preparations containing

L-carnitine, L-acetylcarnitine, or both L-carnitine and L-acetylcarnitine. The benefits of adding carnitine, methionine, and inositol to bovine semen extender were studied in a freeze-thaw model (Bucak et al. 2010). The addition of carnitine and inositol at the 7.5 mMol levels improved post-thawing subjective motility. Methionine and carnitine at 2.5 and 7.5 mMol and inositol at 7.5 mMol decreased the percentage of total spermatozoa abnormalities. The computer-assisted sperm motility analysis (CASA) showed that total motility was improved by carnitine and inositol, and all of the additives decreased DNA damage. A study was done on L-carnitine and pyruvate to improve spermatozoa parameters adversely affected by chilling of stallion semen (Gibb et al. 2015). Semen was collected from Welch or crossbred pony stallions and prepared for storage (room temperature) with the addition of 10 mMol each of L-carnitine, pyruvate, or L-carnitine + pyruvate or without L-carnitine and pyruvate. The semen receiving the addition of L-carnitine and pyruvate maintained acceptable motility (>34%) for 72 h. Supplementing semen with L-carnitine significantly improved motility parameters and reduced oxidative stress. Pyruvate + L-carnitine further improved motility parameters. Poultry spermatozoa are susceptible to lipid peroxidation when cryopreserved. In a freeze-thaw study using semen from Green-legged Partridge chickens (Polish breed of domestic chicken), L-carnitine at 1 mMol and taurine at 1 and 10 mMol significantly increased mitochondrial potential of thawed spermatozoa (Partyka et al. 2017). L-Carnitine and taurine at 1 mMol also significantly reduced apoptosis and membrane reorganization.

7.1.2 Oocytes

In vitro maturation of oocytes has many incentives including cost savings. However, ROS reduces the productivity of in vitro maturation of oocytes. The arrested metaphase II oocytes may shift their metabolism toward using lipid substrates. In vitro matured porcine oocytes were co-incubated with spermatozoa using IVF media plus the addition of L-carnitine at 0, 3, 6, 12, or 24 mMol (Lowe et al. 2017). Blastocyst formation was not decreased with the addition of 3 mMol L-carnitine. Oocytes or spermatozoa were incubated for 1 h with 3 mMol L-carnitine for pre-fertilization for ova or sperm, respectively, or for the first 30 min of in vitro fertilization or the first 5.5 h of in vitro fertilization. The cleavage rate was significantly higher for 1 h pre-fertilization incubation of oocytes and spermatozoa. The blastocysts were vitrified and thawed. The post-warming survival of the blastocysts was increased for the oocytes pre-incubated with 3 mMol L-carnitine. One-hour incubations of oocyte with L-carnitine added to the medium did not significantly change the percentages of sperm-penetrated, fertilized oocytes and pronucleus formation but did significantly increase the cleavage rate of the oocytes. Adding at 0.5 mg L-carnitine/mL to fluids used to mature camel oocytes increased the rate of maturation and fertilization (Fathi and El-Shahat 2017). The rate of embryo development (morula and blastocyst) was increased. The addition of L-carnitine to culture media at 0, 0.3, 0.6, and 1.2 mg/mL was investigated in bovine oocytes (Phongnimitr et al. 2013). Supplementation of the medium with 0.6 mg/mL of L-carnitine significantly improved the survival of blastocysts. The addition of 0.3 and 0.6 mg of L-carnitine to the culture media significantly improved the maturation rate, and the addition of 1.2 mg of L-carnitine significantly decreased the maturation rate. Supplementation of the in vitro maturation fluid for bovine oocytes improved the nuclear maturation and embryo development after in vitro fertilization. However, the addition of L-carnitine did not improve embryo development after vitrification. A study on the effects of L-carnitine on in vitro maturation of bovine oocytes showed that the overall effect of adding L-carnitine to the incubation medium was beneficial (Knitlova et al. 2017). Addition of L-carnitine to incubation medium for the meiotically less competent oocytes showed significantly higher fertilization rates than the control meiotically less competent oocytes. L-Carnitine addition to incubation fluid improved blastocyst yield on days 7 and 8 in oocytes classified as meiotically more competent. A study on L-acetylcarnitine in laboratory matured ovine oocytes to determine the effects on blastocyst rate and effects on intracellular organelles and lipid droplets (Reader et al. 2015). The oocytes were matured in media containing 0.0 or 2.0 mMol L-acetylcarnitine and then fertilized with thawed ram semen in IVF media. The addition of L-acetylcarnitine significantly doubled the blastocyst rate. In the in vitro matured oocytes, there was no difference in mitochondrial volume, numbers of mitochondria, and mitochondrial copy number. L-Acetylcarnitine did increase cytoplasmic volume, causing a trend for increasing vesicle volume and altered distribution of lipid droplets. A study in ovine embryos investigated the effects of L-carnitine at 3.72 mMol to vitrification and warming solutions (Saraiva et al. 2018). Parameters in the study were mortality; cell numbers; autoptic cells and apoptotic index; gene expression for carnitine palmitoyltransferase I and II, carnitine O-acetyltransferase, and peroxiredoxin-1; and levels of ROS. None of these parameters were altered by the addition of L-carnitine.

8 Coconut (*Cocos nucifera* L.)

Coconut water is used as semen extender and as a medium for embryo freezing. An important incentive for using plant-source semen extenders is the likelihood of regulators removing some disease-linked import restrictions on semen. Coconut water-based media compared to media without coconut

water were shown to be a suitable holding solution for bovine immature cumulus-oocyte cell complexes (Cordeiro et al. 2006). Coconut water was investigated as a medium and as an additive to minimum essential media for caprine primordial follicles and was not found to improve survival or follicular diameters (Martins et al. 2005). The effect of a proprietary[18] coconut water extender (sold as a powder) on the fertility of frozen-thawed dog semen was determined (Uchoa et al. 2012a). Following artificial insemination, a 60% conception rate, 50% parturition rate, and a mean birth rate of 3.4 ± 0.6 puppies were reported. A proprietary coconut water extender was used to dilute fresh stored (chilled) dog semen (Uchoa et al. 2012b). Semen was collected, extended with Tris-egg yolk or the proprietary coconut water extender, cooled to 5 °C for 6 h, and then warmed to 37 °C for 30 s and the bitches were inseminated. For semen parameters, there was no significant difference between extenders. All of the bitches inseminated by a stud dog conceived (20/20) verses 18/20 pregnancies for bitches bred by artificial insemination. There were no significant differences for liter size, and the bitches inseminated with semen extended with proprietary coconut water extender gave birth to significantly higher percentage of female puppies. In another study, coconut water-based semen extender was examined for canine semen (Cardoso et al. 2006). The coconut water from green coconuts was filtered. Coconut extender formula was 50% coconut water, 25% ultrapure water, and 25% sodium citrate, and the control extender was an egg yolk extender. Diluted semen was frozen and stored in at –196 °C for 1 week and thawed at 37 °C and semen parameters evaluated. There was no difference between coconut water extender and the egg yolk extender for the semen parameters evaluated. The effects of coconut water on improving the post-thawing parameters were studied in boar semen (Bottini-Luzardo et al. 2013). The treatment groups were using different extender recipes: Group 1, bidistilled water, lactose, and egg yolk; Group 2, bidistilled water, lactose, and egg yolk glycerol and Orvus ET paste (a surfactant); Group 3, coconut deionized water, lactose, and egg yolk; and Group 4, in natura coconut water, lactose, and egg yolk. Coconut water added to the recipes were filtered coconut water without modifications (in natura) and filtered coconut water deionized to remove excess calcium ions. Parameters to evaluate the spermatozoa were motility, acrosomal integrity, membrane integrity, and mitochondrial activity. The semen was evaluated at thawing and 30 and 60 min after thawing. The addition of deionized coconut water significantly improved the post-thawing parameters for boar semen, and the addition of filtered in natura coconut water significantly reduced the post-thawing parameters for boar semen. Reconstituted coconut water from powdered coconut water[19] was studied as a freezing solution for cryopreserved boar semen (Silva et al. 2015). This freeze-thaw study showed that reconstituted coconut water plus dimethylformamide freezing solution produced the highest-quality spermatozoa. The study used an automated cooling control curve for cooling and freezing the semen. Coconut water has been studied as an extender for equine semen (London et al. 2017). Semen extenders used were coconut water, coconut water with a proprietary antioxidant,[20] egg yolk extender, and egg yolk extender with proprietary antioxidant. The diluted semen was frozen and stored in liquid nitrogen (–196 °C). No significant difference in the post-thawing semen parameters were shown between the semen extenders. Virgin coconut oil[21] (VCO) was studied as an additive for chilled and frozen-thawed semen from Brangus-Simmental bulls (Tarig et al. 2017). The semen was diluted with 8% VCO in Tris extender which contained different concentrations 0% (control), 4%, 8%, 12%, 16%, and 20% egg yolk. Semen chilled at 4 °C was evaluated at 24, 72, and 144 h. Frozen semen was thawed and evaluated at 7 and 14 days. For both preservation methods, semen parameters were significantly improved for 8% VCO and 20% egg yolk.

9 Quercetin

Quercetin [2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxychromen-4-one] has been studied as additive to semen extenders and as a testicular protectant. A study in semen from Holstein bulls showed that the addition of quercetin (25, 50, 100, and 200 μg/mL) to extended bull semen significantly improved the tail length, tail DNA, and tail movement (Avdatek et al. 2018). The addition of quercetin and dimethylacetamide to Mahabadi goat semen improved the post-thawing motion kinetics and suppressed lipid peroxidation (Seifi-Jamadi et al. 2017). Quercetin (0.15 mMol) added to sex-sorted and unsorted cryopreserved stallion semen improved post-thaw motility and zona binding ability and reduced DNA fragmentation (Gibb et al. 2013). There were no effects on the motility, acrosome integrity, or viability of sex-sorted spermatozoa. Rats administered 50 mg quercetin/kg body weight/day for 70 days concurrent with 20 mg fenitrothion (pesticide)/kg body weight ameliorated the testicular toxicity of fenitrothion (Saber et al. 2016).

[18] ACP-106c; ACP Servicos Tecnologicos Ltda, ACP Biotecnologia (Fortaleza, Ceara, Brazil)

[19] ACP-103, coconut water from the fruit, sterilized and lyophilized (University of Ceará State, UECE, Brazil)

[20] Oxyrase Corp. (Mansfield, OH)

[21] Nano Xan Sdn. Bhd and Malaysia Agriculture Research and Development Institute (Malaysia)

10 Vitamins and Selenium (Se)

10.1 Background Chemistry

Vitamin E is an antioxidant and is referred to as a radical formation chain breaker because it breaks the cascading propagation of free radical formation (Burton and Ingold 1981). Vitamin E exist in eight analogs (α-, β-, γ-, and δ-tocopherols and α-, β-, γ-, and δ-tocotrienols), and they all have antioxidant activity (Zingg 2015). The vitamin E molecule is irreversibly chemically altered when it reacts as an antioxidant, and the reactive site is the hydroxyl on the chromane moiety. The differences between the vitamin E analogs in their in vivo activity may be analog-specific antioxidant activities, their specific interactions with enzymes, structural proteins, alterations of the physical-structural properties of membrane lipids, enrichment kinetics, and differences in biotransformation products. The α-tocopherol (α-T) form has different kinetics which includes selectively being enriched in mammals by specific proteins that have expression polymorphism between individuals. Vitamin E is absorbed by small intestinal enterocytes from micelles by scavenger receptor class BI and CD36 and exported by assemblage into chylomicrons. α-T is 50 times higher in plasma than the other seven analogs. Important in this enrichment process is the α-T-transport protein, and this transporter also enriches the levels of hepatic α-T. Vitamin E modulates signal transduction of enzymes (reviewed by Zingg 2015). These mechanisms include modulation of signal transduction enzymes by direct binding or altering their redox regulation. Vitamin E, in membranes, may alter signal transduction by counteracting lipid oxidation and, in modulation, by employing different mechanisms and alter lipid raft domains. Vitamin E can compete for lipid mediator binding sites on lipid transport proteins and thereby modulate transport traffic, enzymatic activity, and signaling functions. A common theme for vitamin E activity is enzyme-membrane interaction that modulates or otherwise affects signal transduction and gene expression.

10.2 Vitamin E in Reproduction

Studies have shown that supplementation with vitamin E improves reproductive parameters and excessive vitamin E maybe harmful. In a Japanese study, cows with stillbirths had significantly higher vitamin E and significantly lower levels of vitamin A and Se (Uematsu et al. 2016). No evidence of viral diseases (Akabane, Aino, Chuzan, Peaton, D'Aguilar, Shamonda, BVDV, Parainfluenza 3 virus, and Bovine-Herpesvirus-1) was found. A study in dairy cows showed that administration of vitamin E (DL-α-tocopherol) decreased retained fetal membranes and stillbirths (Pontes et al. 2015). The treated cows were administered 1000 IU of DL-α-tocopherol on days 19.2 ± 4.3, 12.9 ± 3.3, and 6.2 ± 2.9 days before calving. The cows administered vitamin E also had improved nonreturn first service and subsequent decreased pregnancy loss. The diet of Aohan fine-wool rams was supplemented with 0, 20, 200, 1000, or 2400 IU vitamin E/sheep/day for 12 months, and behavior, semen, and testicular parameters were measured (Yue et al. 2010). Supplementing the diet with 200 IU of vitamin E significantly increased libido and total sperm output and increased the activity of superoxide dismutase and glutathione peroxidase in testicular cell membrane. Supplementing with 20 and 200 IU of vitamin E increased sperm motility. All of the rams supplemented with vitamin E had, compared to control rams, had decreased testicular mitochondrial malondialdehyde, and the mitochondrial malondialdehyde in the group supplemented with 200 IU vitamin E was significantly lower than the other treated groups. Vitamin E at 2400 IU increased abnormal spermatozoa. A study in male ferrets (*Mustela nigripes*) showed, for semen parameters, dietary interactions between meat source (beef, horse meat, and whole prey) and vitamin E (Santymire et al. 2015). The daily diets were control horse meat diet, horsemeat supplemented with vitamin E at 400 IU/kg dry matter, horsemeat + whole prey, horsemeat diet + vitamin E daily + whole prey, or beef diet. Diets and blood were assayed for vitamins A and E and Se. Electroejaculates were collected monthly and evaluated for concentration of spermatozoa, morphology, and sperm motility index. Vitamin A in the horsemeat diet was 150% higher than label specification, and the beef diet contained 20% of the label specification for vitamin A. Supplementation with vitamin E increased blood levels of vitamin E. Ferrets fed beef, compared to ferrets fed horse meat, had significantly higher sperm concentration. Ferrets fed beef or horse meat + prey + vitamin E had the largest testicles, and feeding prey tended to increase sperm motility index, testicular size, and percentages of normal acrosomes. Overall, diets did not affect semen volume and percent normal acrosomes. Supplementing with vitamin E tended toward fewer normal spermatozoa. Mating successes and the number of kits born were not influenced by diet. This study shows that diet and the interactions with supplements can have an effect on semen parameters. This study also provides evidence that wild carnivores in habitats with limited dietary choices could have dietary impacts in reproduction. Semen from dogs does not have good post-storage scores. This is generally attributed to the formation of free radicals due to oxidation of fatty acids in the spermatozoa and subsequent disruption of the cell membrane. Stud dogs, in a randomized double-blind study with each dog serving as its own control, were supplemented with vitamin E and selenium (Kirchhoff et al. 2017). Studs of known fertility and normospermia were selected for the study. They were fed a basal diet (control) or a basal diet and randomly assigned to a

group receiving 0.1 mg/Se/dog/day, 100 mg vitamin E/dog/day, or 100 mg vitamin E and 0.1 mg Se/dog/day. The volume of the sperm-rich fraction did not differ between groups. Treatments with vitamin E and selenium reduced the number of sperm-head defects. No other semen parameters were improved.

11 Ancillary Nutraceuticals

Various algae cultures and extracts have been reported to improve reproduction in domestic animals. Boars were given 1.5 mL of *Spirulina platensis* extract/day for 5 or 10 days (Granaci 2007). The author concluded that the *Spirulina* extract increased ejaculate volume, motility, farrowing rate, and number of piglets born. No other reports on *Spirulina* extract in pigs were found. Pen restrained ram lambs were fed a diet supplemented with dried grape pomace (from wine making, *Vitis* spp.) at 0, 5, and 10% of dietary dry matter for 74 days and testicular parameters measured (Zhao et al. 2017). This study investigated offsetting effects of grape seed pomace on the negative effects of restraint effects on testicular size from pen-rearing rams. After feeding grape pomace, the rams were euthanized and the testicles removed. Feeding grape pomace reduces testicular ROS and malondialdehyde and increased testicular weights and testicular length. Evaluation of spermatozoa isolated from the epididymis showed that feeding grape pomace at 10% of dry matter significantly increased spermatozoa numbers by 10% and decreased sperm deformities. Grape pomace at the 10% level significantly increased motility and testicular gene expression of superoxide dismutase. Supplementing the diet of mares with 100 g L-arginine/mare/day for 17 days did not increase the recovery of embryos (Kelley et al. 2014). The mares had spontaneous ovulations and artificial inseminations. Carotenoid terpenoids are found in feedstuffs and added to animal feeds to improve reproduction, reduce oxidative stress, and prevent deficiencies. Deficiencies in vitamin A are known to cause reproductive dysfunctions and other health issues. β-carotene is a precursor for the synthesis of vitamin A. The levels of natural β-carotene in feedstuffs are difficult to predict, and nutritionist recommend that the daily requirements of vitamin A be added to the ration. A 2-year study in Swedish Holsteins cows showed that lactating dairy cows kept under organic farming methods without supplementation of vitamins A and E did not report an increase in occurrences of infertility (Johansson et al. 2014). Italian trotting mares on grass hay and grain supplement were studied peripartum for the effects of a β-carotene[22] supplement on reproduction parameters (Trombetta et al. 2010). Immediately following birthing, the mares were supplemented with 1 g β-carotene/mare for 15 days. In control mares, β-carotene was decreased for 5 days and then stabilized, whereas β-carotene along with vitamin A was increased in the treated mares. Estradiol was significantly increased by β-carotene treatment. There was a suggestion that the parturition to ovulation interval was shortened. In another study, mares were supplemented with 1 g β-carotene[23]/day for 56 days, starting 2 weeks before parturition (Kuhl et al. 2012). β-carotene was excreted in the milk, especially in the colostrum. Mares were bred on the first postpartum heat (foal heat), and 7/7 of the control mares conceived and 5/12 of the β-carotene-supplemented mares conceived. The effects of carotenoids from *Haematococcus pluvialis* microalgae on performance parameters were studied in mink (Korhonen and Huuki 2015). The body weight of the mothers was significantly higher in the females fed the carotenoids from *H. pluvialis*, but the number of kits whelped/female was not increased. The number of barren females was higher in the control group (16 vs. 13) as was the 46-day kit losses (40 vs. 26).

The YiShenJianPi recipe for traditional Chinese medicine has been shown to improve tripterygium glucoside-induced oligoasthenozoospermia in BALB/c mice (Sheng et al. 2017). The decoction was made from the YiShenJianPi recipe which contains 30 g *Cuscuta chinensis* seeds, 30 g *Lycium* spp. fruit, 30 g *Schisandra* spp. fruit, 10 g *Codonopsis pilosula* root, 10 g *Astragalus membranaceus* root (unprocessed), 10 g *Citrus reticulata* skin (dry), 6 g *Bupleurum* spp., 6 g *Cimicifuga foetida* rhizomes, 6 g *Ligusticum chuanxiong* rhizomes, and 6 g *Carthamus tinctorius* flowers. The mice were given tripterygium glucoside orally at a dose of 40 mg/kg body weight for 4 weeks to induce oligoasthenozoospermia. The tripterygium glucosides, from *Tripterygium wilfordii* Hook F. (thunder god vine), have been shown to induce infertility in men and rats (Qian 1987) and are prescribed for arthritis. The doses of YiShenJianPi recipe decoction were 1.35 mg/kg/day and 2.70 mg/kg/day for 4 weeks. The mice receiving the low dose of the YiShenJianPi recipe plus the tripterygium glucoside had improved spermatogenesis and mitochondrial function in spermatozoa.

12 Urinary Diseases

12.1 Background

Urinary tract infections occur when virulent organisms multiply and persist in the urinary tract and cause pathological and pathophysiological issues. Urinary tract infection is associated with breaches in the innate and adaptive

[22] Rovimix® containing 10% β-carotene 10%, Istituto delle Vitamine SpA (Milano, Italy)

[23] Blattiviko beta 8000, Blattin Mineralfutterwerk (Seitschen, Germany)

components of the immune system and is also a complication associated with anatomical and neurological pathologies. Urinary infections, especially in older animals, develop a history of reoccurrence (Raditic 2015). Although the molecular mechanisms that lead to chronic urinary infections are not completely understood, oxidative stress is being identified as one of the "cornerstones" for causation (Ratliff et al. 2016). As the importance of oxidative stress in renal disease becomes better understood, the interest in and use of functional foods and nutraceuticals are increasing. The recurrence of urinary tract infections and uroliths, and resistance of microorganisms to antimicrobial chemotherapeutics, is increasing the use of nutraceuticals in veterinary and human medicine. Infections can be ascending or localized to a specific site such as the urinary bladder or the renal pelvis. Antimicrobial pharmaceuticals are usually effective in treating urinary tract infections, but recurrences following the therapeutic regimen are common. Antibiotics also disrupt the corpus microbiome. *Escherichia coli* accounts for about 50% of the urinary infections in cats and dogs. The type 1 fimbriae of *E. coli* and other *Enterobacteriaceae* that cause urinary tract infections adhere to the mannose sugar residue in mucous and cell membranes. The adherence is enabling for infection to be established and spread. Uroliths are a common urinary disease in companion animal medicine. Infections, especially organisms that are urease positive, can increase the occurrence of struvite crystals (Gomes et al. 2018). The formation of uroliths generally consists of distinct stages. These are supersaturation, nucleation, growth, aggregation, and retention. Calcium oxalate uroliths are precipitated calcium oxalate with the oxalate being from dietary and metabolic sources. There is increasing evidence that the microbiome can affect the incidence of uroliths (Mehta et al. 2016). The finding in human medicine suggests that oxalate uroliths are associated with a microbiome that has decreased oxalate-degrading microorganisms. Nutraceuticals are being studied for their prophylactic effects on preventing kidney damage linked to oxidative stress. Ethanol and petroleum ether extracts of avocado (*Persea americana*), walnut (*Persea americana*), flaxseed (*Linum usitatissimum*), and arugula (*Eruca sativa*; syns. *E. vesicaria* subsp. *sativa*) were shown to protect rats from the toxic effects of cisplatin (Al-Okbi et al. 2014). Each group of rats was orally administered the plant seed extracts at 250 mg/kg body weight for 20 days and then administered 7.5 mg cisplatin/kg body weight. Administration of the nutraceutical extracts reduced plasma malondialdehyde levels (measure of lipid peroxidation) in the rats treated with cisplatin. Pretreatment with the extracts significantly decreased chromosome aberrations compared to the group administered cisplatin only. Histopathology comparison of the control rats (no nutraceutical or cisplatin), cisplatin by itself, and nutraceutical plus cisplatin showed that the arugula extracts (*E. sativa*) provided 70% protection and flaxseed extracts 50% protection against the nephrotoxic effects of cisplatin. In veterinary and human medicine, a diet high in fruits and vegetables has been shown to be protective against kidney disease (Hall et al. 2016; Manfredini et al. 2016).

12.2 Fruits

A diet high in fruits and vegetables has been shown in veterinary and human medicine to be protective against kidney disease (Hall et al. 2016; Manfredini et al. 2016). The increased excretion of citrates and malates are beneficial in reducing the occurrence and recurrence of uroliths.

12.2.1 Cranberry (*Vaccinium* spp.) and Pears (*Pyrus* spp.)

Whole cranberries (*Vaccinium macrocarpon* Aiton, *V. oxycoccos*, and *V. vitis-idaea*) and cranberry juice have been used for hundreds of years as remedies for urinary health issues (Davidson et al. 2014, Anon 2016; Zhao et al. 2018). The levels and chemical profile of type A proanthocyanidins and the efficacy of cranberry-source products all vary with the *Vaccinium* spp. and growing conditions. Cranberry extracts are produced by various methods. There is evidence to show that proanthocyanidin group of phytochemicals in cranberries blocks the adhesion of bacterial types 1 and 2 fimbria to the mannose moiety in urinary tract cells and mucous (Abascal and Yarnell 2008). Cranberry juice also reduces the formation of urinary biofilms. A study, using a small number of animals in client-owned dogs with a history of greater than three recurrent urinary tract infections, showed that oral administration of cranberry extract[24] prevented the development of urinary tract infections. The in vitro portion of this study also showed that the urine from dogs receiving cranberry extract prevented adherence of *Escherichia coli* to Madin-Darby canine kidney cells (Chou et al. 2016). The urine from the cranberry extract treated dogs did not have bacteriostatic activity. The concentrations of proanthocyanidin phytochemicals (polyphenol flavonoids) in the cranberry extract were not reported. Pears are being recommended in human medicine as a prophylactic for uroliths (Manfredini et al. 2016). The organic acids in pears are likely important in the prevention of uroliths. Pears contain antioxidant and anti-inflammatory compounds (Li et al. 2012). The antioxidant compounds are the polyphenols, namely, arbutin, catechin, chlorogenic acid, quercetin, and rutin. The anthocyanins levels were correlated with antioxidant activity, and triterpenoids are strongly correlated to anti-inflammatory activity. No reports in the veterinary literature were found for

[24] Cranimals (West Vancouver, BC, Canada)

the use of pear or its extracts being prescribed as a prophylactic or for treatment of uroliths.

12.3 Herbal Remedies

Goldenseal (*Hydrastis canadensis* L.) contains berberine which has antibacterial properties and is used for cystitis in human medicine (Abascal and Yarnell 2008). It has efficacy for the treatment of infections stemming from *E. coli*. Goldenseal is also used as an ethnotherapeutic to treat canine and feline uterine infections in traditional medicine in British Columbia, Canada (Lans et al. 2009). Berberine in goldenseal blocks fimbriae expression of *E. coli* and adhesion of *Streptococcus pyogenes* to epithelial cells. Buchu Berg. (syn. *Barosma betulina* Bartl.) has historical usage for urinary infections and calculi, and the extracts have antibiotic activity (Abascal and Yarnell 2008; Moolla and Viljoen 2008). High doses cause hepatotoxicity in rats. There appears to be no reports of its use in veterinary medicine. Ethanol extract of *Poria cocos* Wolf. (syn. *Wolfiporia extensa* Peck.) was studied in rats using an adenine-induced renal failure model (Zhao et al. 2013). Concurrent with the adenine treatment, one group of mice was administered 60 mg of the dried *Poria cocos* extract (reconstituted in 1 mL). The authors concluded that ten of the biomarkers studied in a metabonomic profile showed that *Poria cocos* extract was protective to the kidney. Extracts of the epidermis of *Poria cocos* sclerotia were tested in rats for diuretic activity (Feng et al. 2013). The ethyl acetate extract had the most diuretic activity and increased the excretion of sodium and chlorine ions. The ethyl acetate extracts (epidermis of *Poria cocos* sclerotia) were further characterized chemically and studied in vitro in HK-2 cultures for inhibition of fibrosis (Wang et al. 2018). The triterpenes, identified as PZG and PZH, inhibited the upregulation of collagen I expression and podocyte injury. Ethanol extract of *Poria cocos* has antifungal (*A. fumigatus*, *C. albicans*) and antibacterial (*Acinetobacter baumannii*, *S. aureus*) activity (Zhang et al. 2013). An extract containing triterpenes, polysaccharides, and steroids from *Poria cocos* has been tested in vivo for its antioxidant activity and prevention of renal cell injury linked to urolithiasis (Schulman et al. 2016). The authors concluded this extract is a candidate prophylactic for urolithiasis. Raspberry (*Rubus idaeus* L.) has been reported to be effective in human medicine as treatment and prophylactic for uroliths. In Chinese traditional medicine, raspberry fruit is considered a cure for renal disease, and raspberry leaf is considered a mild diuretic. Using a glyoxylate-induced calcium oxalate nephrolithiasis in mice model, the efficacy of raspberry root extract was investigated (Ghalayini et al. 2011). The mice were administered 100 or 200 mg/kg/day of raspberry root extract for 12 days. The kidneys were examined by histopathology and the kidneys analyzed for calcium. The treatment with raspberry root extract reduced calcium oxalate deposits in the kidneys of mice receiving glyoxylate. The renal weights of kidneys from mice receiving glyoxylate were significantly higher from mice receiving glyoxylate plus raspberry root extract. Calcium levels in kidneys from the mice receiving glyoxylate plus raspberry root extract were not significantly different from control mice (no glyoxylate or raspberry root extract treatment). A study in rats using hot water and hot methanol extracts from air-dried wild raspberry fruit had a potassium-conservative diuretic effect (Zhang et al. 2011). The decoctions were dried, and the rats were given the extracts at 3 g/kg body weight, hydrochlorothiazide was administered as a reference standard, and control rats were administered 10 mL of water/kg body weight. The rats administered hydrochlorothiazide had the highest urinary output followed by the rats receiving the methanol extract of wild *Rubus idaeus* fruit. The extracts from cultivated raspberry fruit did not have diuretic properties. Both water and solvent extracts of *Rubus rosaefolius* Sm. leaves have diuretic properties in rats (de Souza et al. 2017). Its folk medical use is to treat hypotension. *Rubus idaeus* does not appear to have use in veterinary medicine for urinary maladies other than in traditional Chinese medicine applied to veterinary medicine. Quercetin is bioflavonoid and is the most abundant polyphenol in fruits and vegetables. It is present as a glycoside in eaten plant material and as the aglycone in supplements and is important in the pharmacokinetics of absorption. Common fruits that contain quercetin are apples, cranberries, cherries, and grapes, and vegetables containing quercetin are onion, peppers, and asparagus. High doses of quercetin (over 1 g/day) may be harmful to preexisting kidney damage. Quercetin may be a promoter of estrogen-dependent neoplastic cells. The pharmacokinetics and toxicology of quercetin have recently been reviewed (Andres et al. 2018). It is considered a candidate drug for renal disease in companion animal and human medicine (Pollen 2001; Nirumand et al. 2018). Concurrent administration of quercetin has been shown to reduce the renal toxicity of ciprofloxacin in rats (Elbe et al. 2016).

12.4 Lipoic Acid

Naturally occurring lipoic acid exists as lipoyllysine and is synthesized de novo in mammalian cells. α-Lipoic acid [(*R*)-5-(1,2-dithiolan-3-yl) pentaenoic acid] is a nutraceutical used as a mitochondrial nutrient, as an antioxidant, and as an equalizer for geriatric-linked decrease in the activity of pyruvate dehydrogenase. The α-lipoic acid molecule is a disulfide derivative of octanoic acid and in the oxidized form has an intermolecular disulfide bond. The 1,2-dithiolate ring is reactive with redox-sensitive molecules. Cells reduce α-lipoic

acid to dihydrolipoic acid and both forms are present extracellularly. Molecule per molecule, α-lipoic acid is more reactive than glutathione and *N*-acetylcysteine (Zhang and McCullough 2016). α-Lipoic acid is amphipathic and is soluble in both the aqueous and lipid environments. Symmetric dimethylarginine is being recognized in veterinary medicine as a biomarker of renal function and an endogenous marker chemical for calculating glomerular filtration rate. Having more sensitive biomarkers available provides sensitive parameters for evaluation of the efficacy of nutraceuticals prescribed as kidney protectorates. The dose of DL-α-lipoic acid ranges from 2.5 to 25 mg/kg/day in dog foods. Extruded dog foods have lower bioavailability of DL-α-lipoic acid (Zicker et al. 2010). There are reports of intoxications by DL-α-lipoic acid in cats and dogs (Hill et al. 2004; Loftin and Herold 2009). Observed toxic doses in dogs are 191–210 mg DL-α-lipoic acid/kg body weight for dogs. Dose levels of 30–60 mg DL-α-lipoic acid/kg body weight are toxic to cats with a dose of 60 mg/kg body weight causing fatalities in cats. A dog ingested an estimated dose of 210 mg DL–αlipoic acid/kg body weight and was presented for veterinary care 60 h later. The clinical observations were hyperesthesia, hypoglycemia, acute hepatic insult, and oliguric renal failure. The dog was euthanized 76 h following ingestion. Treatment in dogs is symptomatic including aggressive treatment of hypoglycemia. Although used, the efficacy of *S*-adenosyl methionine, *N*-acetylcysteine, and silymarin for treatment of DL-α-lipoic acid intoxication in dogs has not been reported. A dog, after ingesting 191 mg DL-α-lipoic acid/kg body weight, was hospitalized and treated symptomatically. It was discharged 4 days later with meds and 4 months later was reported by the owner as asymptomatic. The toxicology of DL-α-lipoic acid has been studied in detail in cats with cats shown to be ten times more sensitive than humans, dogs, or rats. A dose of 30 mg DL-α-lipoic acid/kg body weight is toxic to cats, and a dose of 60 mg/kg can be fatal. The calculated maximum tolerated single oral dose for cats is 13 mg DL-α-lipoic acid/kg body weight. In cats, clinical signs of intoxication include ptyalism, hyperesthesia, and some degree of anorexia. There does not appear to be any distinct gross lesions for DL-α-lipoic acid intoxication. Hepatic histopathology observed in cats is swollen, granular to vesicular cytoplasm, loss of distinct sinusoidal linings, and lack of lipid or glycogen stores in the centrilobular regions of the liver. Histopathology observed by ultrastructural microscopy is expanded space of Disse, loss and disruption of hepatocyte sinusoidal microvilli, disruption of junctional complexes and bile canaliculi, and loss of glycogen stores. In the cell, DL–αlipoic acid is enzymatically transformed to dihydrolipoic acid by glutathione reductase, lipoamide dehydrogenase, and thioredoxin reductase. Dihydrolipoic acid is excreted from cells and then reoxidized or metabolized. Cell culture research has shown dihydrolipoic acid to be a trigger causing a caspase 3 cascade in mitochondria leading to apoptosis.

12.5 Mannose and Methionine

Bacteria, to infect epithelial cells, need to adhere before infiltrating the cells. Some bacteria have specific virulence factors for adherence to uroepithelium. Uropathogenic *E. coli* has adhesin lectin fimbriae H virulence factor that enables the bacteria to adhere to the oligomannosides of the glycoprotein uroplakin Ia on the uroepithelium. D-mannose has been shown to be effective in reducing the recurrence of urinary tract infections in women. An in vitro study showed that D-mannose reduced the adherence of the equine pathogens *E. coli*, *Pseudomonas aeruginosa*, and *Streptococcus zooepidemicus* to equine endometrial cells (King et al. 2000). There are reports of successful use of D-mannose to treat primary and recurrent urinary tract infections in horses, dogs, and cats. The reported side effect is diarrhea. D,L-methionine had been reported as a treatment for infection-linked struvite uroliths in dogs (Raditic 2015). The dose used was 100 mg D,L-methionine/kg body weight every 12 h for up to 4 months.

13 Nutraceutical Recipes and Candidate Nutraceuticals

Herbal remedies and probiotics are used in the treatment of urolithiasis. A desired efficacy of herbal supplements is to dissolve and prevent the recurrence of the uroliths and be efficacious as a treatment and prophylactic for urinary cystitis. CrystalClair[25], a new product based on the Pai Shi Tang formula of traditional Chinese medicine, was developed to treat urolithiasis in dogs and cats (Wen and Johnston 2012). Four to 60 weeks of treatment with 33.3 mg CrystalClair/kg body weight was effective in dissolving uroliths in 19/33 dogs and 7/13 cats. Treatment was effective in 2/8 dogs with calcium oxalate uroliths. This preparation is recommended as a prophylactic in dogs and cats with known history of urolithiasis. No side effects were reported. *Poria cocos* mushroom (Fu-Ling) has been used in traditional Chinese medicines as an ingredient in remedies for renal disorders. This mushroom, considered a candidate nutraceutical for veterinary medicine, grows around pine trees and contains triterpenoids (poriatin), polysaccharides, and steroids. Canephron N[26] is a nutraceutical produced in Germany used for the inflammation of the urinary tract and urolithiasis and as antispasmodic and antibacterial (Naber 2013). The ingredients are centaury (*Centaurium erythraea* Rafn.), lovage (*Levisticum officinale* Koch.), and rosemary

[25] CrystalClair, Natural Solutions, (Speonk, NY). Contains: abutilon, vaccaria, plantain seeds, lygodium, pyrrosia leaf, achyranthes, alisma, lindera, licorice, glechoma, phellodendron, eupolyphaga, earthworm, scorpion, centipede. http://www.naturalsolutionsvet.com/product.html?pid=81

[26] Bionorica SE (Neumarkt, Germany)

(*Rosmarinus officinalis* L.). These plants contain flavonoids, phenolic glycosides, phthalides, secoiridoids, phenolcarboxylic acids, and essential oils. There is one report of liver dysfunction associated with Canephron N (Sychev et al. 2011). In this occurrence, concurrent medications with Canephron N were risperidone and clomipramine, and the Naranjo scale[27] was defined as probable. The conclusion is this Canephron N has benefit and is safe. No reports were found on its use in veterinary medicine. A study was done of the toxicology of *C. erythraea* in mice and rats (Tahraoui et al. 2010). *C. erythraea* plants were harvested in north Morocco during May and June. The plants were air-dried, milled, and extracted with water. Mice were given 1–15 g of extract/kg body weight and no adverse effects were observed. Rats were orally administered 0, 100, 600, and 1200 mg of extract/kg body weight for 90 days. All the hematological parameters except mean corpuscular volume were not significantly different from controls. Liver and urinary parameters were not significantly changed, and in the 600 and 1200 mg/day groups, blood glucose and triglycerides were significantly decreased. *C. erythraea* may be a candidate nutraceutical for use in veterinary medicine. A crossover-designed study on herbal recipes Alisma, San Ren Tang, and Wei Ling Tang in healthy, spayed female cats on treatment and off treatment did not show any differences in urinary parameters (Daniels et al. 2017). Parameters measured were 24 h urinary analyte excretions, 24 h urine volume, urine pH, and 24 h urinary saturation for calcium oxalate or struvite. A canine diet[28] containing fish oil, lipoic acid, vitamins C and E, L-carnitine, fruits, vegetables, and egg and chicken proteins, formulated to control sodium intake and provide protein with a high biological value, was shown to improve renal function in aging dogs (Hall et al. 2016). Renal function was indexed on serum symmetric dimethylarginine and creatinine.

14 Probiotics

The probiotics are being explored in human and veterinary medicine as prophylactic to reduce the risk and recurrence of genitourinary disease. There is substantial interest in the total corpus microbiome and its interactions in chronic urogenital diseases. Oxalate-containing uroliths are difficult to dissolve with various therapies and generally have to be removed by mechanical and surgical procedures. Oxalate is absorbed in all segments of the small intestine, and there is evidence that a large amount of oxalate is absorbed in the large intestine (Peck et al. 2016). Focusing on the gut microbiome, *Oxalobacter formigenes* in the large intestine can induce oxalate secretion by the enterocytes into the lumen where it is metabolized by gut microbes (Miller and Dearing 2013). Oxalate-degrading bacteria, such as *O. formigenes*, *Bifidobacterium* spp., *Porphyromonas gingivalis*, and *Bacillus* spp., degrade oxalate in the gut (Peck et al. 2016). The colon has the ability to regulate absorption and secretion of oxalate and has a major influence on oxalate homeostasis in the body. *O. formigenes*, a Gram-negative and obligate anaerobe, is considered important in maintaining oxalate homeostasis. There is evidence that therapies using antimicrobials likely alter the oxalate homeostasis by altering the gut microbiome. *O. formigenes* may be a candidate probiotic in veterinary medicine. Bacteria[29,30] from two probiotics were studied in vitro for metabolizing oxalate (Cho et al. 2015). The bacteria present in the probiotics were *Lactobacillus* spp., *Bifidobacterium* spp., and *Enterococcus* spp. Two *L. acidophilus* isolates decreased oxalate in the culture; *L. plantarum* increased oxalate in the culture medium. The general conclusion is that *Lactobacillus* spp. and its strains are likely variable in their ability to degrade oxalate. Lactic acid bacteria in the canine gut have in vitro capacity to degrade oxalate (Ren et al. 2011). These isolates are *Leuconostoc mesenteroides* (RL75), *Lactococcus garvieae* (CD2), *Lactococcus lactis* subsp. *lactis* (CS21), *Enterococcus faecium* (CL71 and CL72), and *Enterococcus faecalis* (CD14, CS62, and CD12). Many of these are used in probiotics, but there are no reports of their use in probiotics for urolithiasis. In the management of cystitis, there is increasing interest in using probiotic infusions to alter the microbiology of the urinary bladder to control chronic cystitis.

15 Ethnoveterinary Medicine

The use of plant extracts, decoctions, infusions, tinctures, and other formulations for the treatment of animal maladies is recorded throughout written history. There is an increased interest in investigating the historic and current use of botanicals and other nutraceuticals in veterinary medicine especially in documenting efficacy and safety. Table 1 shows that many rural areas use plant preparations and nutraceuticals. The cost of modern drugs is increasing the interest in researching the efficacy of ethno-nutraceuticals.

[27] https://livertox.nih.gov/Narajo.html

[28] Hill' s Pet Nutrition, Inc. (Topeka, USA)

[29] Vetri-Mega Probiotic, Vetri-Science Laboratories (Essex Junction, USA)

[30] Proviable-DC, Nutramax Laboratories Inc. (Lancaster, USA)

Table 1 Examples of ethno-nutraceuticals used as genitourinary remedies for domestic animals

Condition (species)	Plant scientific name	Plant parts	Route of administration	Reference
Prevent retained placenta and hemorrhage (bovine, ovine)	*Adiantum capillus-veneris* L.	Fronds made into a decoction	Oral	Uncini Manganelli et al. (2001)
Prevent postpartum issues (bovine, ovine)	*Laurus nobilis* L.	Decoction made from leaves	Oral	
	Linum usitatissimum L.	Decoction made from seeds; boiled seeds, can be mixed with other plants	Oral	
	Malus sylvestris L.	Mixed with other seeds and decoctions	Oral	
(Bovine, lagomorph)	*Rubia peregrina* L.	Whole plant without roots	Oral	
(bovine)	*Apium nodiflorum* L.	Infusion made from the whole plant	Oral	
	Parietaria diffusa Mert. et Koch.	Decoction mixed with wheat flower	Oral	
Retained placenta (ruminants)	*Acacia sinuata* Lour., Merr.	Tea from stem, bark, and roots	Oral	Upadhyay et al. (2011)
	Acacia catechu L.	50 g crushed with 3–4 leaves of *Cassia occidentalis* L.	Oral	Kumar and Bharati (2013)
	Curcuma longa L.	Turmeric powder with jiggery oil	Oral	
	Cynodon dactylon L.		Oral	
	Ficus carica L. *Ficus racemosa* L.	Boiled crushed fruits and jiggery; water shake	Oral	
	Butea monosperma Lam.	Crushed root tea; may be mixed with other plants	Oral	
	Flacourtia indica Burm.	Leaf tea with whey	Oral	
	Hordeum vulgare L.	Grains with fodder	Oral	
	Pennisetum americanum L.	Leaves mixed with barley tea	Oral	
	Saccharum officinarum L.	Fresh or boiled leaves in water given with fodder; other variants used	Oral	
	Tridax procumbens L.	Leaves (25 g) mixed with cow dung ashes given with water	Oral	
	Triticum aestivum L.	Given with jaggary		
	Ziziphus nummularia Burm., Wight & Arn.	Crushed roots in water	Oral or topical on uterus	
	Narcissus tazetta L.	0.25 kg leaves mixed with gurr and flour and boiled	Oral	Aziz et al. (2018)
	Visnaga daucoides Gaertn.	Infusion made from fruit for 3 days	Oral	
Retained placenta and uterine infection (ruminants)	*Alchemilla vulgaris* L.	90 mL of tincture from aerial parts	Oral	Lans et al. (2007)
	Hedera helix L.	Handful of aerial parts	Oral	Lans et al. (2007)
Uterine dislocation (ruminants)	*Anisomeles indica* Linn.	Ground root nixed with butter milk	Oral	Upadhyay et al. (2011)
Prolapsed uterus (ruminants)	*Butea monosperma* Lam., Taub.	Crushed root tea; may be mixed with other plants	Oral	Upadhyay et al. (2011)
	Flacourtia indica Burm. f., Merr.	Leaf tea with whey	Oral	
	Ichnocarpus frutescens L.	Leaf paste mixed with ginger and boiled	Oral	
Leucorrhoea (ruminants)	*Bauhinia racemosa*, Lamk.	Plant juice mixed with vegetable oil	Oral	Upadhyay et al. (2011)
Prevent abortion (ruminants)	*Corchorus depressus* L. *Hordeum vulgare* L.	Leaf paste mixed with Hordeum vulgare flour	Oral	Upadhyay et al. (2011)
	Pedalium murex L.	Plant juice	Oral	
Diuretic, abortion (bovine, ovine, porcine)	*Petroselinum sativum* Hoffm.	Fresh plant, increased dose can cause abortion	Oral	Uncini Manganelli et al. (2001)

(continued)

Table 1 (continued)

Condition (species)	Plant scientific name	Plant parts	Route of administration	Reference
Urinary issues (ruminants)	*Dichrostachys cinerea* Wight & Arn.	Stem bark powder	Oral	Upadhyay et al. (2011)
Kidney stone (canine)	*Costus spiralis* Jacq.	Leaf infusion, add to drinking water	Oral	Antonio et al. (2015)
	Echinodorus grandiflorus Cham & Schltdl.	Leaf infusion, add to drinking water	Oral	
	Phyllanthus tenellus Roxb.	Leaf infusion	Oral	
Hematuria (ruminants)	*Abutilon indicum* L.	200 g crushed	Oral	Kumar and Bharati (2013)
	Acacia nilotica subsp. *indica* Benth.	250 g crushed with 4–5 leaves of *Syzygium cumini* (L.), *Mangifera indica* (L.), and *Aegle marmelos* (L.)	Oral	
	Aegle marmelos L.	100 g crushed with 3–4 leaves of *Abutilon indicum* (L.)		
Urinary infection (bovine, ovine)	*Potentilla reptans* L.	Infusion	Oral	Uncini Manganelli et al. (2001)
	Costus spiralis Jacq.			
Postpartum hemorrhage (ruminants)	*Euphorbia microphylla* Lam.	Whole plant with chapatti	Oral	Upadhyay et al. (2011)
Increase fertility (Bovine)	*Allium sativum* L.	Bulb crushed and mixed with whey for 6–8 days	Oral	Aziz et al. (2018)
	Alocasia macrorrhizos L.	About 100 g crushed with a dash of camphor	Oral with bread of *Triticum aestivum* given 3× daily for 2 week	Kumar and Bharati (2013)
(Bovine, ovine, caprine)	*Heracleum candicans*	200 g fresh root mixed with wheat flour and given for 3 days	Oral	Kumar and Bharati (2013)
Abnormal estrus (elephantine)	*Allium cepa* L.	Fresh bulbs with butter	Oral	Jayakumar et al. (2017)
	Aloe vera L., Burm.	Fresh leaves with fodder	Oral	
	Citrullus vulgaris Thunb., Matsum & Nakai.	Fruit	Oral	
	Curcuma longa L.	Fresh rhizome with camphor	Oral	
	Lycopersicum esculantum L.	Fresh fruit	Oral	
	Musa paradisiaca L.	Liquid from fresh stem	Oral	
Musth (elephantine)	*Allium cepa* L.	Fresh bulbs with curds	Oral	Jayakumar et al. (2017)
	Musa paradisiaca L.	Liquid from fresh stem and fruit	Oral	
	Myristica fragrans Houtt.	Fruit powder given with fodder	Oral	
	Piper nigrum L.	Powdered seeds with rock salt	Oral	
Improve fertility (bovine, ovine)	*Avena sativa* L.	Germinated *caryopses*	Oral	Uncini Manganelli et al. (2001)
	Prunus avium L.	Leaf	Oral before breeding	
Increase egg production (gallus)	*Stellaria media* L., Vill.	Leaves added to chicken feed	Oral	
(Poultry)	*Urtica dioica* L.	Seeds	Added to feed	
	Urtica urens L.	Seeds, used with other plants	Added to feed	
Improved heat symptoms and other reproduction parameters (ovine)	*Lepidium meyenii* Walpers.	Tuber	Oral	Thesis cited in Hermann and Bernet (2009)
Higher number of ejaculated sperms with increased motility (bovine)				

16 Concluding Remarks and Future Directions

The efficacy of nutraceuticals is often a controversy. Important in the efficacy debate are unsupported claims for proprietary nutraceuticals. There are studies that have shown nutraceuticals to be efficacious. This is also the rational that veterinarians and physicians, to wisely use nutraceuticals, must be specifically trained in materia medica of nutraceuticals and ethnopharmacology. Nutraceuticals are being increasingly considered in medical management when conventional medical therapeutics are not efficacious. This includes the use of ethnic medical and homeopathic recipes in veterinary medicine. There is also intermingling of conventional and nutraceutical therapies. Nutraceuticals are a mixture of phytochemicals and generally are polyvalent in their actions. The interactions of stimulating-inhibiting multiple receptors with phytochemicals are essentially unknown but generally are considered essential for their efficacy. Future research will likely refine the actions and interactions of phytochemicals and increased refinement of phytochemicals considered to be the most potent and efficacious. Oxidative stress is being investigated, and its contribution to disease and methods of prevention are being elucidated. There is increasing evidence that in ovo and in utero exposures to phytochemicals in nutraceuticals have epigenetic effects on the offspring. The most likely advancement is prescribing nutraceuticals for prevention and remission of chronic debilitating diseases and in understanding mechanisms of action for phytochemicals at the molecular level.

References

Abascal K, Yarnell E (2008) Botanical medicine for cystitis. Altern Complement Ther 14(2):69–77

Abd-Elrazek AM, Ahmed-Farid OAH (2018) Protective effect of L-carnitine and L-arginine against busulfan-induced oligospermia in adult rat. Andrologia 50(1):e12806

Abuelo A, Hernandez J, Benedito JL et al (2015) The importance of the oxidative status of dairy cattle in the periparturient period: revisiting antioxidant supplementation. J Anim Physiol Anim Nutr (Berl) 99 (6):1003–1016

Abuelo A, Alves-Nores V, Hernandez J et al (2016) Effect of parenteral antioxidant supplementation during the dry period on postpartum glucose tolerance in dairy cows. J Vet Intern Med 30(3):892–898

Adeva-Andany MM, Carneiro-Freire N, Seco-Filgueira M et al (2018) Mitochondrial beta-oxidation of saturated fatty acids in humans. Mitochondrion. https://doi.org/10.1016/j.mito.2018.02.009

Agarwal A, Sengupta P, Durairajanayagam D (2018) Role of L-carnitine in female infertility. Reprod Biol Endocrinol 16(1):5

Alizadeh A, Esmaeili V, Shahverdi A et al (2014) Dietary fish oil can change sperm parameters and fatty acid profiles of ram sperm during oil consumption period and after removal of oil source. Cell J 16 (3):289–298

Al-Okbi SY, Mohamed DA, Hamed TE et al (2014) Prevention of renal dysfunction by nutraceuticals prepared from oil rich plant foods. Asian Pac J Trop Biomed 4(8):618–627

Andres S, Pevny S, Ziegenhagen R et al (2018) Safety aspects of the use of quercetin as a dietary supplement. Mol Nutr Food Res 62 (1):1700447

Anon (2016) Cranberry products or topical estrogen-based therapy for the prevention of urinary tract infections: a review of clinical effectiveness and guidelines. Canadian Agency for Drugs and Technologies in Health, Ottawa, ON. https://www.ncbi.nlm.nih.gov/books/NBK401598/

Antonio RL, Souza RM, Furlan MR et al (2015) Investigation of urban ethnoveterinary in three veterinary clinics at east zone of Sao Paulo City, Brazil. J Ethnopharmacol 173:183–190

Arnao MB, Hernandez-Ruiz J (2018) The potential of phytomelatonin as a nutraceutical. Molecules 23(1):238

Arruda RP, Silva DF, Alonso MA et al (2010) Nutraceuticals in reproduction of bulls and stallions. Revista Bras Zootec 39 (Suppl):339–400

Aurich C, Ortega Ferrusola C, Pena Vega FJ et al (2018) Seasonal changes in the sperm fatty acid composition of Shetland pony stallions. Theriogenology 107:149–153

Avdatek F, Yeni D, Inanc ME et al (2018) Supplementation of quercetin for advanced DNA integrity in bull semen cryopreservation. Andrologia. https://doi.org/10.1111/and.12975

Aziz MA, Khan AH, Adnan M et al (2018) Traditional uses of medicinal plants used by indigenous communities for veterinary practices at Bajaur Agency, Pakistan. J Ethnobiol Ethnomed 14(1):11

Balercia G, Regoli F, Armeni T et al (2005) Placebo-controlled double-blind randomized trial on the use of L-carnitine, L-acetylcarnitine, or combined L-carnitine and L-acetylcarnitine in men with idiopathic asthenozoospermia. Fertil Steril 84(3):662–671

Beharry S, Heinrich M (2018) Is the hype around the reproductive health claims of maca (*Lepidium meyenii* Walp.) justified? J Ethnopharmacol 211:126–170

Birkenfeld C, Kluge H, Eder K (2006) L-carnitine supplementation of sows during pregnancy improves the suckling behaviour of their offspring. Br J Nutr 96(2):334–342

Bongalhardo DC, Leeson S, Buhr MM (2009) Dietary lipids differentially affect membranes from different areas of rooster sperm. Poult Sci 88(5):1060–1069

Bottini-Luzardo M, Centurion-Castro F, Alfaro-Gamboa M et al (2013) Effect of addition of coconut water (*Cocos nucifera*) to the freezing media on post-thaw viability of boar sperm. Trop Anim Health Prod 45(1):101–106

Brinsko SP, Varner DD, Love CC et al (2005) Effect of feeding a DHA-enriched nutriceutical on the quality of fresh, cooled and frozen stallion semen. Theriogenology 63(5):1519–1527

Brockus KE, Hart CG, Fleming BO et al (2016) Effects of supplementing Holstein heifers with dietary melatonin during late gestation on growth and cardiovascular measurements of their offspring. Reprod Domest Anim 51(2):240–247

Brooks DE (1979) Carnitine, acetylcarnitine and the activity of carnitine acyltransferases in seminal plasma and spermatozoa of men, rams and rats. J Reprod Fertil 56(2):667–673

Bucak MN, Tuncer PB, Sariozkan S et al (2010) Effects of antioxidants on post-thawed bovine sperm and oxidative stress parameters: antioxidants protect DNA integrity against cryodamage. Cryobiology 61(3):248–253

Burton G, Ingold KU (1981) Autoxidation of biological molecules. 1. The antioxidant activity of vitamin E and related chain-breaking phenolic antioxidants in vitro. J Am Chem Soc 103(21):6472–6647

Campbell MLH, Hampshire D, Hamstead LE et al (2017) The effects of intrauterine infusion of peanut oil on endometrial health, salivary cortisol and interovulatory period in mares. Theriogenology 102:116–125

Cardoso RDS, Silva AR, da Silva LDM (2006) Comparison of two dilution rates on canine semen quality after cryopreservation in a coconut water extender. Anim Reprod 92(3–4):384–391

Castellano CA, Audet I, Laforest JP et al (2011) Fish oil diets alter the phospholipid balance, fatty acid composition, and steroid hormone concentrations in testes of adult pigs. Theriogenology 76 (6):1134–1145

Cerovsky J, Frydrychova S, Lustkova A et al (2009) Semen characteristics of boars receiving control diet and control diet supplemented with L-carnitine. Czeh J Anim Sci 54(9):417–425

Cho SW, Cha YS (2005) Pregnancy increases urinary loss of carnitine and reduces plasma carnitine in Korean women. Br J Nutr 93 (5):685–691

Cho JG, Gebhart CJ, Furrow E et al (2015) Assessment of *in vitro* oxalate degradation by *Lactobacillus* species cultured from veterinary probiotics. Am J Vet Res 76(9):801–806

Chou HI, Chen KS, Wang HC et al (2016) Effects of cranberry extract on prevention of urinary tract infection in dogs and on adhesion of *Escherichia coli* to Madin-Darby canine kidney cells. Am J Vet Res 77(4):421–427

Clement C, Kneubuhler J, Urwyler A et al (2010) Effect of maca supplementation on bovine sperm quantity and quality followed over two spermatogenic cycles. Theriogenology 74(2):173–183

Clement C, Witschi U, Kreuzer M (2012) The potential influence of plant-based feed supplements on sperm quantity and quality in livestock: a review. Anim Reprod Sci 132(1–2):1–10

Cordeiro MS, Silva EHS, Miranda MS et al (2006) The use of coconut water solution (*Cocos nucifera*) as a holding medium for immature bovine oocytes for *in vitro* embryo production. Anim Reprod 3 (3):376–379

Daniels M, Bartges JW, Raditic DM et al (2017) Evaluation of three herbal compounds used for the management of lower urinary tract disease in healthy cats: a pilot study. J Feline Med Surg. https://doi.org/10.1177/1098612X17748241

da Rocha AA, da Cunha IC, Ederli BB et al (2009) Effect of daily food supplementation with essential fatty acids on canine semen quality. Reprod Domest Anim 44(Suppl 2):313–315

Davidson E, Zimmermann BF, Jungfer E, Chrubasik-Hausmann S (2014) Prevention of urinary tract infections with vaccinium products. Phytother Res 28(3):465–470

Deichsel K, Palm F, Koblischke P et al (2008) Effect of a dietary antioxidant supplementation on semen quality in pony stallions. Theriogenology 69(8):940–945

De Koster JD, Opsomer G (2013) Insulin resistance in dairy cows. Vet Clin North Am Food Anim Pract 29(2):299–322

Del Prete C, Tafuri S, Ciani F et al (2018) Influences of dietary supplementation with *Lepidium meyenii* (Maca) on stallion sperm production and on preservation of sperm quality during storage at 5 degrees C. Andrology 6(2):351–361

de Souza P, Boeing T, Somensi LB et al (2017) Diuretic effect of extracts, fractions and two compounds 2alpha,3beta,19alpha-trihydroxy-urs-12-en-28-oic acid and 5-hydroxy-3,6,7,8,4′-pentamethoxyflavone from *Rubus rosaefolius* Sm. (Rosaceae) leaves in rats. Naunyn Schmiedebergs Arch Pharmacol 390 (4):351–360

Diel de Amorim M, Nielsen K, Cruz RK et al (2016) Progesterone levels and days to luteolysis in mares treated with intrauterine fractionated coconut oil. Theriogenology 86(2):545–550

Dietz BM, Hajirahimkhan A, Dunlap TL et al (2016) Botanicals and their bioactive phytochemicals for women's health. Pharmacol Rev 68(4):1026–1073

Eder K (2009) Influence of l-carnitine on metabolism and performance of sows. Br J Nutr 102(5):645–654

El Allali K, Sghiri A, Bouaouda H et al (2018) Effect of melatonin implants during the non-breeding season on the onset of ovarian activity and the plasma prolactin in dromedary camel. Front Vet Sci 5:44

Elbe H, Dogan Z, Taslidere E et al (2016) Beneficial effects of quercetin on renal injury and oxidative stress caused by ciprofloxacin in rats: a histological and biochemical study. Hum Exp Toxicol 35 (3):276–281

Esmaeili V, Shahverdi AH, Alizadeh AR et al (2014) Saturated, omega-6 and omega-3 dietary fatty acid effects on the characteristics of fresh, frozen-thawed semen and blood parameters in rams. Andrologia 46(1):42–49

Fathi M, El-Shahat KH (2017) L-carnitine enhances oocyte maturation and improves in vitro development of embryos in dromedary camels (*Camelus dromedaries*). Theriogenology 104:18–22

Feng YL, Lei P, Tian T et al (2013) Diuretic activity of some fractions of the epidermis of *Poria cocos*. J Ethnopharmacol 150(3):1114–1118

Fischer M, Varady J, Hirche F et al (2009) Supplementation of L-carnitine in pigs: absorption of carnitine and effect on plasma and tissue carnitine concentrations. Arch Anim Nutr 63(1):1–15

Freitas ML, Bouéres CS, Pignataro TA et al (2016) Quality of fresh, cooled, and frozen semen from stallions supplemented with antioxidants and fatty acids. J Equine Vet Sci 46:1–6

Garcia-Ispierto I, Abdelfatah A, Lopez-Gatius F (2013) Melatonin treatment at dry-off improves reproductive performance postpartum in high-producing dairy cows under heat stress conditions. Reprod Domest Anim 48(4):577–583

Garmsir AK, Shahneh AZ, Jalali SMA et al (2014) Effects of dietary thyme (*Thymus vulgaris*) and fish oil on semen quality of miniature Caspian horse. J Equine Vet Sci 34:1069–1075

Gessner DK, Schlegel G, Ringseis R et al (2014) Up-regulation of endoplasmic reticulum stress induced genes of the unfolded protein response in the liver of periparturient dairy cows. BMC Vet Res 10:46

Ghalayini IF, Al-Ghazo MA, Harfeil MN (2011) Prophylaxis and therapeutic effects of raspberry (*Rubus idaeus*) on renal stone formation in Balb/c mice. Int Braz J Urol 37(2):259–266

Gibb Z, Butler TJ, Morris LH et al (2013) Quercetin improves the postthaw characteristics of cryopreserved sex-sorted and nonsorted stallion sperm. Theriogenology 79(6):1001–1009

Gibb Z, Lambourne SR, Quadrelli J et al (2015) L-carnitine and pyruvate are prosurvival factors during the storage of stallion spermatozoa at room temperature. Biol Reprod 93(4):104

Gomes VDR, Ariza PC, Borges NC et al (2018) Risk factors associated with feline urolithiasis. Vet Res Commun 42(1):87–94

Gonzales GF, Gasco M, Cordova A et al (2004) Effect of *Lepidium meyenii* (Maca) on spermatogenesis in male rats acutely exposed to high altitude (4340 m). J Endocrinol 180(1):87–95

Gonzales-Arimborgo C, Yupanqui I, Montero E et al (2016) Acceptability, safety, and efficacy of oral administration of extracts of black or red maca (*Lepidium meyenii*) in adult human subjects: a randomized, double-blind, placebo-controlled study. Pharmaceuticals (Basel) 9 (3):49

Granaci V (2007) Achievements in the artificial insemination of swine. Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca. Anim Sci Biotechnol (Bull USAMV-CN) 63–64:382–386

Gulliver CE, Friend MA, King BJ et al (2012) The role of omega-3 polyunsaturated fatty acids in reproduction of sheep and cattle. Anim Reprod Sci 131(1–2):9–22

Hall JA, MacLeay J, Yerramilli M et al (2016) Positive impact of nutritional interventions on serum symmetric dimethylarginine and creatinine concentrations in client-owned geriatric dogs. PLoS One 11(4):e0153653

Hermann M, Bernet T (2009) The transition of maca from neglect to market prominence. Agricultural Biodiversity and Livelihood Papers 1. Biodiversity International, Rome, Italy

Hill AS, Werner JA, Rogers QR et al (2004) Lipoic acid is 10 times more toxic in cats than reported in humans, dogs or rats. J Anim Physiol Anim Nutr (Berl) 88(3–4):150–156

Holst L, Haavik S, Nordeng H (2009) Raspberry leaf—should it be recommended to pregnant women? Complement Ther Clin Pract 15 (4):204–208

Jacyno E, Kolodziej A, Kamyczek M et al (2007) Effect of L-carnitine supplementation on boar semen quality. Acta Vet Brno 75:595–600
Jayakumar S, Sathiskumar S, Baskaran N et al (2017) Ethnoveterinary practices in Southern India for captive Asian elephant ailments. J Ethnopharmacol 200:182–204
Jeong JK, Choi IS, Moon SH et al (2018) Effect of two treatment protocols for ketosis on the resolution, postpartum health, milk yield, and reproductive outcomes of dairy cows. Theriogenology 106:53–59
Johansson B, Persson Waller K, Jensen SK et al (2014) Status of vitamins E and A and beta-carotene and health in organic dairy cows fed a diet without synthetic vitamins. J Dairy Sci 97 (3):1682–1692
Johnson JR, Makaji E, Ho S et al (2009) Effect of maternal raspberry leaf consumption in rats on pregnancy outcome and the fertility of the female offspring. Reprod Sci 16(6):605–609
Jones WE (1997) Nutraceuticals for equine practice. Equine Pract 17 (11):562–572
Kargar R, Forouzanfar M, Ghalamkari G et al (2017) Dietary flax seed oil and/or vitamin E improve sperm parameters of cloned goats following freezing-thawing. Cryobiology 74:110–114
Kelley D, LeBlanc MM, Warren LK et al (2014) Influence of L-arginine supplementation on reproductive blood flow and embryo recovery rates in mares. Theriogenology 81(5):752–757
Keralapurath MM, Corzo A, Pulikanti R et al (2010) Effects of *in ovo* injection of L-carnitine on hatchability and subsequent broiler performance and slaughter yield. Poult Sci 89(7):1497–1501
Khoshvaght A, Towhidi A, Zare-Shahneh A et al (2016) Dietary n-3 PUFAs improve fresh and post-thaw semen quality in Holstein bulls via alteration of sperm fatty acid composition. Theriogenology 85 (5):807–812
Kidd MT, McDaniel CD, Peebles ED et al (2005) Breeder hen dietary L-carnitine affects progeny carcass traits. Br Poult Sci 46(1):97–103
King SS, Young DA, Nequin LG et al (2000) Use of specific sugars to inhibit bacterial adherence to equine endometrium in vitro. Am J Vet Res 61(4):446–449
Kirchhoff KT, Failing K, Goericke-Pesch S (2017) Effect of dietary vitamin E and selenium supplementation on semen quality in Cairn Terriers with normospermia. Reprod Domest Anim 52(6):945–952
Knitlova D, Hulinska P, Jeseta M et al (2017) Supplementation of l-carnitine during *in vitro* maturation improves embryo development from less competent bovine oocytes. Theriogenology 102:16–22
Korhonen HT, Huuki H (2015) Effect of carotenoid supplement on production performance in mink (*Neovison vison*). Open J Vet Med 5(4):73–79
Kozink DM, Estienne MJ, Harper AF et al (2004) Effects of dietary L-carnitine supplementation on semen characteristics in boars. Theriogenology 61(7–8):1247–1258
Kuhl J, Aurich JE, Wulf M et al (2012) Effects of oral supplementation with beta-carotene on concentrations of beta-carotene, vitamin A and alpha-tocopherol in plasma, colostrum and milk of mares and plasma of their foals and on fertility in mares. J Anim Physiol Anim Nutr (Berl) 96(3):376–384
Kumar R, Bharati KA (2013) New claims in folk veterinary medicines from Uttar Pradesh, India. J Ethnopharmacol 146(2):581–593
Kutzler MA (2015) Alternative methods for feline fertility control: use of melatonin to suppress reproduction. J Feline Med Surg 17 (9):753–757
Lans C, Turner N, Khan T et al (2007) Ethnoveterinary medicines used for ruminants in British Columbia, Canada. J Ethnobiol Ethnomed 3:11
Lans C, Turner N, Brauer G et al (2009) Medicinal plants used in British Columbia, Canada for reproductive health in pets. Prev Vet Med 90 (3–4):268–273
Lee MS, Lee HW, You S et al (2016) The use of maca (*Lepidium meyenii*) to improve semen quality: a systematic review. Maturitas 92:64–69
Lemley CO, Vonnahme KA (2017) Physiology and endocrinology symposium: alterations in uteroplacental hemodynamics during melatonin supplementation in sheep and cattle. J Anim Sci 95 (5):2211–2221
Lenzi A, Lombardo F, Sgro P et al (2003) Use of carnitine therapy in selected cases of male factor infertility: a double-blind crossover trial. Fertil Steril 79(2):292–300
Leon J (1964) The "Maca" (*Lepidium meyenii*), a little known food plant of Peru. Econ Bot 18(2):122–127
Li X, Zhang JY, Gao WY et al (2012) Chemical composition and anti-inflammatory and antioxidant activities of eight pear cultivars. J Agric Food Chem 60(35):8738–8744
Lisboa FL, Hartwig FP, Maziero RRD et al (2012) Use of L-carnitine and acetyl-L-carnitine in cooled-stored stallion semen. J Equine Vet Sci 32(8):493–494
Loftin EG, Herold LV (2009) Therapy and outcome of suspected alpha lipoic acid toxicity in two dogs. J Vet Emerg Crit Care (San Antonio) 19(5):501–506
London KT, Christensen BW, Scott CJ et al (2017) The effects of an oxygen scavenger and coconut water on equine sperm cryopreservation. J Equine Vet Sci 58:51–57
Longobardi V, Salzano A, Campanile G et al (2017) Carnitine supplementation decreases capacitation-like changes of frozen-thawed buffalo spermatozoa. Theriogenology 88:236–243
Lowe JL, Bartolac LK, Bathgate R et al (2017) Cryotolerance of porcine blastocysts is improved by treating *in vitro* matured oocytes with L-carnitine prior to fertilization. J Reprod Dev 63(3):263–270
Manfredini R, De Giorgi A, Storari A et al (2016) Pears and renal stones: possible weapon for prevention? A comprehensive narrative review. Eur Rev Med Pharmacol Sci 20(3):414–425
Martins FS, Van den Hurk R, Santos RR et al (2005) Development of goat primordial follicles after in vitro culture of ovarian tissue in minimal essential medium supplemented with coconut water. Anim Repord 2(2):106–113
Masoudi R, Sharafi M, Zare Shahneh A et al (2016) Effect of dietary fish oil supplementation on ram semen freeze ability and fertility using soybean lecithin- and egg yolk-based extenders. Theriogenology 86 (6):1583–1588
Mavangira V, Sordillo LM (2018) Role of lipid mediators in the regulation of oxidative stress and inflammatory responses in dairy cattle. Res Vet Sci 116:4–14
Mayasari N, Chen J, Ferrari A et al (2017) Effects of dry period length and dietary energy source on inflammatory biomarkers and oxidative stress in dairy cows. J Dairy Sci 100(6):4961–4975
Mehta M, Goldfarb DS, Nazzal L (2016) The role of the microbiome in kidney stone formation. Int J Surg 36(Pt D):607–612
Miller AW, Dearing D (2013) The metabolic and ecological interactions of oxalate-degrading bacteria in the mammalian gut. Pathogens 2 (4):636–652
Moallem U, Shafran A, Zachut M et al (2013) Dietary alpha-linolenic acid from flaxseed oil improved folliculogenesis and IVF performance in dairy cows, similar to eicosapentaenoic and docosahexaenoic acids from fish oil. Reproduction 146(6):603–614
Moolla A, Viljoen AM (2008) 'Buchu'—*Agathosma betulina* and *Agathosma crenulata* (Rutaceae): a review. J Ethnopharmacol 119 (3):413–419
Morris L, Gibb Z (2016) Oral supplementation with L-carnitine improves stallion fertility. J Equine Vet Sci 43:S81
Mourvaki E, Cardinali R, Dal Bosco A et al (2010) Effects of flaxseed dietary supplementation on sperm quality and on lipid composition of sperm subfractions and prostatic granules in rabbit. Theriogenology 73(5):629–637
Mousa-Balabel TM (2011) Using light and melatonin in the management of New Zealand White rabbits. Open Vet J 1(1):1–6
Mura MC, Luridiana S, Farci F et al (2017) Melatonin treatment in winter and spring and reproductive recovery in Sarda breed sheep. Anim Reprod Sci 185:104–108

Murphy EM, Stanton C, Brien CO et al (2017) The effect of dietary supplementation of algae rich in docosahexaenoic acid on boar fertility. Theriogenology 90:78–87

Musser RE, Goodband RD, Tokach MD et al (1999) Effects of L-carnitine fed during gestation and lactation on sow and litter performance. J Anim Sci 77(12):3289–3295

Naber KG (2013) Efficacy and safety of the phytotherapeutic drug Canephron$^{(R)}$ N in prevention and treatment of urogenital and gestational disease: review of clinical experience in Eastern Europe and Central Asia. Res Rep Urol 5:39–46

Neuman SL, Lin TL, Heste PY (2002) The effect of dietary carnitine on semen traits of White Leghorn roosters. Poult Sci 81(4):495–503

Ng CM, Blackman MR, Wang C et al (2004) The role of carnitine in the male reproductive system. Ann N Y Acad Sci 1033:177–188

Nirumand MC, Hajialyani M, Rahimi R et al (2018) Dietary plants for the prevention and management of kidney stones: preclinical and clinical evidence and molecular mechanisms. Int J Mol Sci 19 (3):765

Partyka A, Rodak O, Bajzert J et al (2017) The effect of L-carnitine, hypotaurine, and taurine supplementation on the quality of cryopreserved chicken semen. Biomed Res Int 2017:7279341

Paulenz H, Taugbol O, Hofmo PO et al (1995) A preliminary study on the effect of dietary supplementation with cod liver oil on the polyunsaturated fatty acid composition of boar semen. Vet Res Commun 19(4):273–284

Peck AB, Canales BK, Nguyen CQ (2016) Oxalate-degrading microorganisms or oxalate-degrading enzymes: which is the future therapy for enzymatic dissolution of calcium-oxalate uroliths in recurrent stone disease? Urolithiasis 44(1):45–50

Peebles ED, Kidd MT, McDaniel CD et al (2007) Effects of breeder hen age and dietary L-carnitine on progeny embryogenesis. Br Poult Sci 48(3):299–307

Phongnimitr T, Liang Y, Srirattana K et al (2013) Effect of L-carnitine on maturation, cryo-tolerance and embryo developmental competence of bovine oocytes. Anim Sci J 84(11):719–725

Pollen SM (2001) Renal disease in small animals: a review of conditions and potential nutrient and botanical interventions. Altern Med Rev 6 (Suppl):S46–S61

Pontes GC, Monteiro PL Jr, Prata AB et al (2015) Effect of injectable vitamin E on incidence of retained fetal membranes and reproductive performance of dairy cows. J Dairy Sci 98(4):2437–2449

Pruneda A, Yeung CH, Bonet S et al (2007) Concentrations of carnitine, glutamate and myo-inositol in epididymal fluid and spermatozoa from boars. Anim Reprod Sci 97(3–4):344–355

Qian SZ (1987) *Tripterygium wilfordii*, a Chinese herb effective in male fertility regulation. Contraception 36(3):335–345

Quiroz-Rocha GF, LeBlanc S, Duffield T et al (2009) Evaluation of prepartum serum cholesterol and fatty acids concentrations as predictors of postpartum retention of the placenta in dairy cows. J Am Vet Med Assoc 234(6):790–793

Raditic DM (2015) Complementary and integrative therapies for lower urinary tract diseases. Vet Clin North Am Small Anim Pract 45 (4):857–878

Ramanau A, Kluge H, Spilke J et al (2002) Reproductive performance of sows supplemented with dietary L-carnitine over three reproductive cycles. Arch Tierernahr 56(4):287–296

Ramanau A, Kluge H, Eder K (2005) Effects of L-carnitine supplementation on milk production, litter gains and back-fat thickness in sows with a low energy and protein intake during lactation. Br J Nutr 93 (5):717–721

Ratliff BB, Abdulmahdi W, Pawar R et al (2016) Oxidant mechanisms in renal injury and disease. Antioxid Redox Signal 25(3):119–146

Reader KL, Cox NR, Stanton JA et al (2015) Effects of acetyl-L-carnitine on lamb oocyte blastocyst rate, ultrastructure, and mitochondrial DNA copy number. Theriogenology 83(9):1484–1492

Rebouche CJ (2004) Kinetics, pharmacokinetics, and regulation of L-carnitine and acetyl-L-carnitine metabolism. Ann N Y Acad Sci 1033:30–41

Ren Z, Pan C, Jiang L et al (2011) Oxalate-degrading capacities of lactic acid bacteria in canine feces. Vet Microbiol 152(3–4):368–373

Ringseis R, Keller J, Eder K (2018) Regulation of carnitine status in ruminants and efficacy of carnitine supplementation on performance and health aspects of ruminant livestock: a review. Arch Anim Nutr 72(1):1–30

Risso AL, Pellegrino FJ, Corrada Y et al (2017) Effect of fish oil and vitamin E on sperm lipid peroxidation in dogs. J Nutr Sci 6:e48

Rodrigues AC, Ruiz CM, De Nardo CD et al (2017) Effect of dietary supplementation with omega-3 and -6 on fresh and frozen/thawed sperm quality of dogs. Semina Ciênc Agrár 38(5):3069–3076

Saber TM, Abd El-Aziz RM, Ali HA (2016) Quercetin mitigates fenitrothion-induced testicular toxicity in rats. Andrologia 48 (5):491–500

Santymire RM, Lavin SR, Branvold-Faber H et al (2015) Effect of dietary vitamin E and prey supplementation on semen quality in male black-footed ferrets (*Mustela nigripes*). Theriogenology 84 (2):217–225

Saraiva H, Batista R, Alfradique VAP et al (2018) L-carnitine supplementation during vitrification or warming of *in vivo*-produced ovine embryos does not affect embryonic survival rates, but alters CrAT and PRDX1 expression. Theriogenology 105:150–157

Saxena VK (2017) Physiology of melatonin, melatonin receptors, and their role in the regulation of reproductive behavior in animals. In: Ravishankara GA, Ravishankara AR (eds) Serotonin and melatonin their functional role in plants, food, phytomedicine, and human health. CRC, Boca Raton, FL

Schafer-Somi S (2017) Effect of melatonin on the reproductive cycle in female cats: a review of clinical experiences and previous studies. J Feline Med Surg 19(1):5–12

Schmid-Lausigk Y, Aurich C (2014) Influences of a diet supplemented with linseed oil and antioxidants on quality of equine semen after cooling and cryopreservation during winter. Theriogenology 81 (7):966–973

Schulman A, Chaimowitz M, Choudhury M et al (2016) Antioxidant and renoprotective effects of mushroom extract: implication in prevention of nephrolithiasis. J Clin Med Res 8(12):908–915

Seifi-Jamadi A, Ahmad E, Ansari M et al (2017) Antioxidant effect of quercetin in an extender containing DMA or glycerol on freezing capacity of goat semen. Cryobiology 75:15–20

Shah SM, Ali S, Zubair M et al (2016) Effect of supplementation of feed with flaxseed (*Linum usitatissimum*) oil on libido and semen quality of Nilli-Ravi buffalo bulls. J Anim Sci Technol 58:25

Sheikh N, Goodarzi M, Bab Al-Havaejee H et al (2007) L-carnitine level in seminal plasma of fertile and infertile men. J Res Health Sci 7 (1):43–48

Sheng W, Zhang YS, Li YQ et al (2017) Effect of Yishenjianpi recipe on semen quality and sperm mitochondria in mice with oligoasthenozoospermia induced by tripterygium glycosides. Afr J Tradit Complement Altern Med 14(4):87–95

Showell MG, Mackenzie-Proctor R, Jordan V et al (2017) Antioxidants for female subfertility. Cochrane Database Syst Rev 7:CD007807

Silva CG, Cunha ER, Blume GR et al (2015) Cryopreservation of boar sperm comparing different cryoprotectants associated in media based on powdered coconut water, lactose and trehalose. Cryobiology 70(2):90–94

Simpson M, Parsons M, Greenwood J et al (2001) Raspberry leaf in pregnancy: its safety and efficacy in labor. J Midwifery Womens Health 46(2):51–59

Smith CA (2010) Homoeopathy for induction of labour (review). Cochrane database of systematic reviews, vol 2003. Cochrane Collaboration, Hoboken, NJ. http://www.cochranelibrary.com/

Sprando RL, Collins TF, Black TN et al (2000a) The effect of maternal exposure to flaxseed on spermatogenesis in F(1) generation rats. Food Chem Toxicol 38(4):325–334

Sprando RL, Collins TF, Wiesenfeld P et al (2000b) Testing the potential of flaxseed to affect spermatogenesis: morphometry. Food Chem Toxicol 38(10):887–892

Stradaioli G, Sylla L, Zelli R et al (2000) Seminal carnitine and acetylcarnitine content and carnitine acetyltransferase activity in young Maremmano stallions. Anim Reprod Sci 64 (3–4):233–245

Stradaioli G, Sylla L, Zelli R et al (2004) Effect of L-carnitine administration on the seminal characteristics of oligoasthenospermic stallions. Theriogenology 62(3–4):761–777

Sychev DA, Semenov AV, Polyakova IP (2011) A case of hepatic injury suspected to be caused by Canephron N, a Centaurium Hill containing phytotherapeutics. Int J Risk Saf Med 23(1):5–6

Tahraoui A, Israil ZH, Lyoussi B (2010) Acute and sub-chronic toxicity of a lyophilised aqueous extract of *Centaurium erythraea* in rodents. J Ethnopharmacol 132(1):48–55

Tarig AA, Wahid H, Rosnina Y et al (2017) Effect of different concentrations of egg yolk and virgin coconut oil in tris-based extenders on chilled and frozen-thawed bull semen. Anim Reprod Sci 182:21–27

Trombetta MF, Accorsi PA, Falaschini A (2010) Effect of beta-carotene supplementation on Italian Trotter mare peripartum. J Equine Sci 21 (1):1–6

Uchoa DC, Silva TF, Mota Filho AC et al (2012a) Intravaginal artificial insemination in bitches using frozen/thawed semen after dilution in powdered coconut water (ACP-106c). Reprod Domest Anim 47 (Suppl 6):289–292

Uchoa DC, da Silva TF, Cardoso Jde F et al (2012b) Favoring the birth of female puppies after artificial insemination using chilled semen diluted with powdered coconut water (ACP-106c). Theriogenology 77(9):1959–1963

Uematsu M, Kitahara G, Sameshima H et al (2016) Serum selenium and liposoluble vitamins in Japanese Black cows that had stillborn calves. J Vet Med Sci 78(9):1501–1504

Uncini Manganelli RE, Camangi F, Tomei PE (2001) Curing animals with plants: traditional usage in Tuscany (Italy). J Ethnopharmacol 78(2–3):171–191

Upadhyay B, Singh KP, Kumar A (2011) Ethnoveterinary uses and informants consensus factor of medicinal plants of Sariska Region, Rajasthan, India. J Ethnopharmacol 133(1):14–25

Van Blerkom J (2011) Mitochondrial function in the human oocyte and embryo and their role in developmental competence. Mitochondrion 11(5):797–813

Vrolijk MF, Opperhuizen A, Jansen EH et al (2015) The shifting perception on antioxidants: the case of vitamin E and beta-carotene. Redox Biol 4:272–278

Wang M, Chen DQ, Chen L et al (2018) Novel RAS inhibitors poricoic acid ZG and poricoic acid ZH attenuate renal fibrosis via a Wnt/beta-Catenin pathway and targeted phosphorylation of smad3 signaling. J Agric Food Chem 66(8):1828–1842

Wathes DC, Abayasekara DR, Aitken RJ (2007) Polyunsaturated fatty acids in male and female reproduction. Biol Reprod 77 (2):190–201

Wen JJ, Johnston K (2012) Treatment of urolithiasis in 33 dogs and 13 cats with a novel Chinese herbal medicine. Am J Tradit Chin Vet Med 7(2):39–45

Wilsher S, Allen WR (2011) Intrauterine administration of plant oils inhibits luteolysis in the mare. Equine Vet J 43(1):99–105

Winkler A, Gessner DK, Koch C et al (2015) Effects of a plant product consisting of green tea and curcuma extract on milk production and the expression of hepatic genes involved in endoplasmic stress response and inflammation in dairy cows. Arch Anim Nutr 69 (6):425–441

Wonnacott KE, Kwong WY Hughes J et al (2010) Dietary omega-3 and -6 polyunsaturated fatty acids affect the composition and development of sheep granulosa cells, oocytes and embryos. Reproduction 139(1):57–69

Xi L, Brown K, Woodworth J et al (2008) Maternal dietary L-carnitine supplementation influences fetal carnitine status and stimulates carnitine palmitoyltransferase and pyruvate dehydrogenase complex activities in swine. J Nutr 138(12):2356–2362

Yeste M, Sancho S, Briz M et al (2010) A diet supplemented with L-carnitine improves the sperm quality of Pietrain but not of Duroc and Large White boars when photoperiod and temperature increase. Theriogenology 73(5):577–586

Yoshida K, Ohta Y, Kawate N et al (2018) Long-term feeding of hydroalcoholic extract powder of *Lepidium meyenii* (maca) enhances the steroidogenic ability of Leydig cells to alleviate its decline with ageing in male rats. Andrologia 50(1):e12803

Yue D, Yan L, Luo H et al (2010) Effect of vitamin E supplementation on semen quality and the testicular cell membranal and mitochondrial antioxidant abilities in Aohan fine-wool sheep. Anim Reprod Sci 118(2–4):217–222

Zeyner A, Harmeyer J (1999) Metabolic functions of L-carnitine and its effects as feed additive in horses. A review. Arch Tierernahr 52 (2):115–138

Zhai W, Neuman SL, Latour MA et al (2007) The effect of dietary L-carnitine on semen traits of White Leghorns. Poult Sci 86 (10):2228–2235

Zhai W, Neuman S, Latour MA et al (2008a) The effect of in ovo injection of L-carnitine on hatchability of white leghorns. Poult Sci 87(3):569–572

Zhai W, Neuman SL, Latour MA et al (2008b) The effect of male and female supplementation of L-carnitine on reproductive traits of white leghorns. Poult Sci 87(6):1171–1181

Zhang J, McCullough PA (2016) Lipoic acid in the prevention of acute kidney injury. Nephron 134(3):133–140

Zhang Y, Zhang Z, Yang Y et al (2011) Diuretic activity of *Rubus idaeus* L (Rosaceae) in rats. Trop J Pharm Res 10(3):243–248

Zhang L, Ravipati AS, Koyyalamudi SR et al (2013) Antifungal and antibacterial activities of ethanol extracts of selected traditional Chinese medicinal herbs. Asian Pac J Trop Med 6(9):673–681

Zhao YY, Li HT, Feng YL et al (2013) Urinary metabonomic study of the surface layer of *Poria cocos* as an effective treatment for chronic renal injury in rats. J Ethnopharmacol 148(2):403–410

Zhao J, Jin Y, Du M et al (2017) The effect of dietary grape pomace supplementation on epididymal sperm quality and testicular antioxidant ability in ram lambs. Theriogenology 97:50–56

Zhao S, Liu H, Gu L (2018) American cranberries and health benefits—an evolving story of 25 years. J Sci Food Agric. https://doi.org/10.1002/jsfa.8882

Zheng BL, He K, Kim CH et al (2000) Effect of a lipidic extract from *Lepidium meyenii* on sexual behavior in mice and rats. Urology 55 (4):598–602

Zicker SC, Avila A, Joshi DK et al (2010) Pharmacokinetics of orally administered DL-alpha-lipoic acid in dogs. Am J Vet Res 71 (11):1377–1383

Zingg JM (2015) Vitamin E: a role in signal transduction. Annu Rev Nutr 35:135–173

Nutraceuticals in Obesity and Metabolic Disorders

Rhian B. Cope

Abstract

This chapter examines a number of proposed nutraceuticals. Functional foods are not specifically addressed. Each section of the chapter is divided into an evaluation of the "proof-of-concept" studies that purport to demonstrate efficacy, an examination of the claimed modes and mechanisms of action of each nutraceutical, and, where available, a brief discussion of any identified key adverse effects. Nutraceutical agents covered include curcumin, *Lagenaria siceraria* (bottle gourd), *Trigonella foenum-graecum* (fenugreek), *Emblica officinalis* (Indian gooseberry), *Murraya koenigii* (curry tree), *Vigna* sp. (black gram), *Camellia sinensis* (tea), *Hibiscus sabdariffa*, *Hypericum perforatum* (St. John's wort), avocado, capsicum, and rosemary. While potential nutraceutical products and ingredients continue to be a frequent source of "proof-of-concept" scientific publications, high-quality human clinical trial data is often lacking. Substantial translational scientific work is still needed for many nutraceuticals in terms of assessing their safety and demonstrating their efficacy in humans and animals.

Keywords

Veterinary nutraceuticals · Obesity · Metabolic disorders

1 Introduction

In this chapter, the term nutraceuticals will be considered separately from the functional foods concept. A nutraceutical is defined as a pharmaceutical-grade and standardized nutrient (or dietary supplement or food additive), whereas a functional food is a food given an additional function by the existence or addition of a specific food component. Foods can, of course, be fortified with a specific nutraceutical, and such products would then be considered a functional food; however, for reasons of expediency and simplicity, this chapter will focus on specific plant species from which various claimed anti-obesity nutraceutical extracts have been derived. Common foods which claim to have anti-obesity effects such as peanuts, citrus fruits, and pomegranates are not covered by this chapter. This chapter is not intended as a review of the pathophysiology of obesity and metabolic syndrome(s); however, a brief introduction to some key concepts in the pathogenesis of these diseases that are relevant to nutraceuticals is provided. Current proposed nutraceuticals are particularly those with claimed effects on dietary fat bioavailability, antioxidative and anti-inflammatory effects, modification of lipogenesis and pre-adipocyte maturation, increased lipid metabolism, and satiety. However, many critical modes of action remain incompletely understood.

2 Curcumin ((1E,6E)-1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione)

Curcumin is the major curcuminoid (linear diarylheptanoid) found in turmeric. Human phase I and phase II clinical trials have demonstrated that this food coloring and flavoring agent are relatively well tolerated (Soleimani et al. 2018). Some trial subjects developed diarrhea, headache, skin rashes, and yellow stools. Overall, oral curcumin dosing at 6 g/day for 4 to 7 weeks was reasonably well tolerated in humans. Orally bioavailable curcumin formulations were reasonably well tolerated following a dosage of 500 mg BID for 30 days. However, there is more limited information on curcumin nano-formulations. Curcumin was not genotoxic in classical assays, was not a reproductive toxin in animals following oral dosing studies, showed no toxic effects in humans, and was

R. B. Cope (✉)
Australian Pesticides and Veterinary Medicines Authority, Armidale, NSW, Australia
e-mail: rhian.Cope@health.gov.au

R. C. Gupta et al. (eds.), *Nutraceuticals in Veterinary Medicine*, https://doi.org/10.1007/978-3-030-04624-8_34

safe at the dose of 6 g/day orally for 4–7 weeks. While curcumin has multiple pharmacological actions, it has poor oral bioavailability and is rapidly excreted (Purpura et al. 2018). Phospholipidation and nanoparticle dose forms co-dosed with piperine have been used to increase oral bioavailability. Curcumin inhibits several CYP450 enzymes, glutathione-*S*-transferases, and UDP glucuronosyltransferases. Thus, its use may be associated with drug interaction effects.

Due to its anti-inflammatory actions, curcumin treatment has been applied to a wide range of inflammatory conditions, including obesity and metabolic disorders. Within the inflammation, signaling milieu curcumin interacts directly with cyclooxygenase 2, DNA polymerase, lipoxygenase, glycogen synthase kinase 3b, and tumor necrosis factor alpha. It also modulates the actions of a number of signal transducers (AP-1, beta-catenin, STAT, and PPARc).

The antioxidative and anti-inflammatory effects of curcumin have been evaluated in several small human clinical trials. In a human double-blind placebo-controlled trial, the effect of curcumin and phospholipidated curcumin on antibody titers to heat shock protein 27 (a biomarker of inflammation and oxidative stress) was evaluated in patients with metabolic syndrome (Mohammadi et al. 2018). Dosing with 1 g/day for 6 weeks with curcumin or phospholipidated curcumin had no significant ($p > 0.6$) effect on heat shock protein 27 titers. However, oral dosing of patients with metabolic syndrome with curcumin (1 g/day, with or without 10 mg/day of piperine) for 6–8 weeks resulted in a significant ($p < 0.05$) increase in serum prooxidant-antioxidant balance (Ghazimoradi et al. 2017; Panahi et al. 2015); however, curcumin dosing had no beneficial effects on circulating levels of vitamin E (Mohammadi et al. 2017). A human double-blind placebo-controlled trial that evaluated the effect of curcumin or phospholipidated curcumin on serum copper, zinc, and zinc/copper ratio demonstrated that, compared with placebo, both curcumin and phospholipidated curcumin complex, given at a dose of 1 g per day for 6 weeks, were associated with a significant ($p < 0.05$) increase in serum zinc and serum zinc/copper ratio in patients with metabolic syndrome (Safarian et al. 2018). In this trial, phospholipidated curcumin (1 g = 200 mg of curcumin) was significantly ($p < 0.05$) more efficacious in this effect of curcumin. This is likely due to the higher bioavailability of the phospholipidated dose form. Based on the results of a double-blind randomized control trial, the effects of curcumin (1 g/day) combined with piperine (10 mg/day) resulted in increased serum adiponectin, decreased serum leptin, and decreased leptin/adiponectin ratio (a biomarker of atherosclerosis) (Panahi et al. 2016, 2017). These changes were claimed to reflect a decrease in the level of systemic inflammation as measured by circulating TNF-alpha levels.

A limited number of human clinical trials have also examined the effects of curcumin on obesity and dyslipidemias. A small, preliminary human randomized clinical trial demonstrated that patients who did not lose body weight (<2% reduction) after 30 days of diet and lifestyle intervention had increased weight loss (about 2–5%) and increased reductions in BMI (about 2–6%) cf. controls after daily oral dosing with 800 mg/day of curcumin complexed with sunflower phospholipids and 8 mg of piperine. The plasma lipid-modifying effects of a bioavailable curcumin complex (1 g/day with 10 mg of piperidine, dosing for 8 weeks) were evaluated in patients who had been diagnosed with metabolic syndrome (Panahi et al. 2014). In this trial, curcumin was more effective than placebo in reducing serum LDL-C, non-HDL-C, total cholesterol, triglycerides, and Lp(a) and elevating HDL-C concentrations. Similar effects were observed in a second trial which utilized TID dosing at 630 mg/day (Yang et al. 2014).

Overall, while there is reasonable quality preliminary, small-scale human clinical trial evidence that bioavailable forms of curcumin may have beneficial effects on metabolic syndrome, obesity, and dyslipidemias, evidence of overall long-term beneficial outcomes in patients with metabolic syndrome and obesity is lacking (Hariri and Haghighatdoost 2018).

3 *Lagenaria siceraria* (Bottle Gourd)

Bottle gourd, a plant native to India, has a long history of ethnopharmacological use. Traditional medical uses of the plant include (1) as an aliuretic, purgative, and laxative; (2) as a diuretic for cardiogenic ascites; (3) for the relief of headache and toothache; (4) as an anticonvulsant; (5) as a treatment for boils, tetanus, fever, wounds, and rheumatism; (6) as a treatment for asthma and coughs; (7) as an anticancer agent; (8) as a treatment for alopecia; and (9) as an antidote for poisoning. The plant is reputed to have cardioprotective, antihyperlipidemic, antioxidative, antihyperglycemic, analgesic, anti-inflammatory, immunomodulatory, and hepatoprotective effects in humans. Fresh bottle gourd juice is considered an effective treatment for obesity in India.

Small controlled studies have claimed that consuming freshly prepared bottle gourd fruit extract once/day for 90 days reduced plasma total cholesterol (marginal), plasma triglycerides, plasma low-density lipoproteins, and BMI as well as having antioxidative properties in humans (Kaur et al. 2015; Katare et al. 2014). These findings are supported by studies in rats where oral dosing of bottle gourd methanol extracts at 100–400 mg/kg body wt/day resulted in dose-dependent reductions in plasma total cholesterol, triglycerides, low-density lipoproteins levels, and increased

plasma high-density lipoproteins (Ghule et al. 2006, 2009). Based on the results of this small study, fresh bottle gourd juice may have some limited beneficial effects on dyslipidemias in humans.

Overall, while there is reasonable preliminary exploratory data on the possible beneficial effects of bottle gourd products on dyslipidemia in humans, evidence of long-term beneficial outcomes in patients is lacking.

4 *Trigonella foenum-graecum* (Fenugreek)

One of the world's oldest medicinal plants, fenugreek, originated in India and North Africa (Kaur et al. 2015). There are three groups of medicinal compounds found in the plant: steroidal saponins, galactomannans, and isoleucine. Fenugreek is an important source of the drug precursor diosgenin which is used in the synthesis of steroidal drugs.

While fenugreek is generally considered a safe product, patients with allergies to the plant or allergies to chick peas (cross-reactive with fenugreek) should avoid it (Kaur et al. 2015). Consumption of fenugreek is known to cause dizziness, transient diarrhea, and flatulence. Hypoglycemia and reduced T3 levels have been reported in some patients. Fenugreek contains coumarin and consumption may result in increased prothrombin times and a bleeding tendency. Fenugreek should be avoided during pregnancy because it stimulates uterine contractions in animal studies. Because fenugreek is rich in dietary fiber it may decrease the oral bioavailability of drugs. Since fenugreek induces hypoglycemia, concurrent use with other hypoglycemic agents may exacerbate their pharmacological effects.

The ability of fenugreek preparations to attenuate postprandial glycemia and insulinemia is reputed to be due to its soluble dietary fiber effects (Repin et al. 2017). These effects are claimed to be due to modification of ingesta viscosity and delaying of gastric emptying. The effects of fenugreek soluble dietary fiber on these types of parameters are about the same as with other sources of soluble dietary fiber.

The fenugreek steroidal saponins are claimed to have antihypercholesterolemia and anti-hyperlipidemia effects in humans (Kassaian et al. 2009). The mechanism of these effects is not completely understood but may be a manifestation of increased soluble dietary fiber intake.

Galactomannans are polymers of galactose and mannose present in the seeds of fenugreek. They are hydrophilic and form viscous aqueous solutions at low concentrations (Kaur et al. 2015) and have been used as gastric "fillers" in an attempt to stimulate satiety and decrease caloric intake. Isoleucine is an amino acid precursor which regulates insulin secretion.

5 *Emblica officinalis* (syn. *Phyllanthus emblica*; Indian Gooseberry)

Indian gooseberry is an important dietary source of vitamin C and minerals in many areas of India and has a long history of use as a traditional medicine (treatment of fever, anti-inflammatory, diuretic, hair and liver tonic, and digestive aid) (Kaur et al. 2015). The fruit also has religious significance and has been used to make ink due to its high tannin content.

Preparations of Indian gooseberry have been demonstrated to decrease serum cholesterol, low-density lipoprotein, and triglycerides in humans and are PPAR-alpha agonists (Kaur et al. 2015; Akhtar et al. 2011). They are also reported to exert some of their effects due to their antioxidant and free radical scavenging properties (D'souza et al. 2014).

6 *Murraya koenigii* (Curry Tree)

The leaves of this plant are commonly used in Indian cooking (typically in curries). The plant also has a long history of use as a traditional medicine, and it is known for having antioxidant, antitumor, anti-inflammatory, antihyperglycemic, hypoglycemic, and hypolipidemic effects (Kaur et al. 2015). Curry tree products act as hypoglycemics by increasing hepatic glycogen storage and decreasing gluconeogenesis. Part of the nutraceutical effects of the curry tree may be due to the actions of the carbazole alkaloid mahanimbine. In mice, mahanimbine inhibits the obesogenic effects of a high-fat diet including reducing body weight gain; preventing hyperlipidemia, fat accumulation in adipose tissue, and hepatic steatosis; improving glucose clearance; and improving insulin responsiveness (Jagtap et al. 2017).

7 *Vigna* sp. (Black Gram, Black Lentil, Cow Pea, Black-Eyed Pea, Adzuki Bean, Mung Bean)

Black lentils (*Vigna mungo*) have been used as a safe, high-protein traditional food source in Nepal and India for millennia. The claimed benefits of consuming black lentils are (Kaur et al. 2015):

- The high-fiber, low glycemic index properties of this food are supposedly able to modulate lipid homeostasis in people with a high saturated fat diet.
- The high-fiber, low glycemic index properties of this food reputedly help to maintain blood glucose control in people with diabetes mellitus.

- Diets reputedly high in black lentils have some benefit in controlling body weight since they are claimed to have satiety effects, thus limiting overall food consumption.
- Black lentils inhibit alpha amylase. Alpha amylase inhibition is known to delay carbohydrate absorption and to reduce peak postprandial plasma glucose concentration.

Other members of the *Vigna* genus are reputed to have similar effects. In a human randomized double-blind crossover trial, consumption of *V. unguiculata* protein reduced total cholesterol (12%), LDL cholesterol (18.9%), non-HDL cholesterol (16%), and apoB (14%) and increased HDL cholesterol (+2.7%); however, no effects on the serum inflammasome were noted (Frota Kde et al. 2015). Adzuki bean consumption by mice with high-fat diet-induced nonalcoholic liver disease resulted in reduced fasting blood and hepatic triglyceride and cholesterol levels with evidence of effects on hepatic lipogenesis and decreased inflammation (Kim et al. 2015).

8 *Camellia sinensis* (Tea)

Claims regarding the health benefits of the three main types of tea, black, oolong, and green, extend back in history for thousands of years. More research effort has been devoted to *Camellia sinensis* than any other potential nutraceutical. However, despite this substantial research effort, a definitive consensus on the health benefits and appropriate uses of tea as nutraceuticals and complimentary medicines has not yet been reached (Jurgens et al. 2012).

There are three main categories of claimed beneficial nutraceutical compounds in tea:

- Xanthine bases and methylxanthines (caffeine, theophylline, and theobromine)
- Essential oils
- Polyphenolic compounds (notably epigallocatechin, epigallocatechin gallate, epicatechin gallate, and gallocatechin gallate)

Various studies have demonstrated that tea phytochemicals appear to have beneficial effects on obesity and metabolic syndrome in high-fat diet animal models. The following studies are representative of the findings in rodents. Green tea phytochemicals administered in drinking water to Wistar rats at concentrations up to 3.2 g/L over a period of 26 weeks reduced visceral adipose tissue accumulation by about 40% versus control (no dose response) (Tian et al. 2013). The effect was claimed to be due to modulation of the erk1/2-PPARγ-adiponectin pathway. Oral dosing of mice fed a high-fat diet containing 400 mg/kg body wt/day of green tea extract (containing 15 μg/mg epigallocatechin, 95 μg/mg epigallocatechin gallate, 20.8 μg/mg epicatechin gallate, and 4.9 μg/mg gallocatechin gallate) for 8 weeks was associated with an increase in hormone-sensitive lipase and perilipin in mesenteric adipose tissue, thus reputedly increasing lipolysis and reducing low-grade inflammation (as measured by TNF-alpha levels and reductions in TLR4, MYD88, and TRAF6 proinflammatory signaling) and reducing body weight by about 16% versus control (Cunha et al. 2013).The anti-obesity effects of tea are not only associated with leaf and leaf extract products. Ethanolic extracts of the fruit peel of the tea plant which is normally regarded as an agricultural waste product have been demonstrated to have anti-obesity effects in rats (Chaudhary et al. 2014). In this study, administration of 100 mg/kg body wt/day of an ethanolic green tea fruit peel extract as part of a high-fat diet fed to rats for 50 days resulted in about a 25% reduction in body weight and a 74% reduction in adipose tissue fat pad weights versus control.

Suggested modes of action of tea extracts on obesity and metabolic syndromes include inhibition of pancreatic lipase, appetite-repression activity, downregulation of adipogenesis, thermogenesis, alterations in lipid metabolism including increased lipolysis and decreased lipogenesis, increased antioxidant defenses, changes in gut microflora, increased whole-body fat utilization, increased fat oxidation, and anti-inflammatory effects (Grove et al. 2012; Yuda et al. 2012; Moon et al. 2007; Wolfram et al. 2006; Lu et al. 2012; Axling et al. 2012; Basu et al. 2010; Park et al. 2011).

The presumed major phytochemical of tea is (−)-epigallocatechin 3-gallate within the polyphenol fraction, responsible for anti-obesity effect.

The safety properties of tea have been best studied for green tea preparations. The consumption of moderate amounts of green tea as a beverage is currently regarded as safe. Concentrated great tea extracts have reportedly been associated with hepatotoxicity in a small number of people. However, the US National Center for Complementary and Integrative Health has concluded that in the case of green tea "definite conclusions cannot yet be reached on whether green tea is helpful for most of the purposes for which it is used" (National Center for Complementary and Integrative Health 2016).

9 *Hibiscus sabdariffa*

This well-studied plant is commonly prepared as a soft drink and is used to reduce inflammation, hypertension, and liver disorders. In the monosodium glutamate-induced obese mouse model, *Hibiscus sabdariffa* calyces aqueous extract administered at 120 mg/kg body wt/day for 60 days resulted

in an approximately 22% reduction in body weight gain versus control and reduced liver steatosis (Alarcon-Aguilar et al. 2007). Reductions in body weight and adipose tissue weight versus control have also been demonstrated in obese rats treated with extracts from this plant (Gamboa-Gómez et al. 2015). Similar results have been achieved in other animal models. Promising results have also been noted in human clinical trials where consumption of *H. sabdariffa* extract for 12 weeks resulted in weight loss, reduced BMI, reduced body fat, reduced waist-to-hip ratio, and reduced liver steatosis in subjects with a BMI $\geq$ 27 and those between the ages of 16 and 65 years (Chang et al. 2014).

The critical phytochemicals identified in this plant are phenolic and flavonoid compounds (Peng et al. 2011). Multiple putative modes of action relating to the plant's anti-obesity, antimetabolic disorder, and hepatoprotective effects have been proposed including downregulation of the genes coding for free fatty acid synthase and sterol regulatory element-binding proteins, inhibition of lipid droplet accumulation in adipocytes and adipocyte hypertrophy, antioxidant effects, anti-inflammatory effects, and downregulation of pancreatic lipases (Gamboa-Gómez et al. 2015; Kim et al. 2003).

10 *Hypericum perforatum* (St. John's Wort)

A limited database of studies suggests that St. John's Wort may have anti-obesity effects in addition to its well-known uses as a sedative, an anxiolytic, and an antidepressant. Oral dosing of high-fat diet-induced obese rats with a St. John's Wort extract at 100 and 200 mg/kg body wt/day for 15 days resulted in 12% and 15% reductions in body weight (respectively) versus control as well as reducing blood total cholesterol and low-density cholesterol in normal rats (Husain et al. 2011). The same extract normalized dyslipidemia and improved insulin sensitivity in fructose fed rats. Similar results with extracts of other *Hypericum* sp. have been reported by other investigators (García-de la Cruz et al. 2013).

Possible relevant modes of action for *Hypericum* sp. extracts have included modulation of the serotonin system which, in turn, may reduce food intake, suppress appetite, and reduce anxiety (Ganji et al. 2017). Reduced oxidative stress, inhibition of inflammation, inhibition of adipogenesis, attenuation of insulin-sensitive glucose uptake by adipocyte, and limiting the differentiation of pre-adipocytes have also been associated with the anti-obesity effects (Richard et al. 2012).

The specific anti-obesogenic compounds in St. John's Wort have not been fully described but may include hyperforin, hypericin, flavonoids, and condensed tannins.

The safety and drug interactional properties of St. John's Wort extracts are well known with the most important ones being serotonin syndrome-like effects and drug-drug interactional effects due to induction of CYP3A4 and CYP1A2 (reviewed in Coppock and Dziwenka 2016; Gupta et al. 2018).

11 *Persea americana* (Avocado)

Anti-obesity effects associated with avocados have been reported for fruit, leaf, and oil extract products (Brai et al. 2007). Eight weeks of dosing of rats with hypercholesterolemia induced by a high saturated fat diet with 10 mg/kg body wt/day of aqueous and methanolic extracts of avocado leaves resulted in a 25% reduction in body wt gain versus controls. Likewise, rats fed defatted avocado fruit pulp (100 g/kg of diet) for 28 days had a 26% reduced body weight gain and reduced total hepatic fat levels versus controls; however, when this diet and cholesterol were co-fed, higher serum cholesterol occurred (Naveh et al. 2002). In the case of avocado pulp, the anti-obesity effects were attributed to reduced food consumption. Similar effects have been reported for alcoholic fruit extracts (100 mg/kg body wt/day for 14 weeks) and hydroalcoholic extracts (Padmanabhan and Arumugam 2014; Monika and Geetha 2016). These effects were associated with antioxidant effects, changes in adiponectin and PPARγ, alterations in key lipid metabolic enzymes, and lower blood LDL and lipid peroxides. The key anti-obesity compounds in avocado products have not yet been defined. Avocados are a safe food in humans (Mahmassani et al. 2018); however, they are toxic to a variety of domestic animals and birds (Hargis et al. 1994; Stadler et al. 1991; Burger et al. 1994).

12 *Capsicum annuum* (Capsicum)

Capsicum extracts have been reported to be anti-adipogenic in animal models. A dietary 13.35% capsicoside G fraction (dosed at 10 and 100 mg/kg body wt/day) isolated from capsicum seeds modulated the obesogenic effects of a high-fat diet in C57BL/6J mice (Sung et al. 2016). Key effects included reduced epididymal adipose tissue weight, reduced adipocyte hypertrophy, reduced hepatic steatosis, and reduced expression of adipocyte differentiation regulators (PPARγ, CCATα, and sterol regulatory element-binding protein 1c). Likewise, C57BL/6 mice fed a high-fat diet with a green pepper juice supplement (10 mL/kg body wt/day) had lower weight gain, decreased serum triglycerides, reduced serum cholesterol and LDL, and lower abdominal fat (subcutaneous and visceral) volume versus controls (Kim and Park 2015).

Anti-obesogenic effects of capsaicinoids from *Capsicum* sp. have also been demonstrated in a limited human clinical trial. A dose of 6 mg/day for 12 weeks resulted in a small reduction in abdominal adiposity versus placebo.

Various modes of action have been described for the anti-obesity effects of capsicum including inhibition of adipogenesis, changes in gut microflora with a resulting reduction in chronic low-grade inflammation, antioxidant effects, modulation of inflammation, and alteration in lipoprotein metabolism (Kim et al. 2018; Kang et al. 2017; Marrelli et al. 2016; Yu et al. 2012).

13 *Rosmarinus officinalis* (Rosemary)

Rosemary is a well-known medicinal spice with a long history of use as an herbal abortifacient; however, there is now an expanding body of animal data demonstrating potential efficacy in obesity and metabolic disorders. Dosing of high-fat diet-induced obese mice with 200 mg/kg body wt/day of a rosemary leaf extract resulted in reduced weight gain by about 64% accompanied by an approximate 57% reduction in fat mass gain (Harach et al. 2010). This was accompanied by increased fecal lipid excretion and an approximate 39% reduction in hepatic triglyceride levels. Similar results were obtained when the rosemary leaf extract was standardized to a 20% carnosic acid content and dosed at 500 mg/kg body wt/day of the extract (Ibarra et al. 2011). Several investigators have now replicated these findings (Zhao et al. 2015).

The best investigated active anti-obesity compound in rosemary is carnosic acid which has a number of potentially relevant modes of action including inhibition of gastric lipase, modulation of genes involved in metabolism (SIRT1, PPARγ coactivator 1α, glucose-6-phosphatase, ACC, and low-density lipoprotein receptor), modulation of PPARγ, activation of the AMPK and PPAR pathways, increased hepatic glycolysis and fatty acid oxidation, decreasing inflammation, decreasing leptin, increasing adiponectin, modifying the cecal microbiome, inhibition of cecal beta-glucosidase, increasing fecal short-chain fatty acid and fiber excretion, stimulation of muscle glucose uptake via modulation of the PME-1/PP2A/PKB signaling axis, modulation of TLR4-MyD88 signaling in adipocytes, and reducing hepatic lipogenesis while increasing hepatic lipolysis (Romo-Vaquero et al. 2012, 2014a, b; Tu et al. 2013; Park and Mun 2014). Carnosic acid inhibits TLR4-MyD88 signaling pathway in LPS-stimulated 3T3-L1 adipocytes (Park and Sung 2015).

Rosmarinic acid, another bioactive compound in rosemary, has also been shown to suppress adipogenesis, lipolysis, and inflammation in adipocytes. Therefore, it appears to be a promising natural product for improving adipose mobilization in obesity (Rui et al. 2017).

The safety properties of rosemary and rosemary extract have been well-documented. The key effects are on male fertility, establishment/maintenance of pregnancy, and changes in estrogen metabolism.

14 Concluding Remarks and Future Directions

While obesity and metabolic disorders continue to be an ever-increasing, worldwide public health problem, a market will soon exist for nutraceutical products that target these diseases. Accordingly, molecule mining in the nutraceutical area is likely to continue in the foreseeable future. While there is an increasing body of proof-of-concept-type literature in various animal models, there remain relatively few large, high-quality clinical trials. Mechanistic data is also often of a preliminary, explorative nature. Given the current status quo, substantially more translational research is required to fully establish the therapeutic credibility of many of these materials.

References

Akhtar M, Ramzan A, Ali A et al (2011) Effect of Amla fruit (Emblica officinalis Gaertn.) on blood glucose and lipid profile of normal subjects and type 2 diabetic patients. Int J Food Sci Nutr 62 (6):609–616

Alarcon-Aguilar F, Zamilpa A, Perez-Garcia M et al (2007) Effect of Hibiscus sabdariffa on obesity in MSG mice. J Ethnopharmacol 114 (1):66–71

Axling U, Olsson C, Xu J et al (2012) Green tea powder and Lactobacillus plantarum affect gut microbiota, lipid metabolism and inflammation in high-fat fed C57BL/6J mice. Nutr Metab (Lond) 9(1):105

Basu A, Sanchez K, Leyva M et al (2010) Green tea supplementation affects body weight, lipids, and lipid peroxidation in obese subjects with metabolic syndrome. J Am Coll Nutr 29(1):31–40

Brai B, Odetla A, Agomo P (2007) Effects of Persea americana leaf extracts on body weight and liver lipids in rats fed hyperlipidaemic diet. Afr J Biotechnol 6:1007–1011

Burger WP, Naudé TW, van Rensburg IB et al (1994) Cardiomyopathy in ostriches (*Struthio camelus*) due to avocado (*Persea americana* var. guatemalensis) intoxication. J South Afr Vet Assoc 65 (3):113–118

Chang H, Peng C, Yeh D et al (2014) Hibiscus sabdariffa extract inhibits obesity and fat accumulation, and improves liver steatosis in humans. Food Funct 5(4):734–739

Chaudhary N, Bhardwaj J, Seo H et al (2014) Camellia sinensis fruit peel extract inhibits angiogenesis and ameliorates obesity induced by high-fat diet in rats. J. Funct Foods 7:479–486

Coppock RW, Dziwenka M (2016) St. John's Wort. In: Gupta RC (ed) Nutraceuticals: efficacy, safety and toxicity. Academic/Elsevier, Amsterdam, pp 619–631

Cunha C, Lira F, Rosa Neto J et al (2013) Green tea extract supplementation induces the lipolytic pathway, attenuates obesity, and reduces low-grade inflammation in mice fed a high-fat diet. Mediators Inflamm 2013:635470

D'souza J, D'souza P, Fazal F et al (2014) Anti-diabetic effects of the Indian indigenous fruit Emblica officinalis Gaertn: active constituents and modes of action. Food Funct *5*(4):635–644

Frota Kde M, dos Santos Filho R, Ribeiro V et al (2015) Cowpea protein reduces LDL-cholesterol and apolipoprotein B concentrations, but does not improve biomarkers of inflammation or endothelial dysfunction in adults with moderate hypercholesterolemia. Nutr Hosp *31*(4):1611–1619

Gamboa-Gómez C, Rocha-Guzmán N, Gallegos-Infante J et al (2015) Plants with potential use on obesity and its complications. EXCLI J *14*:809–831

Ganji A, Salehi I, Sarihi A et al (2017) Effects of Hypericum Scabrum extract on anxiety and oxidative stress biomarkers in rats fed a long-term high-fat diet. Metab Brain Dis *32*(2):503–511

García-de la Cruz L, Galvan-Goiz Y, Caballero-Caballero S et al (2013) Hypericum silenoides Juss. and Hypericum philonotis Cham. & Schlecht. extracts: in-vivo hypolipidaemic and weight-reducing effects in obese rats. J Pharm Pharmacol 65(4):591–603

Ghazimoradi M, Saberi-Karimian M, Mohammadi F et al (2017) The effects of curcumin and curcumin-phospholipid complex on the serum pro-oxidant-antioxidant balance in subjects with metabolic syndrome. Phytother Res *31*(11):1715–1721

Ghule B, Ghante M, Saoji A et al (2006) Hypolipidemic and antihyperlipidemic effects of Lagenaria siceraria (Mol.) fruit extracts. Indian J Exp Biol 44(11):905–909

Ghule B, Ghante M, Saoji A et al (2009) Antihyperlipidemic effect of the methanolic extract from Lagenaria siceraria Stand. fruit in hyperlipidemic rats. J Ethnopharmacol 124(2):333–337

Grove K, Sae-Tan S, Kennett M et al (2012) Epigallocatechin-3-gallate inhibits pancreatic lipase and reduces body weight gain in high fat-fed obese mice. Obesity *20*:2311–2313

Gupta RC, Srivastava A, Lall R (2018) Toxicity potential of nutraceuticals. In: Nicolotti O (ed) Computational toxicology: methods and protocols. Springer, New York, pp 367–394

Harach T, Aprikian O, Monnard I et al (2010) Rosemary (Rosmarinus officinalis L.) leaf extract limits weight gain and liver steatosis in mice fed a high-fat diet. Planta Med 76(6):566–571

Hargis AM, Stauber E, Casteel S et al (1994) Avocado (*Persea americana*) intoxication in caged birds. J Am Vet Med Assoc 194 (1):64–66

Hariri M, Haghighatdoost F (2018) Effect of curcumin on anthropometric measures: a systematic review on randomized clinical trial. J Am College Nutr. https://doi.org/10.1080/07315724.2017.1392263

Husain G, Chatterjee S, Singh P et al (2011) Hypolipidemic and antiobesity-like activity of standardised extract of Hypericum perforatum L. in rats. Pharmacology 2011:505247

Ibarra A, Cases J, Roller M et al (2011) Carnosic acid-rich rosemary (Rosmarinus officinalis L.) leaf extract limits weight gain and improves cholesterol levels and glycaemia in mice on a high-fat diet. Br J Nutr 106(8):1182–1189

Jagtap S, Khare P, Mangal P et al (2017) Effect of mahanimbine, an alkaloid from curry leaves, on high-fat diet-induced adiposity, insulin resistance, and inflammatory alterations. Biofactors *43* (2):220–231

Jurgens T, Whelan A, Killian L et al (2012) Green tea for weight loss and weight maintenance in overweight or obese adults. Cochrane Database Syst Rev 1465–1858. https://doi.org/10.1002/14651858.CD008650.pub2

Kang C, Wang B, Kaliannan K et al (2017) Gut microbiota mediates the protective effects of dietary capsaicin against chronic low-grade inflammation and associated obesity induced by high-fat diet. MBIO 8(4)

Kassaian N, Azadbakht L, Forghani B et al (2009) Effect of fenugreek seeds on blood glucose and lipid profiles in type 2 diabetic patients. Int J Vitam Nutr Res 79(1):34–39

Katare C, Saxena S, Agrawal S et al (2014) Lipid-lowering and antioxidant functions of bottle gourd (Lagenaria siceraria) extract in human dyslipidemia. J Evid Based Complement Alternat Med *19* (2):112–118

Kaur G, Mukundan S, Wani V et al (2015) Nutraceuticals in the management and prevention of metabolic syndrome. Austin J Pharmacol Ther *3*(1):1–6

Kim N, Park S (2015) Evaluation of green pepper (Capsicum annuum L.) juice on the weight gain and changes in lipid profile in C57BL/6 mice fed a high-fat diet. J Sci Food Agric 95(1):79–87

Kim M, Kim J, Kim H et al (2003) Hibiscus extract inhibits the lipid droplet accumulation and adipogenic transcription factors expression of 3T3-L1 preadipocytes. J Altern Complement Med *9*(4):499–504

Kim S, Hong J, Jeon R et al (2015) Adzuki bean ameliorates hepatic lipogenesis and proinflammatory mediator expression in mice fed a high-cholesterol and high-fat diet to induce nonalcoholic fatty liver disease. Nutr Res *36*(1):90–100

Kim H, You M, Wang Z et al (2018) Red pepper seed inhibits differentiation of 3T3-L1 cells during the early phase of adipogenesis via the activation of AMPK. Am J Chin Med *46*(1):107–118

Lu C, Zhu W, Shen C et al (2012) Green tea polyphenols reduce body weight in rats by modulating obesity-related genes. PLoS One *7*: e38332

Mahmassani HA, Avendano EE, Raman G et al (2018) Avocado consumption and risk factors for heart disease: a systematic review and meta-analysis. Am J Clin Nutr 107:523–536

Marrelli M, Menichini F, Conforti F (2016) Hypolipidemic and antioxidant properties of hot pepper flower (*Capsicum annuum* L.). Plant Foods Hum Nutr 71(3):301–306

Mohammadi A, Sadeghnia H, Saberi-Karimian M et al (2017) Effects of curcumin on serum vitamin E concentrations in individuals with metabolic syndrome. Phytother Res *31*(4):657–662

Mohammadi F, Ghazi-Moradi M, Ghayour-Mobarhan M et al (2018) The effects of curcumin on serum heat shock protein 27 antibody titers in patients with metabolic syndrome. J Diet Suppl *29*:1–10

Monika P, Geetha A (2016) Effect of hydroalcoholic fruit extract of Persea americana Mill. on high fat diet induced obesity: a dose response study in rats. Indian J Exp Biol 54(6):370–378

Moon H, Chung C, Lee H et al (2007) Inhibitory effect of (-)epigallocatechin-3-gallate on lipid accumulation of 3T3-L1 cells. Obeisty *15*:2571–2582

National Center for Complementary and Integrative Health (2016) Green tea. Retrieved from Health Topics. https://nccih.nih.gov/health/greentea

Naveh E, Werman M, Sabo E et al (2002) Defatted avocado pulp reduces body weight and total hepatic fat but increases plasma cholesterol in male rats fed diets with cholesterol. J Nutr *132* (7):2015–2018

Padmanabhan M, Arumugam G (2014) Effect of Persea americana (avocado) fruit extract on the level of expression of adiponectin and PPAR-γ in rats subjected to experimental hyperlipidemia and obesity. J Complement Integr Med *11*(2):107–119

Panahi Y, Khalili N, Hosseini M et al (2014) Lipid-modifying effects of adjunctive therapy with curcuminoids-piperine combination in patients with metabolic syndrome: results of a randomized controlled trial. Complement Ther Med *22*(5):851–857

Panahi Y, Hosseini M, Khalili N et al (2015) Antioxidant and anti-inflammatory effects of curcuminoid-piperine combination in subjects with metabolic syndrome: a randomized controlled trial and an updated meta-analysis. Clin Nutr *34*(6):1101–1108

Panahi Y, Hosseini M, Khalili N et al (2016) Effects of supplementation with curcumin on serum adipokine concentrations: a randomized controlled trial. Nutrition *32*(10):1116–1122

Panahi Y, Khalili N, Sahebi E et al (2017) Curcuminoids plus piperine modulate adipokines in type 2 diabetes mellitus. Curr Clin Pharmacol *12*(4):253–258

Park M, Mun S (2014) Carnosic acid inhibits TLR4-MyD88 signaling pathway in LPS-stimulated 3T3-L1 adipocytes. Nutr Res Pract *8* (5):516–520

Park M, Sung M (2015) Carnosic acid attenuates obesity-induced glucose intolerance and hepatic fat accumulation by modulating genes of lipid metabolism in C57BL/6J-*ob/ob* mice. J Sci Food Agric 95 (4):828–835

Park H, DiNatale D, Chung M et al (2011) Green tea extract attenuates hepatic steatosis by decreasing adipose lipogenesis and enhancing hepatic antioxidant defenses in ob/ob mice. J Nutr Biochem *22* (4):393–400

Peng C, Chyau C, Chan K et al (2011) Hibiscus sabdariffa polyphenolic extract inhibits hyperglycemia, hyperlipidemia, and glycation-oxidative stress while improving insulin resistance. J Agric Food Chem *59*(18):9901–9909

Purpura M, Lowery RP, Wilson JM et al (2018) Analysis of different innovative formulations of curcumin for improved relative oral bioavailability in human subjects. Eur J Nutr 57:929–938

Repin N, Kay B, Cui SW et al (2017) Investigation of mechanisms involved in postprandial glycemia and insulinemia attenuation with dietary fibre consumption. Food Funct *8*(6):2142–2154

Richard A, Amini Z, Ribnicky D et al (2012) St. John's Wort inhibits insulin signaling in murine and human adipocytes. Biochim Biophys Acta 1822(4):557–563

Romo-Vaquero M, Yáñez-Gascón M, García Villalba R et al (2012) Inhibition of gastric lipase as a mechanism for body weight and plasma lipids reduction in Zucker rats fed a rosemary extract rich in carnosic acid. PLoS One 7(6):e39773

Romo-Vaquero M, Larrosa M, Yáñez-Gascón M et al (2014a) A rosemary extract enriched in carnosic acid improves circulating adipocytokines and modulates key metabolic sensors in lean Zucker rats: critical and contrasting differences in the obese genotype. Mol Nutr Food Res *58*(5):942–953

Romo-Vaquero M, Selma M, Larrosa M et al (2014b) A rosemary extract rich in carnosic acid selectively modulates caecum microbiota and inhibits β-glucosidase activity, altering fiber and short chain fatty acids fecal excretion in lean and obese female rats. PLoS One 9(4):e94687

Rui Y, Tong L, Cheng J et al (2017) Rosmarinic acid suppresses adipogenesis, lipolysis in 3T3-L1 adipocytes, lipopolysaccharide-stimulated tumor necrosis factor-α secretion in macrophages, and inflammatory mediators in 3T3-L1 adipocytes. Food Nutr Res 61:1330096

Safarian H, Parizadeh S, Saberi-Karimain M et al (2018) The effect of curcumin on serum copper and zinc and Zn/Cu ratio in individuals with metabolic syndrome: a double-blind clinical trial. J Diet Suppl 18

Soleimani V, Sahebkar A, Hosseinzadeh H (2018) Turmeric (Curcuma longa) and its major constituent (curcumin) as nontoxic and safe substances: Review. Phytother Res *32*(6):985–995

Stadler P, van Rensberg IB, Naudé TE et al (1991) Suspected avocado (*Persea americana*) poisoning in goats. J South Afr Vet Assoc 62 (4):186–188

Sung J, Jeong H, Lee J (2016) Effect of the capsicoside G-rich fraction from pepper (*Capsicum annuum* L.) seeds on high-fat diet-induced obesity in mice. Phytother Res 30(11):1848–1855

Tian C, Ye X, Zhang R et al (2013) Green tea polyphenols reduced fat deposits in high fat-fed rats via erk1/2-PPARγ-adiponectin pathway. PLos One 8(1):e53796

Tu Z, Moss-Pierce T, Ford P et al (2013) Rosemary (Rosmarinus officinalis L.) extract regulates glucose and lipid metabolism by activating AMPK and PPAR pathways in HepG2 cells. J Agric Food Chem 61(11):2803–2810

Wolfram S, Wang Y, Thielecke F (2006) Anti-obesity effects of green tea: from bedside to bench. Mol Nutr Food Res *50*:176–187

Yang Y, Su Y, Yang H et al (2014) Lipid-lowering effects of curcumin in patients with metabolic syndrome: a randomized, double-blind, placebo-controlled trial. Phytother Res 28(12):1770–1777

Yu Q, Wang Y, Yu Y et al (2012) Expression of TRPV1 in rabbits and consuming hot pepper affects its body weight. Mol Biol Rep *39* (7):7583–7589

Yuda N, Tanaka M, Suzuki M et al (2012) Polyphenols extracted from black tea (Camellia sinensis) residue by hot-compressed water and their inhibitory effect on pancreatic lipase in vitro. J Food Sci *77*: H254–H261

Zhao Y, Sedighi R, Wang P et al (2015) Carnosic acid as a major bioactive component in rosemary extract ameliorates high-fat-diet-induced obesity and metabolic syndrome in mice. J Agric Food Chem 63(19):4843–4852

Nutraceuticals for Diabetes in Dogs and Cats

Ramesh C. Gupta, Rajiv Lall, Anita Sinha, and Ajay Srivastava

Abstract

Diabetes is a serious health issue in dogs and cats that is usually treated through the use of injectable insulin. There are more than 90 million pet dogs in the United States, and 1 in every 400–500 dogs suffers from diabetes. Diabetes typically occurs later in life, usually around 7–10 years of age, with certain breeds being at a higher risk of developing this disease. Traditional diabetic management in dogs and cats includes food monitoring, glucose monitoring (which is done with the use of a canine-specific glucometer), moderate exercise, medication, and insulin therapy. Often times, dog and cat owners decline diabetic treatment because they either do not feel confident in giving insulin injections or because they simply cannot afford treatment, as traditional insulin therapy and medication are expensive. Therefore, it is beneficial to determine the efficacy and safety of a potential oral formulation for diabetic management. An oral formulation of nutraceutical(s) may provide an easier administration (noninvasive) method and provide lower costs to dog and cat owners by not requiring additional supplies such as needles and syringes. This chapter describes various plant extracts and nutraceuticals that have potential for anti-diabetic and antihyperglycemic effects in diabetic dogs and cats.

Keywords

Nutraceuticals · Diabetes · Hyperglycemia · Veterinary medicine · Insulin resistance · Insulin sensitivity

R. C. Gupta (✉)
Toxicology Department, Breathitt Veterinary Center, Murray State University, Hopkinsville, KY, USA
e-mail: rgupta@murraystate.edu

R. Lall · A. Sinha · A. Srivastava
Vets Plus Inc., Menomonie, WI, USA

1 Introduction

In humans, type 2 diabetes mellitus (T2DM) is a chronic metabolic disorder that has become the fourth leading cause of death in developed countries (Lee 2016). More than 415 million individuals with diabetes were reported in 2015, with five million deaths in 2015 (WHO 2016). Clinically, about 90% of diabetic patients are reported to be T2DM, which is characterized by insulin resistance and β-cell dysfunction. Among animal species, dogs suffer with diabetes more often than other species. Currently, the global dog population is approximately 900 million. There are more than 90 million pet dogs in the United States. Statistics show that approximately one in every 200 cats and one in every 400–500 dogs are diagnosed with diabetes. Diabetes typically occurs later in life, usually around 7–10 years of age in dogs, with certain breeds (dachshunds, schnauzers, poodles, cairn and Australian terriers, springer spaniels, keeshonds, samoyeds, and golden retrievers) being at a higher risk of developing this disease. It is interesting to note that in keeshonds, diabetes is inherited as an autosomal recessive trait. Seventy percent of dogs with diabetes are female and 30% are male. Unlike dogs, male cats are more prone to diabetes. Burmese cats have been reported to have a higher risk of diabetes than other breeds of cats. T2DM is characterized by pancreatic β-cell dysfunction, which causes hyperglycemia and may lead to several secondary complications, such as neuropathy, nephropathy, and cardiac failure.

Traditional diabetic management in dogs and cats may include diet monitoring, moderate exercise, and therapy with insulin and other glucose-lowering drugs to control blood glucose concentrations. However, therapeutic drugs may produce side effects, including weight gain, GI disturbances, and heart failure. Often times, dog owners decline diabetic treatment because they either do not feel confident in giving insulin injections or because they simply cannot afford treatment. Recently, glucagon-like peptide-1

R. C. Gupta et al. (eds.), *Nutraceuticals in Veterinary Medicine*, https://doi.org/10.1007/978-3-030-04624-8_35

(GLP1) has been reported to be effective against T2DM following oral or parenteral administration (Lee 2016).

It is beneficial to determine the efficacy and safety of a potential oral formulation of natural alternative medicines for diabetic management. An oral formulation of nutraceutical (s) may provide an easier administration (noninvasive) method and provide lower costs to dog and cat owners by not requiring additional supplies such as needles and syringes.

Commonly used plant extracts which have anti-diabetic potential may include *Berberis vulgaris*, *Trigonella foenum-graecum*, *Momordica charantia*, *Curcuma longa*, *Moringa oleifera*, *Gymnema sylvestre*, *Azadirachta indica*, *Aloe vera*, *Panax ginseng*, and many others. Extracts of these and other plants may exert anti-diabetic effects by multiple mechanisms (Patel et al. 2012; Nair et al. 2013; Gupta et al. 2017). Nutraceuticals have also been implicated in diabetes-associated secondary complications, such as nephropathy, cardiopathy, retinopathy, pancreatitis, and wounds. Additionally, nutraceuticals can prevent or reverse T2DM-induced alterations in microbiota marked by enterobacteria performance and a decrease in the number of beneficial bacteria (Villarruel-López et al. 2018).

This chapter describes various plant extracts and nutraceuticals that have potential for anti-diabetic and antihyperglycemic effects in dogs and cats.

2 Pathophysiology of Diabetes Mellitus

The pathophysiology of diabetes mellitus (DM) is very complex, and the exact mechanism of action is yet to be established. Type 2 diabetes mellitus (T2DM) is the most common form of diabetes. Insulin resistance in target tissues is a characteristic feature and major contributing factor to T2DM (Araki and Miyazaki 2007; DeFronzo and Tripathy 2009; Kang et al. 2016). Mitochondrial dysfunction and oxidative stress (due to increased ROS production) have been linked to the development of insulin resistance in diabetes (Kaneto et al. 2007; Kim et al. 2008; Matsuzawa-Nagata et al. 2008; Kangralkar et al. 2010; Raza et al. 2011). Additionally, impaired phosphatidylinositol 3-kinase (PI3K)/AKT pathway in diabetes is one of the main mechanisms of insulin resistance induced by the increased level of ROS (Wang et al. 2011; Naveen and Baskaran 2018). An increase in insulin resistance leads to elevated gluconeogenesis and reduced glycogen synthesis in the liver, thereby causing decreased insulin secretion from pancreatic β cells, and hyperglycemia (reviewed in Yan et al. 2018). The PI3K/AKT pathway plays an important role in the insulin signaling pathway, which is considered the key regulator relevant to gluconeogenesis and glycogen synthesis (Whiteman et al. 2002). Critical targets in DM include α-amylase, α-glucosidase, dipeptidyl peptidase-IV (DPP-IV), aldolase reductase, peroxisome proliferator-activated receptor gamma (PPAR-γ), 5′ adenosine monophosphate-activated protein kinase (AMP kinase), and glucose transporter type 4 (GLUT4) (Naveen and Baskaran 2018). Naveen and Baskaran (2018) have classified DM-associated complications into two categories: (1) microvascular (nephropathy, neuropathy, and retinopathy) and (2) macrovascular (heart attack, stroke, and peripheral vascular disease).

Alterations of endothelial homeostasis, due primarily to proinflammatory cytokines and reduced adiponectin secretion, appear to be two key events in insulin resistance and hyperglycemia. These conditions are associated with altered gene expression and cell signaling in the vascular endothelium, thereby affecting release of endothelium-derived factors, activation of NADPH oxidase, uncoupling of endothelial NOS (eNOS), and the expression of endothelin-1. This cascade of events often results in an imbalance between the production of vasodilator and vasoconstrictor mediators and the induction of adhesion molecules.

3 Experimental Diabetes Models

A number of animal models are used to experimentally induce DM by injecting diabetogenic chemicals (Reed et al. 2000; Etuk 2010; King 2012). Streptozotocin (STZ) is the most commonly used drug for induction of diabetes in rats and mice (Balamurugan et al. 2003). A single dose of streptozotocin (80 mg/kg in rats and 150 mg/kg in mice) is given intraperitoneally to DM. Diabetes develops gradually and may be assessed after a few days, approximately 4 days for mice and 7 days for rats (Etuk 2010). Usually, a serum glucose level of about 180–500 mg/dL indicates the induction of DM. In rats, DM can also be induced by intraperitoneal (IP) injection of alloxan (100 mg/kg of body weight). Lenzen (2008) induced hyperglycemia (>200 mg/dL) in rats after 72 h by injecting alloxan monohydrate (150 mg/kg, IP). Alloxan has been used to produce experimental diabetes in rats, mice, rabbits, and dogs (Etuk 2010). With proper dose selection, either drug can induce type 1 or type 2 DM.

Either of these two drugs, with half a dose when given alone, can also be given in combination with a high-fat diet (Reed et al. 2000; Parveen et al. 2011). In a rat model of diabetes, a high-fat diet (40% of calories as fat) is often given for 2 weeks before streptozotocin or alloxan injection (Reed et al. 2000).

STZ causes selective pancreatic islet β-cell cytotoxicity mediated through the release of nitric oxide (NO) and oxidative/nitrosative stress (Raza et al. 2011). NADPH oxidase type 4 (NOX4) overexpression has been reported in livers of STZ-induced rats. This results in rapid reduction in pancreatic islet pyridine nucleotide concentration and subsequent

β-cell necrosis. The action of STZ on mitochondria generates superoxide dismutase (SOD) anions, which leads to diabetic complications. STZ partly destroys the β cells bringing about inadequate insulin discharge and creating type 2 diabetes (reviewed in Jayaprasad et al. 2015). Alloxan is a urea derivative which causes selective necrosis of the pancreatic islet β cells. Szkudelski (2001) and Lenzen (2008) described in detail the mechanism of STZ and alloxan in the pancreas and β cells.

4 Diabetic Monitoring

Blood glucose measurement is usually carried out with a canine- or feline-specific blood glucometer and test kit. Clinically normal dogs and cats have blood glucose values ranging from 79–103 mg/dL and 74–216 mg/dL, respectively. Recently, Corradini et al. (2016) suggested the use of a flash glucose monitoring system (FGMS) to measure interstitial glucose (IG) concentrations continuously for days or weeks. The method is painless, but mild erythema at the site of the application was found in five out of ten dogs at the end of the wearing period, i.e., 14 days. The FGMS is found to be easy to use and accurate for IG glucose measurement in diabetic dogs. Using a commercially available kit, glycosylated hemoglobin A1 was measured in the blood of dogs and cats for monitoring the status of diabetes, but the test was not found to be of any value (Delack and Stogdale 1983). In humans, glycosylated HbA1c is a routine index of T2DM, as it is a biomarker for identifying stress hyperglycemia and diabetic hyperglycemia. A number of factors can affect blood glucose levels' monitoring (Ginsberg 2009). In dogs and cats, however, HbA1c does not correlate well with diabetes, so glycated serum protein assay (GSP; glycated albumin; Diazyme Laboratories, Poway, CA, USA) is recommended (Kouzuma et al. 2002). Animal fasting blood glucose levels above 200 mg/dL are considered diabetic.

5 Prevention and Management of Diabetes in Animals

Like humans (Brouns 2018), moderate exercise, low-carbohydrate high-fat diet, and therapeutic drug medications may be recommended to prevent and manage diabetes in dogs and cats. Feinman et al. (2015) reported that dietary carbohydrate restriction is the best approach in diabetes management by improving insulin sensitivity and significant weight loss. Wyk et al. (2016) and Brouns (2018) further emphasized that quality but not quantity of carbohydrates appears to be a key aspect to be considered. When glucose availability is in decline due to low-carbohydrate diet, two metabolic processes (gluconeogenesis and ketogenesis) come into play (Wyk et al. 2016; Hall 2017). Switching from a high-carbohydrate to a low-carbohydrate high-fat (LCHF) diet may lead to weight loss, reduction of appetite, improved insulin sensitivity, fewer fluctuations in blood glucose levels, and lower fasting blood glucose levels (Brouns 2018). Also, the metabolic effects of LCHF diets may lead to improvements of various disease risk factors, including cardiovascular diseases (Westman et al. 2007; Accurso et al. 2008; Volek et al. 2008; Feinman et al. 2015).

5.1 Common Plants/Nutraceuticals with Anti-diabetic and Antihyperglycemic Potential

Commonly used plant extracts which have anti-diabetes potential may include *Berberis vulgaris*, *Trigonella foenum-graecum*, *Momordica charantia*, *Curcuma longa*, *Moringa oleifera*, *Gymnema sylvestre*, *Azadirachta indica*, *Aloe vera*, *Panax ginseng*, and many others. Extracts of these plants may exert anti-diabetic effects by multiple mechanisms: (1) increase insulin secretion, (2) activation of glycogenolysis, (3) increase hepatic glycolysis, (4) adrenomimeticism, (5) pancreatic β-cell potassium channel blocker activity, (6) cAMP activation, and (7) modulation of glucose absorption from the intestine (Patel et al. 2012; Gupta et al. 2017). Some important plants and their active principles with anti-diabetic potential are discussed here in brief, while others are listed in Table 1.

5.1.1 Berberine

Berberine (BBR) is an isoquinoline alkaloid commonly obtained from plants of genera *Berberis*, *Coptis*, *Hydrastis*, and *Argemone*. BBR has a molecular weight of 336.361 and its structural formula is shown in Fig. 1.

The pharmacokinetics and PBPK aspect of berberine have been described in detail in chapter "Glucosinolates and Organosulfur Compounds" of this book. In a number of studies, BBR has been shown to exert antihyperglycemic and anti-diabetic effects (Chen and Xie 1986; Dong et al. 2012). Mechanisms involved in antihyperglycemic effect of BBR may include:

1. Decreased glucose absorption by inhibiting α-glucosidase
2. Reduced glucose transport through the intestinal epithelium
3. Activation of adenosine 5′-monophosphate-activated protein kinase (AMPK)
4. Increased glucokinase activity
5. Induction of glucose transport by enhancing GLUT1 gene expression

Table 1 Nutraceuticals and plant extracts with potential of hypoglycemic and anti-diabetic effects

Nutraceuticals/plants	Active ingredient	Effect	References
Acacia arabica, *Acacia nilotica*	Flavonoids, saponins	Hypoglycemic	Rather et al. (2015)
Acacia karroo Hayne	Phenols, flavonoids, tannins, saponins, and cardiac glycosides	Hypoglycemic, anti-diabetic, α-amylase inhibitor, suppressed phosphorylation of Akt1, antioxidative	Njanje et al. (2017)
Aconitum carmichaelii	Aconitan A	Hypoglycemic	Hikino et al. (1989)
Achyranthes rubrofusca	–	Anti-diabetic	Geetha et al. (2011)
Adansonia digitata	–	Hypoglycemic, anti-diabetic	Tanko et al. (2008)
Aloe vera (*Aloe barbadensis*)	β-sitosterol, campesterol, anthraquinones, saponins	Anti-diabetic, antioxidative, anti-inflammatory, immunomodulatory	Can et al. (2004)
Argemone mexicana, *Berberis aristata*, *Berberis aquifolium*, *Berberis vulgaris*, *Coptis chinensis*, *Coptis trifolia*, *Hydrastis canadensis*	Berberine	Anti-diabetic, hypoglycemic	Chen and Xie (1986), Kong et al. (2009), Dong et al. (2011, 2012), Xia et al. (2011), Kumar et al. (2015), Lan et al. (2015), Pang et al. (2015)
Artanema sesamoides Benth	Alkaloids, flavonoids, saponins	Anti-diabetic, antihyperglycemic, antioxidative, cytoprotective	Selvan et al. (2008)
Artocarpus altilis	β-sitosterol, polyphenol, lutein	Anti-diabetic	Nair et al. (2013), Ragasa et al. (2014)
Artocarpus heterophyllus (jackfruit)	Artocarpin, artocarpecin, morin, dihydromorin, cycloartinone, cynomacurin	Anti-diabetic, anti-inflammatory, antioxidative, immunomodulatory	Prakash et al. (2009), Nair et al. (2013)
Balanites aegyptiaca Del. (desert date)	Furostanol, balanitines (1–7)	Anti-diabetic, antioxidative	Chothani and Vaghasiya (2011), Abou Khalil et al. (2016)
Barleria prionitis Linn.	Berlerinoside	Anti-diabetic, antihyperglycemic	Dheer and Bhatnagar (2010)
Beta vulgaris L. var cicla (chard)	Pectin, saponins, flavonoids, polysaccharides, ascorbic acid	Hypoglycemic, anti-diabetic	Yanarday and Colak (1998)
Bovine casein hydrolysate	Eleven casein medium-sized peptides, amino acid leucine	Insulinotropic, antihyperglycemic	Drummond et al. (2018)
Butea monosperma	Butrin, isobutrin, butin, palasitrin, butein	Hypoglycemic, anti-diabetic	Deore et al. (2008)
Caralluma edulis	–	Antioxidative, antihyperglycemic, anti-diabetic	Wadood et al. (1989)
Cassia kleinii	Terpenoids, coumarins, saponin	Anti-diabetic, antihyperglycemic	Babu et al. (2002, 2003a, b)
Catharanthus roseus	Catharanthine, ursolic acid, daucosterol, β-sitosterol, lochnericine, quercetin, kaempferol	Anti-diabetic	Singh et al. (2001), Wu et al. (2017)
Chloroxylon swietenia	Iridoid glucoside	Anti-diabetic	Jayaprasad et al. (2015)
Chromium trivalent	Chromium trivalent	Antihyperglycemic, anti-diabetic	Ryan et al. (2003), Biswas et al. (2010), Rains and Jain (2011), Hua et al. (2012), Fleck et al. (2014)
Cinnamomum zeylanicum Cinnamon	(E)-Cinnamaldehyde	Anti-diabetic, anti-obesity	SubashBabu et al. (2007), Anand et al. (2010), Nair et al. (2013), Soliman et al. (2012, 2013), Muhammad and Dewettinck (2017)
Cissus sicyoides	Tannins, alkaloids, steroid-triterpenes, amino acids, kaempferol, quercetin, saponins, quinones, phenolics, sabadin	Anti-diabetic, hypoglycemic, anti-inflammatory	Viana et al. (2004)
Citrus sinensis L. and other citrus fruits' peel	Phenolics, flavonoid diosmin	Anti-diabetic, antihyperglycemic, antioxidative, anti-inflammatory, insulinotropic, cytoprotective, increased expression of PPARγ	Cova et al. (1992), Jain et al. (2014), Hsu et al. (2017), Sathiyabama et al. (2018)
Clitoria ternatea	Polyphenols	Antioxidative, postprandial glucose suppression	Chusak et al. (2018)

(continued)

Table 1 (continued)

Nutraceuticals/plants	Active ingredient	Effect	References
Coscinium fenestratum	Not yet identified	Anti-diabetic	Shirwaikar et al. (2005)
Costus pictus D. Don, *Costus igneus* Nak (insulin plant)	Ergastanol, stigmasterol, lupeol, quercetin, diosgenin	Anti-diabetic, antioxidative	Jayasri et al. (2008), Hegde et al. (2014)
Curcuma longa (turmeric)	Curcumin and curcuminoids	Anti-diabetic, antioxidative, anti-inflammatory, anti-apoptosis, anti-diabetic cardiomyopathy, antihyperlipidemic	Javeri and Chand (2016), Rao and Najam (2016), de Melo et al. (2018), Zheng et al. (2018)
Ferocactus latispinus	–	Antihyperglycemic, antihyperlipidemic	Perez-Gutierrez and Mota Flores (2010)
Ferocactus histrix	–	Antihyperglycemic, antihyperlipidemic	Perez-Gutierrez and Mota Flores (2010)
Fish oil	Eicosapentaenoic acid, docosahexaenoic acid	Improved insulin sensitivity, antihyperglycemic	Menchetti et al. (2018)
Fructus arctii	Lignans	Anti-diabetic	Xu et al. (2008)
Galega officinalis L.	Diterpenes, triterpenes, phytosterols, and flavonoids	Anti-diabetic, hypoglycemic, immunomodulating, and lymphocyte apoptosis inhibiting	Nagalievska et al. (2018)
Hippophae rhamnoides L. (sea buckthorn)	Proanthocyanidins, isorhamnetin, quercetin	Hypoglycemic, sensory properties	Zhang et al. (2010c), Ma et al. (2017)
Hypoxis hemerocallidea	Phytosterols, sterolin	Anti-diabetic, antinociceptive, anti-inflammatory	Mahomed and Ojewole (2003), Ojewole (2006)
Lagerstroemia speciosa (L) Pers (banaba)	Corosolic acid, ellagitannins, lagerstroemin, flosin B, and reginin A	Hypoglycemic, antihyperglycemic, anti-diabetic	Mishra et al. (1990), Tanquilut et al. (2009)
Momordica charantia (bitter melon)	Glycosides (momordins I and II), saponins, alkaloids, triterpenes, steroids	Antihyperglycemic, anti-diabetic	Ahmed et al. (2004), Miura et al. (2004)
Mangifera indica	Antioxidants	Anti-diabetic, antioxidative, anti-inflammatory, immunomodulatory	Bhowmik et al. (2009), Etuk and Muhammed (2010), Irondi et al. (2016)
Melatonin	Melatonin	Anti-diabetic, antioxidative	Shieh et al. (2009), Sharman and Bondy (2016)
Moringa oleifera Lam (drum stick tree or horse rider tree)	Polyphenols (quercetin-3-glycoside, rutin, and kaempferol)	Antihyperglycemic, anti-diabetic, α-amylase, and α-glucosidase inhibiting	Jain et al. (2010), Gupta et al. (2012), Yassa and Tohamy (2014), Stohs and Hartman (2015), Khan et al. (2017), Villarruel-López et al. (2018)
Mucuna pruriens leaf extract	Monoterpenes and alkaloids	Anti-diabetic	Tanko et al. (2012)
Origanum vulgare L. (oregano)		Antioxidative, anti-obesity, anti-diabetic	Sabino et al. (2018)
Panax ginseng, *P. japonica*, *P. notoginseng*, *P. quinquefolius*, *P. vietnamensis* (ginseng)	Ginsenosides Rb2 and Rh2, protopanaxatriol, (20R)-protopanaxadiol, Rg1, Rc, Rd, Re, Rf, Rg2, Rh1, Rb1, pyroglutamic acid, peptidoglycan panaxan B	Antihyperglycemic, hypoglycemic	Tomoda et al. (1985), Cho et al. (2006), Lee et al. (2006), Yang and Wu (2016)
Persea americana Mill	Saponins, tannins, phlobatannins, flavonoids, alkaloids, polysaccharides	Hypoglycemic	Antia et al. (2005)
Phyllanthus niruri		Anti-diabetic	Okoli et al. (2011)
Piper betle		Anti-diabetic	Nair et al. (2013)
Polygonatum odoratum	Homoisoflavonoids (apigenin, luteolin, quercetin, maringenin, hesperitin, genistein), phloretin, polygonatumoside F, polygonatumoside G	Glucose transporter 2 inhibiting, antihyperglycemic	Liu et al. (2018), Wang et al. (2018)
Quercus robur L. *Quercus ilex*	Quercitol, cyclitols	Hypoglycemic, anti-diabetic	Worawalai et al. (2018)

(continued)

Table 1 (continued)

Nutraceuticals/plants	Active ingredient	Effect	References
Rehmannia glutinosa	Catalpol	Anti-diabetic, antihyperglycemic, antioxidative, anti-apoptotic, anti-insulin resistance, anti-gluconeogenesis, elevating glycogen synthesis	Shieh et al. (2011), Zhou et al. (2015), Yan et al. (2018)
Resveratrol	Resveratrol	Antioxidative, antihyperglycemic, anti-diabetic	Bhatt et al. (2012), Rouse et al. (2014)
Royal jelly	Royal jelly proteins	Antihyperglycemic	Fujii et al. (1990)
Ruta graveolens	Rutin	Antihyperglycemic, antihyperlipidemic, antioxidative	Ahmed et al. (2010)
Sarcopoterium spinosum	Triterpenes, tannins	Hypoglycemic	Reher et al. (1991)
Shilajit	Dibenzo-α-pyrones, dibenzo-α-pyrones chromoproteins, fulvic acid	Anti-diabetic, antioxidative, anti-inflammatory, immunomodulatory	Bhavsar et al. (2016)
Sida cordifolia	Alkaloids, flavonoids, lignin, glycosides, saponins, phytosterols	Anti-diabetic, anti-hypercholesterolemic, anti-inflammatory, hypoglycemic, hepatoprotective	Kaur et al. (2011), Sivapalan (2015)
Sophora tonkinensis	Flavonoids	Anti-diabetic	Huang et al. (2016)
Sphaeranthus indicus	β-sitosterol	Anti-diabetic	Prabhu et al. (2008)
Stevia rebaudiana Bertoni (stevia)	Diterpene glycosides (stevioside, steviolbioside, rebaudiosides (A,B, C,D,E), and dulcoside A) and polyphenols	Anti-diabetic, antioxidative, hepatoprotective, renoprotective, immunomodulatory, antihypertensive	Geuns et al. (2007), Stoyanova et al. (2011), Shivanna et al. (2013), Saleh et al. (2016), Ahmad and Ahmad (2018), Nichol et al. (2018)
Strychnos potatorum L.f.	Isomotiol, sitosterol, compesterol, galactomannan, galactan	Anti-diabetic, antioxidative	Dhasarathan and Theriappan (2011)
Terminalia arjuna	Phytosterol, lactones, flavonoids, phenolics, tannins, glycosides	Anti-diabetic	Parveen et al. (2011)
Trigonella foenum-graecum (Fenugreek)	Diosgenin, trigonelline, galactomannan	Antihyperglycemic	Zia et al. (2001), Garg (2016)
Vernonia amygdalina	Alkaloids, saponins, tannins, flavonoids	Anti-diabetic, antihyperglycemic	Etuk and Muhammed (2010)
Viburnum dilatatum	Polyphenols	Antihyperglycemic, α-glucosidase inhibitory	Iwai et al. (2006)
Virgin olive oil	Polyphenols, tyrosol, hydroxytyrosol, secoiridoids	Anti-insulin resistance, anti-mitochondrial dysfunction, anti-diabetic, antioxidative, anti-inflammatory	Lama et al. (2017), Celano et al. (2018)
Wheat-based rusks	Amylose	Antihyperglycemic, anti-diabetic	Vetrani et al. (2018)
Zingiber officinale (ginger)	Gingerol, gingerenone A, shogaols, paradol, 1-dehydro-10-gingerdione	Antioxidant, anti-inflammatory, antihyperglycemic, anti-diabetic, anti-diabetic nephropathy	Rafieian-Kopaei and Nasri (2014), Rahmani et al. (2014), Roufogalis (2014)

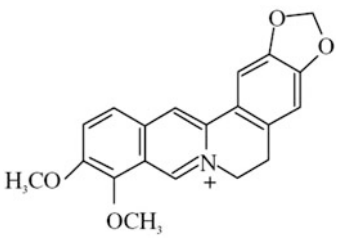


Fig. 1 Structural formula of berberine

6. Increased glucose uptake from blood to target organs, such as skeletal muscle and adipose tissue
7. Enhanced glucose metabolism by stimulation of glycolysis
8. Inhibition of gluconeogenesis in liver
9. Increased insulin receptor (*InsR*) expression and improved insulin secretion and sensitivity
10. Inhibition of the intracellular accumulation of ROS/RNS, cellular apoptosis, and inflammation that characterize vascular injury

In clinical trials, BBR has been shown to significantly lower fasting blood glucose, hemoglobin A_{1c}, triglyceride, total cholesterol, and LDL-C and increase insulin receptor expression in patients with type 2 diabetes receiving BBR (1 g daily or 500 mg three times a day) (Li 2007; Yin et al. 2008; Zhang et al. 2010a, b). It is interesting to note that BBR has positive effects in treating diabetes-associated complications such as nephropathy, neuropathy, and cardiomyopathy (Pang et al. 2015; Imenshahidi and Hosseinzadeh 2015; Zhang and Chen 2012; Lan et al. 2015). Clinical trials have yet to be carried out in dogs and cats to establish the right dose and efficacy of BBR against diabetes and associated complications.

5.1.2 Curcumin

The root of *Curcuma longa* is the source of turmeric (yellow powder), which consists of a group of phytoconstituents commonly called curcuminoids. Curcuminoids include curcumin (diferuloylmethane), demethoxycurcumin, and bisdemethoxycurcumin (BMC). Turmeric powder and the structural formula of curcumin (enol and keto form) are shown in Fig. 2.

Chemically, curcumin is a diarylheptanoid, a natural phenol responsible for turmeric's yellow color. Curcumin's tautomerism exists in the enolic form in organic solvents and in the keto form in water (Manolova et al. 2014).

So far, curcumin has not led to any drug development due to several factors, such as chemical instability, water insolubility, lack of selective target activity, low bioavailability, limited tissue distribution, and extensive metabolism (Anand et al. 2013; Metzler et al. 2013; Nelson et al. 2017). However, it is a very popular ingredient in many nutraceuticals/dietary supplements around the world. Presently, its largest market is in North America.

Curcumin appears to prevent and ameliorate diabetes and its complications (nephropathy, retinopathy, and cardiopathy) by multiple mechanisms (Abo-Salem et al. 2014; Wu et al. 2014; Javeri and Chand 2016). Inflammation of micro and macro blood vessels and peripheral nerves in the body plays a major role in the pathophysiology of diabetes. Long-term low-dose feeding of curcumin may prevent and ameliorate progression and prevalence of diabetes in humans and animals. Castro et al. (2014) rationalized the therapeutic efficacy of curcumin in diabetes by modulating immune cells responsible for β-cell death in a mouse model of type 1 diabetes.

Curcumin has been shown to significantly increase the expression of AMPK and PPARγ, and reduced the expression of Il-6 and NF-κB, thereby ameliorating chronic inflammation in diabetic mice (Abo-Salem et al. 2014; Jiménez-Flores et al. 2014). Additional benefits of curcumin in diabetes may be due to its antioxidative property (Menon and Sudheer 2007; Javeri and Chand 2016).

Recently, Hariri and Haghighatdoost (2017) suggested that curcumin in a more soluble and bioavailable form can reduce body weight and obesity, but long-term clinical trials need to be carried out. In a number of other studies, various formulations of curcumin have been developed for improved

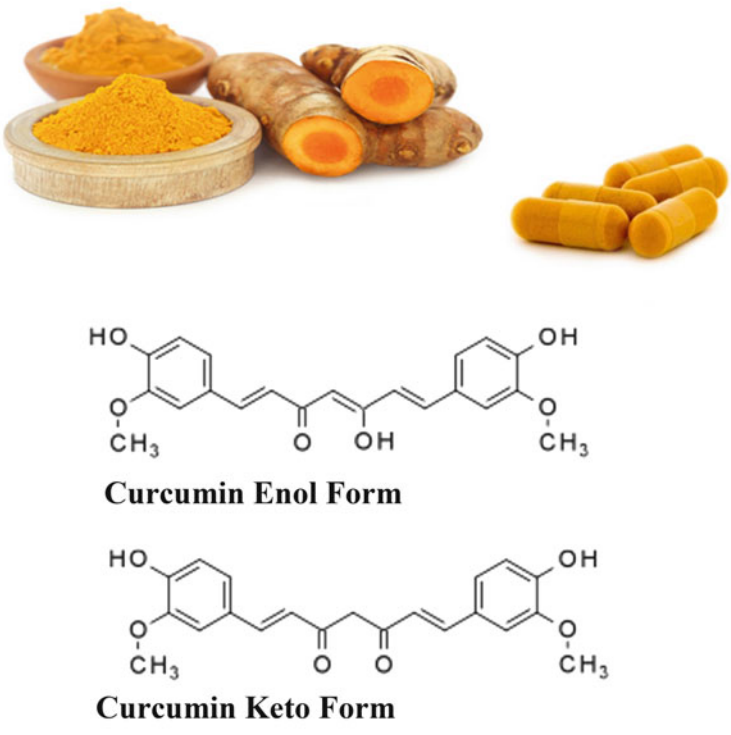


Fig. 2 Structural formula of curcumin (upper, enol form; lower, keto form)

bioavailability (Liu et al. 2006; Antony et al. 2008; Cuomo et al. 2011; Sasaki et al. 2011; Jager et al. 2014; Yallapu et al. 2015). Purpura et al. (2018) demonstrated that γ-cyclodextrin curcumin formulation (CW8) significantly improved the absorption of curcuminoids in healthy humans.

Clinical studies in dogs and cats have yet to be carried out to establish the therapeutic dose, efficacy, and safety in diabetic dogs and cats. In preliminary clinical studies, patients receiving curcumin up to 8 g/day for 3–4 months showed no toxicity, though some subjects complained mild nausea and diarrhea (Hsu and Cheng 2007). Details on various aspects of curcumin, including its beneficial health effects in other diseases, can be found in chapter "Standardized Turmeric and Curcumin" of this book.

5.1.3 Fenugreek

Fenugreek seeds are obtained from the *Trigonella foenum-graecum* L. plant which is commonly cultivated in India, China, and the Mediterranean and North African regions. The seeds are known to contain many phytoconstituents, including flavonoids, steroidal saponins diosgenin, galactomannan fiber, amino acid 4-hydroxyisoleucine, vitamins, and the alkaloid trigonelline. Ribes et al. (1986) reported that the defatted part of the fenugreek seed, called subfraction "a" which contains the seed parts like testa (seed coat) and endosperm (a source of stored food), has been associated with the antihyperglycemic effect in diabetic dogs. The structural formula of trigonelline and diosgenin is shown in Figs. 3 and 4, respectively.

In addition to trigonelline, the seeds of fenugreek contain other alkaloids such as gentianine and carpaine. 4-Hydroxyisoleucine has been shown to cause an increase in glucose-dependent insulin secretion (Sauvaire et al. 1998) and lower elevated plasma triglycerides and total cholesterol. Haeri et al. (2012) observed antihyperglycemic and antihyperlipidemic (triglycerides and total cholesterol) effects in STZ-treated rats. The antihyperglycemic effect from fenugreek seeds can also be due to galactomannan and diosgenin (reviewed in Garg 2016). All findings taken together from various studies show that phytoconstituents in seeds of fenugreek can exert antihyperglycemic effect by multiple mechanisms (such as antioxidative, decreased lipid peroxidation, anti-inflammatory, increased insulin secretion, decreased insulin resistance, decreased gluconeogenesis, increased hepatic glycogen content, and others) and therefore present fenugreek seeds as a nutraceutical for diabetic dogs and cats. One drawback with fenugreek for anti-diabetic effect is that a relatively high dose for the antihyperglycemic effect is required. For further details on therapeutic efficacy and safety of fenugreek, readers are referred to Garg (2016) and chapter "Fenugreek for Animals, Birds, and Fish Health" by Kumar and co-authors in this book.

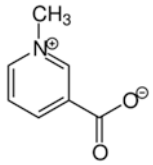


Fig. 3 Structural formula of trigonelline

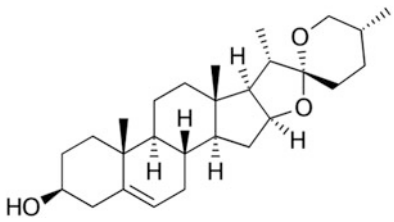


Fig. 4 Structural formula of diosgenin

Fig. 5 *Left*: Dried cinnamon bark and powder obtained from *Cinnamomum* tree. *Right*: Structural formula of (E)-cinnamaldehyde

5.1.4 Cinnamon

Cinnamon is an aromatic spice in powder form (Fig. 5) obtained from the dried bark of several species of the *Cinnamomum* tree (*Cinnamomum zeylanicum* Blume). In the United States, two varieties of cinnamon (Ceylon and cassia) are available. Ceylon cinnamon is considered a true cinnamon and is quite expensive. Although cinnamon bark powder contains many phytoconstituents, (E)-cinnamaldehyde is the main component. Cinnamon bark powder and the structural formula of (E)-cinnamaldehyde are shown in Fig. 5.

Cinnamon powder contains the second highest amount of antioxidants next to cloves. In prediabetics, consumption of 500 mg cinnamon/day for 12 weeks may reduce oxidative stress by 14%. In addition, cinnamon may increase insulin secretion, increase insulin sensitivity, and lower blood glucose by moving glucose into cells. However, the effects of cinnamon on hemoglobin A1C are conflicting. Shen et al. (2010) demonstrated that streptozotocin-induced diabetic rats receiving aqueous extract of *C. zeylanicum* (30 mg/kg/day for 22 days) were rescued from hyperglycemia and nephropathy. These rats were found to have upregulation of uncoupling

protein-1 (UCP-1) and glucose transporter 4 (GLUT4) in their brown adipose tissues as well as in their muscles. In 3T3-L1 adipocytes, cinnamon extract upregulated GLUT4 translocation and increased glucose uptake. Cinnamon extract exhibited its anti-diabetic effect independently from insulin by at least two mechanisms: (1) upregulation of mitochondrial UCP-1 and (2) enhanced translocation of GLUT4 in the muscle and adipose tissues.

In humans, the daily dose of cinnamon is from 1 to 4 g. No clinical trials have yet been undertaken in dogs and cats.

5.1.5 *Stevia rebaudiana*

Stevia rebaudiana (commonly called stevia) is native to Paraguay. Leaves of this plant contain multiple active principles including diterpene glycosides (stevioside; steviolbioside; rebaudiosides A, B, C, D, and E) and dulcoside A. A sweet taste is attributed to stevioside and rebaudiosides. Antihyperglycemic effect occurs due to stevioside. The structural formula of stevioside is shown in Fig. 6.

Recently, Ahmad and Ahmad (2018) reported that diabetic rats receiving an aqueous extract of *Stevia rebaudiana* leaf (200, 300, 400, and 500 mg/kg body wt) for 8 weeks showed (in a dose-dependent manner) a decrease in blood glucose (–73.24%), fasting blood glucose (–66.09%), and HbA1c hemoglobin (5.32%), while insulin (17.82 μIU/mL) and liver glycogen (45.02 mg/g) levels were significantly improved. In another study, Saleh et al. (2016) demonstrated that stevioside administration in diabetic rats exhibited an insulinomimetic effect. Stevioside increased insulin and leptin secretion, normalized hepatic and renal biomarkers, and restored alterations in antioxidant activity and lipid profiles. These authors also reported that for mRNA expression, stevioside upregulated the expression of PK and IRS-1 genes, which are downregulated in diabetic rats, and was very effective in the downregulation of CPT-1 mRNA expression. At the cellular level, stevioside normalized the histopathological changes induced in the pancreas.

Stevia is known to exert antioxidative, antihyperglycemic, antihyperlipidemic, immunomodulatory, hepatoprotective, renoprotective, and antihypertensive effects (Jeppesen et al. 2003; Gregersen et al. 2004; Lailerd et al. 2004; Chen et al. 2005; Hossain et al. 2011; Stoyanova et al. 2011; Lemus-Mondaca et al. 2012; Shivanna et al. 2013). Recently, in a meta-analysis, Nichol et al. (2018) compared the glycemic impact of non-nutritive sweetener (NNS) stevioside with other NNS (aspartame, saccharin, and sucralose) and concluded that the glycemic impact of these sweeteners did not differ by type of NNS. In conclusion, stevia exerts insulin-like effects and appears to be a promising nutraceutical for the management of diabetes and its associated complications.

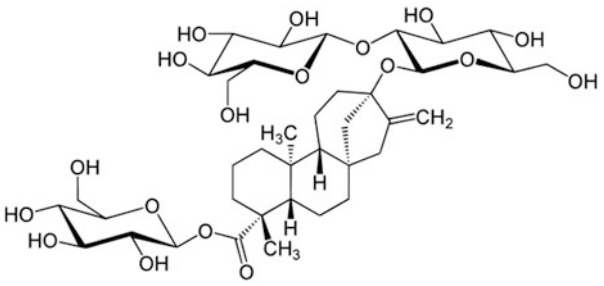


Fig. 6 Structural formula of stevioside

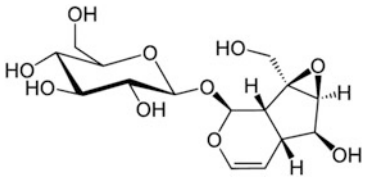


Fig. 7 Structural formula of catalpol

5.1.6 Catalpol

Catalpol is a natural product (an iridoid glucoside) isolated from the root of *Rehmannia glutinosa*. The structural formula of catalpol is shown in Fig. 7.

Catalpol exerts antioxidative, anti-inflammatory, anti-apoptosis, and anti-diabetes properties (Shieh et al. 2011; Zhou et al. 2015). Catalpol has been shown to have beneficial effects against glucose/lipid metabolic disorder and insulin resistance in diabetes (Yan et al. 2018). Zhou et al. (2015) reported that catalpol ameliorated insulin resistance in high-fat diet (HFD)-induced diabetes in mice. Shieh et al. (2011) and Bao et al. (2016) demonstrated that catalpol could improve insulin resistance, decrease blood glucose levels, and promote glucose uptake through increasing the protein expression of glucose transporter-4 (GLUT4) in skeletal muscle and adipose tissues in db/db mice and STZ-induced rats. Catalpol reduced high-fat diet-induced insulin resistance and adipose tissue inflammation by suppressing the JNK and NF-kappaB pathways. Recently, Yan et al. (2018) found that catalpol (100–200 mg/kg/day)-treated T2DM mice via gavage ameliorated hepatic insulin resistance by acting on the AMP-activated protein kinase (AMPK)/NADPH oxidase type 4 (NOX4)/phosphatidylinositol 3-kinase (PI3K)/AKT pathway. The suppressive effect of catalpol on glucosamine-induced NOX4 overexpression was weakened by a knockdown of AMPK with short interfering RNA (siRNA). Catalpol exhibited the effects of decreasing hepatic gluconeogenesis and increasing hepatic glycogen synthesis both in vivo and in vitro (Yan et al. 2018). These findings suggest that catalpol could be a novel nutraceutical to manage hyperglycemia and T2DM in animals and humans.

5.1.7 *Moringa oleifera* Lam

Moringa oleifera is an Indian tree that has been cultivated in many other countries including Mexico. This tree is commonly referred to as the "drum stick tree" or the "horse riding tree." The flowers, leaves, and seeds of *M. oleifera* have been found to be of medicinal value. The therapeutic use of *M. oleifera* leaves has been evaluated in diabetes because the leaves lower blood glucose concentrations after ingestion as they contain polyphenols such as quercetin-3-glycoside, rutin, kaempferol, and glycoside (Jaiswal et al. 2009; Arora et al. 2013; Edoga et al. 2013; Al-Malki and El Rabey 2015). Divi et al. (2012) reported a decrease in fasting blood glucose, oral glucose tolerance test, and postprandial glucose in diabetic rats following *M. oleifera* therapy. Furthermore, the anti-diabetic effect of *M. oleifera* seed powder has been observed in rat models with the decreased blood glucose, the amelioration of lipid peroxide level, and the diminished levels of IL-6 and immunoglobulins A in comparison with diabetic positive control in both insulin-resistant and insulin-deficient bioassays (Anudeep et al. 2016; Gopalakrishnan et al. 2016). Recently, Villarruel-López et al. (2018) reported that consumption of the leaves of *M. oleifera* (50 mg/day) by alloxan monohydrate (150 mg/kg, IP)-induced hyperglycemic/diabetic rats showed hypoglycemia. These authors found that *M. oleifera* leaf powder revealed no adverse effect. The beneficial effects of *M. oleifera* might also be due to its antioxidants, vitamins, and a protease-resistant glycoprotein that functions as dietary fiber (Georgewill et al. 2010; Gupta et al. 2012; Vongsak et al. 2013; Khan et al. 2017). The *M. oleifera* extract has been found effective against diabetes-related complications such as retinopathy (Kumar Gupta et al. 2013). Recently, Khan et al. (2017) suggested that the aqueous extracts of the *M. oleifera* leaf can be used as phytopharmaceuticals for the management of diabetes by using them as adjuvants or alone. In a number of studies, *M. oleifera* has been evaluated for its safety and toxicity (Asare et al. 2012; Awodele et al. 2012; Stohs and Hartman 2015).

M. oleifera has a great potential for its use as anti-diabetic nutraceutical, if it is used in a recommended dose, which is yet to be established through clinical trials in dogs and cats.

5.1.8 *Momordica charantia*

Momordica charantia (bitter melon) is a commonly used vegetable in India, China, and many other countries. Phytoconstituents present in the fruits of this plant include alkaloids, glycosides, saponins, triterpenes, steroids, and fixed oils. Joseph and Jini (2013) reported that consumption of bitter melon lowered blood glucose concentrations by increasing hepatic utilization of glucose and decreasing hepatic glucose output. In a number of studies, bitter melon has shown antihyperglycemic activity, and in normoglycemic rats it produced hypoglycemic activity (Ojewole et al. 2006). Therefore, bitter melon should be used with a great deal of caution in diabetic patients, especially if given as an adjuvant to glucose-lowering drugs (Chan et al. 2016). In addition to hypoglycemia, bitter melon may produce other side effects, such as diarrhea, abdominal pain, headache, fever, atrial fibrillation, and urinary incontinence. In pregnant women, bitter melon has been shown to cause bleeding, contractions, stimulation of menstruation, and miscarriage (reviewed in Gupta et al. 2018).

5.1.9 Diosmin

Diosmin (diosmetin 7-rutinoside) is one of the natural flavone glycosides present in citrus, hyssop, and rosemary. The structural formula of diosmin is shown in Fig. 8.

Diosmin has been demonstrated to improve glucose metabolism in diabetes (Hsu et al. 2017). Its pharmacokinetic profile indicates that diosmin is transformed by gut microbiota to diosmetin, which is absorbed and rapidly distributed throughout the body with a long half-life (26–43 h) in healthy volunteers (Cova et al. 1992). In type 1 DM (T1DM) patients, a diosmin-containing flavonoid mixture showed a decrease in glycosylated hemoglobin and an increase in glutathione peroxidase. Pari and Srinivasan (2010) demonstrated that in T2DM rats, diosmin affected the rate-limiting enzymes of carbohydrate metabolism and was found to reverse the abnormalities in glycoprotein components. In a number of studies, endogenous and exogenous β-endorphin has been shown to increase circulating insulin in humans and animals with DM (Curry et al. 1987; Cheng et al. 2002; Liu and Cheng 2011; Hsu et al. 2017). In essence, diosmin exerts antioxidative, anti-inflammatory, increased insulin secretion by enhanced β-endorphin, decreased glycosylated hemoglobin, and antihyperglycemic activities. Therefore, it has the potential to be a promising nutraceutical for diabetic humans and animals after proper clinical trials in dogs and cats.

5.1.10 Ginseng

Ginseng is the root of plants (genus *Panax*), including Chinese ginseng (*P. notoginseng*), Japanese ginseng (*P. japonica*), Korean ginseng (*P. ginseng*), Vietnamese ginseng (*P. vietnamensis*), and American ginseng (*P. quinquefolius*). Ginseng contains many active ingredients, called ginsenosides

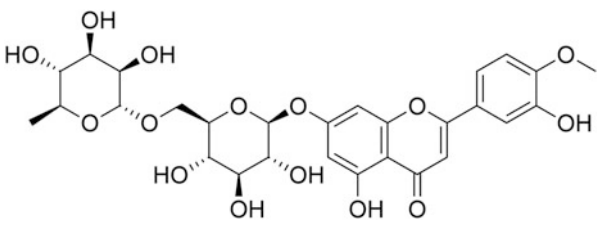


Fig. 8 Structural formula of diosmin

(ginseng saponins). Ginseng has been reported to exert hypoglycemic effect since the early 1970s. Red ginseng was found to be superior over white ginseng for its hypoglycemic effect. The ginsenosides Rb2 and Rh2, protopanaxatriol, (20R)-protopanaxadiol, Rg1, Rc, Rd, Re, Rf, Rg2, Rh1, and Rb1, acidic peptide, adenosine, pyroglutamic acid, and peptidoglycan panaxan B may be responsible for the glucose-lowering effect in diabetes (reviewed in Yang and Wu 2016). Ginsenoside Rh2 exhibited its efficacy in improving DM through reducing blood glucose level and increasing insulin sensitivity in STZ-induced diabetic rats (Lai et al. 2006; Lee et al. 2007). In another study, Xiang et al. (2008) showed that ginsenoside Re had an anti-diabetic effect in mice. In many clinical studies, T2DM patients receiving ginseng have shown an antihyperglycemic effect (Kim et al. 2011). Currently, the recommended anti-diabetic dose of ginseng is 200 mg/day (Natural Medicines Comprehensive Database 2014). Ginseng can be indicated as an alternative natural therapy for diabetic dogs and cats after proper long-term clinical trials. For further details on ginseng, readers are referred to Yang and Wu (2016).

5.1.11 *Costus pictus* and *Costus igneus*

Costus pictus D. Don and *Costus igneus* Nak (commonly called insulin plants) are native to Central and South America. Currently, these plants are also used as an herbal cure for diabetes in America and India. Phytochemical analysis of these plants has revealed the presence of various antioxidants (ascorbic acid, α-tocopherol, β-carotene, terpenoids, and flavonoids), triterpenoids, alkaloids, tannins, saponins, steroids, etc. Devi and Urooj (2008a, b) reported that people with diabetes in India consume one leaf a day to keep their blood glucose under control. In a number of experimental and clinical studies, extracts of these plants have shown antioxidative and anti-diabetic effects (Devi and Urooj 2008a, b; Elavarasi and Saravanan 2012; Hegde et al. 2014). In a cross-sectional study, patients consuming either one fresh leaf or one tablespoon of shade-dried powder/day of *C. igneus*, in conjunction with other modalities of treatment, had effectively produced glycemic control in diabetes (Shetty et al. 2010). Such clinical trials need to be conducted in diabetic dogs and cats before these plant extracts or their constituents can be indicated as nutraceuticals.

5.1.12 Touchi Extract

Touchi extract is a fermented soybean product used for thousands of years in China and Japan. It lowers blood glucose level by inhibiting intestinal α-glucosidase activity. Currently, the recommended dose of Touchi extract is 300 mg before each meal.

5.1.13 Herbal Products Used in Combination Against Diabetes

In complementary and alternative medicine, it is a common practice to use nutraceuticals/plant extracts in combination to attain optimal therapeutic effects. For an antihyperglycemic effect in diabetes, the extracts of *Curcuma longa* and *Eugenia jambolana* (Rao and Najam 2016), or *Cinnamomum tamala* and *Aloe vera* (Singh et al. 2015), or *Balanites aegyptiaca* (desert date) and *Petroselinum sativum* (parsley) provide superior results over when they are used alone (Abou Khalil et al. 2016). In a recent investigation, Rouse et al. (2014) found that resveratrol (0.1–10 μmol/L) and curcumin (1–100 pmol/L) regulated insulin secretion under glucose-stimulated conditions. Additionally, treating β-cell lines and human islets with these polyphenols led to increased intracellular cAMP levels in a manner similar to 3-isobutyl-1-methylxanthine, a classic phosphodiesterase (PDE) inhibitor. Resveratrol and curcumin treatment significantly downregulated the mRNA expression of most of the 11 PDE isozymes, including PDE3B, PDE8A, and PDE10A, which have been inked to regulation of insulin secretion in islets. Both resveratrol and curcumin, in addition to antioxidative and anti-inflammatory activities, inhibited PDE activity in a dose-dependent manner and enhanced pancreatic β-cell function. Currently, many other nutraceuticals/plant alkaloids are used in combination to ameliorate antihyperglycemic and anti-diabetic effects in humans and animals.

5.2 Herbo-Mineral/Metal Nutraceuticals

5.2.1 Shilajit

Shilajit is a pale-brown to blackish-brown herbo-mineral exudate from the sedimentary rocks of the Himalayan Mountains at an altitude of about 10,000 ft. Shilajit has been used for more than 3000 years for both preventative health and treating many diseases in humans, including diabetes, arthritis, hypertension, immune dysfunction, cognition impairment, and arthritis (Agarwal et al. 2007; Lawley et al. 2013). It consists of humus and organic plant (*Styrax officinalis*, *Trifolium repens*, and others) materials. In a number of studies, shilajit has been found to be effective against diabetes. Bhattacharya (1995) reported that oral administration of 50–100 mg/kg of a processed and standardized shilajit attenuated streptozotocin-induced diabetes in rats. Shilajit also increased pancreatic islet superoxide dismutase activity, leading to a decrease in free radical production. Kanikkannan et al. (1994) found that processed shilajit (1.0 mg/kg, sc) prevented streptozotocin-induced diabetes in rats. Shilajit

(100 mg/kg) produced a significant reduction in blood glucose levels and also produced beneficial effects on the lipid profile. The anti-diabetic effect of shilajit could be due to its major constituents (dibenzo-α-pyrones (DBP), DBP-chromoproteins, and fulvic acids with DBP core) exerting antioxidative, anti-inflammatory, and inhibition of alpha-glucosidase and alpha-amylase activity (Aparna et al. 2014; Bhavsar et al. 2016). For further details on shilajit, readers are referred to chapter "Neem Extract" in this book.

5.2.2 Chromium

The use of trivalent chromium in TM2D humans (especially insulin resistant) has become more common because its mechanism of action for antihyperglycemic effect is well explained at the molecular level (Biswas et al. 2010; Hua et al. 2012), and it has been found successful in clinical trials (Ryan et al. 2003). In addition to alleviating insulin resistance, the potential effect of chromium in regulating oxidative stress, and inflammation has been reported (Rains and Jain 2011). A daily dose of 120 μg trivalent chromium is recommended for humans. The FDA allows up to 1000 μg chromium/day. Above this dose, renal toxicity may ensue. In a randomized double-blinded study, moderately osteoarthritic dogs receiving 500 μg trivalent chromium twice daily for a period of 150 days showed no adverse effect (Fleck et al. 2014). Trivalent chromium can be a good adjunct to nutraceutical therapy in diabetic dogs and cats after valid clinical trials.

6 Concluding Remarks and Future Directions

Diabetes in canines and felines is a serious health concern around the world. Etiopathologies and underlying mechanisms of action in diabetes are very complex. Currently, management of diabetes, hyperglycemia, hyperlipidemia, and diabetes-associated complications (cardiac, renal, and nerve damage) relies on diet monitoring, moderate exercise, and pharmacotherapeutic medicines, including insulin injections. Due to the high cost and side effects of synthetic medicines, alternative complementary medicines/nutraceuticals are receiving enormous interest in the scientific community as well as from pet owners. For a large number of nutraceuticals, in vitro and in vivo studies have been conducted for efficacy and safety evaluation, while large-scale clinical trials are completely lacking in dogs and cats. Some of the nutraceuticals have shown strong potential for an anti-diabetic effect and deserve further evaluation in clinical trials. Also, when nutraceuticals are given in conjunction with conventional therapeutic medicines, they need to be evaluated for any possible toxic interactions.

Acknowledgment The authors would like to thank Ms. Robin B. Doss for her technical assistance in preparation of this chapter.

References

Abo-Salem OM, Harisa GI, Ali TM (2014) Curcumin ameliorates streptozotocin-induced heart injury in rats. J Biochem Mol Toxicol 28:263–270

Abou Khalil NS, Abou-Elhamd Alaa S, Wasfy Salwa IA et al (2016) Antidiabetic and antioxidant impacts of desert date (*Balanites aegyptiaca*) and parsley (*Petroselinum sativum*) aqueous extracts: lessons from experimental rats. J Diab Res 2016:ID 8408326

Accurso A, Bernstein RK, Dahlqvist A et al (2008) Dietary carbohydrate restriction in type 2 diabetes mellitus and metabolic syndrome: time for a critical appraisal. Nutr Metab 5(1):9

Agarwal SP, Khanna R, Karmarkar R et al (2007) Shilajit: a review. Phytother Res 21:401–405

Ahmad U, Ahmad RS (2018) Anti-diabetic property of aqueous extract of *Stevia rebaudiana* leaves in streptozotocin-induced diabetes in albino rats. BMC Complement Altern Med 18:179

Ahmed I, Adehgate E, Cummings E et al (2004) Beneficial effects and mechanisms of action of *Momordica charantia* juice in the treatment of streptozotocin-induced diabetes mellitus in rat. Mol Cell Biochem 261:63–70

Ahmed OM, Abdel-Moneim A, Abulyazid I et al (2010) Antihyperglycemic, anti-hyperlipidemic and antioxidant effects and the probable mechanisms of action of *Ruta graveolens* and rutin in nicotinamide/streptozotocin diabetic albino rats. Diabetol Croat 39:15–32

Al-Malki AL, El Rabey HA (2015) The antidiabetic effect of low doses of *Moringa oleifera* Lam. Seeds on streptozotocin induced diabetes and diabetic nephropathy in male rats. BioMed Res Int. https://doi.org/10.1155/2015/381040

Anand P, Murali KY, Tandon V et al (2010) Insulinotropic effect of cinnamaldehyde on transcriptional regulation of pyruvate kinase, phosphoenolpyruvate carboxykinase and GLUT4 translocation in experimental diabetic rats. Chem Biol Interact 186:72–81

Anand P, Kunnumakkara AB, Newman RA et al (2013) Bioavailability of curcumin: problems and promises. Mol Pharm 4(6):807–818

Antia BS, Okokon JE, Okon PA (2005) Hypoglycemic effect of aqueous leaf extract of *Persea americana* Mill on alloxan-induced diabetic rats. Indian J Pharmacol 37:325–326

Antony B, Marina B, Iyer VS et al (2008) A pilot cross-over study to evaluate human oral bioavailability of BCM-95CG (Biocurcumax), a novel bioenhanced preparation of curcumin. Indian J Pharm Sci 70 (4):445–449

Anudeep S, Prasana VK, Adya SM et al (2016) Characterization of soluble dietary fiber from *Moringa oleifera* seeds and its immunomodulatory effects. Int J Biol Macromol 91:656–662

Aparna A, Rege A, Chowdhary AS (2014) Evaluation of alpha-amylase and alpha-glucosidase inhibitory activity of Shilajit. Int J Adv Res 2:735–740

Araki E, Miyazaki J (2007) Metabolic disorders in diabetes mellitus: impact of mitochondrial function and oxidative stress on diabetes and its complications. Antioxid Redox Signal 9(3):289–291

Arora DS, Onsare JG, Kaur H (2013) Bioprospecting of *Moringa* (*Moringaceae*): microbiological perspective. J Pharmacogn Phytochem 1(6):193–215

Asare GA, Gyan B, Bugyei K et al (2012) Toxicity potentials of the nutraceutical *Moringa oleifera* at supra-supplementation levels. J Ethnopharmacol 139:265–272

Awodele O, Oreagba IA, Odoma S et al (2012) Toxicological evaluation of the aqueous leaf extract of *Moringa oleifera* Lam. (Moringaceae). J Ethnopharmacol 139:330–336

Babu V, Gangadevi T, Subramonium A (2002) Anti-hyperglycemic activity of cassia kleinii leaf extract in glucose fed normal rats and alloxan-induced diabetic rats. Indian J Pharmacol 34:409–415

Babu V, Gangadevi T, Subramonium A (2003a) Anti-hyperglycemic activity of *Cassia kleinii* leaf extract in glucose fed normal and alloxan-induced diabetic rats. Indian J Pharmacol 34:409–415

Babu V, Gangadevi T, Subramonium A (2003b) Antidiabetic activity of ethanol extract of *Cassia kleinii* leaf in streptozotocin-induced diabetic rats and isolation of an active fraction and toxicity evaluation of the extract. Indian J Pharmacol 35(5):290–296

Balamurugan AN, Miyamoto M, Wang W et al (2003) Streptozotocin (STZ) used to induce diabetes in animal models. J Ethnopharmacol 26:102–103

Bao X, Shen L, Qian C et al (2016) Anti-diabetic activities of catalpol in db/db mice. Korean J Physiol Pharmacol 20(2):153–160

Bhatt JK, Thomas S, Nanjan MJ (2012) Resveratrol supplementation improves glycemic control in type 2 diabetes mellitus. Nutr Res 32:534–541

Bhattacharya SK (1995) Shilajit attenuates streptozotocin induced diabetes mellitus and decreases pancreatic islet superoxide dismutase activity in rats. Phytother Res 9:41–44

Bhavsar SK, Thaker AM, Malik JK (2016) Shilajit. In: Gupta RC (ed) Nutraceuticals: efficacy, safety and toxicity. Academic/Elsevier, Amsterdam, pp 707–716

Bhowmik A, Khan LA, Akhter M et al (2009) Studies on the antidiabetic effects of *Mangifera indica* stem-barks and leaves on nondiabetic, type 1 and type 2 diabetic model rats. Bangladesh J Pharmacol 4:110–114

Biswas TK, Polley G, Pandit S et al (2010) Effects of adjunct therapy of proprietary herbo-chromium supplement in type 2 diabetes: a randomized clinical trial. Int J Diab Develop Countr 30:153–161

Brouns F (2018) Overweight and diabetes prevention: is a low-carbohydrate-high-fat diet recommendable? Eur J Nutr 57:1301–1312

Can A, Akev N, Ozsoy N et al (2004) Effect of *Aloe vera* leaf gel and pulp extracts on the liver in type-II diabetic rat models. Biol Pharm Bull 27:694–698

Castro CN, Barcala Tabarrozzi AE, Winnewisser J et al (2014) Curcumin ameliorates autoimmune diabetes. Evidence in accelerated murine models of type 1 diabetes. Clin Exp Immunol 177:149–160

Celano R, Piccinelli AL, Pugliese A et al (2018) Insights into the analysis of phenolic secoiridoids in extra virgin olive oil. J Agric Food Chem 66:6053–6063

Chan N, Li S, Perez E (2016) Interactions between Chinese nutraceuticals and Western medicines. In: Gupta RC (ed) Nutraceuticals: efficacy, safety and toxicity. Academic/Elsevier, Amsterdam, pp 875–882

Chen QM, Xie MZ (1986) Studies on the hypoglycemic effect of *Coptis chinensis* and berberine. Acta Pharm Sin 21:401–406

Chen TH, Chen SC, Chan P et al (2005) Mechanism of the hypoglycemic effect of stevioside, a glycoside of *Stevia rebaudiana*. Plant Med 71:108–113

Cheng JT, Liu IM, Tzeng TF et al (2002) Plasma glucose-lowering effect of beta-endorphin in streptozotocin-induced diabetic rats. Horm Metab Res 34:570–576

Cho WC, Chung WS, Lee SK et al (2006) Ginsenoside Re of *Panax ginseng* possesses significant antioxidant and antihyperlipidemic efficacies in streptozotocin-induced diabetic rats. Eur J Pharmacol 550(1–3):173–179

Chothani DL, Vaghasiya HU (2011) A review on *Balanites aegyptiaca* Del (desert date): phytochemical constituents, traditional uses, and pharmacological activity. Pharmacogn Rev 5(9):55–62

Chusak C, Thilavech T, Henry CJ et al (2018) Acute effect of *Clitoria ternatea* flower beverage on glycemic response and antioxidant capacity in healthy subjects: a randomized crossover trial. BMC Complement Altern Med 18:6

Corradini S, Pilosio B, Dondi F et al (2016) Accuracy of a flash glucose monitoring system in diabetic dogs. J Vet Int Med 30 (4):983–988

Cova D, De Angelis L, Giavarini F et al (1992) Pharmacokinetics and metabolism of oral diosmin in healthy volunteers. Int J Clin Pharmacol Ther Toxicol 30:29–33

Cuomo J, Appendino G, Dern AS et al (2011) Comparative absorption of a standardized curcuminoid mixture and its lecithin formulation. J Nat Prod 74(4):664–669

Curry DL, Bennett LL, Li CH (1987) Stimulation of insulin secretion by beta-endorphins (1-27 and 1-31). Life Sci 40:2053–2058

DeFronzo RA, Tripathy D (2009) Skeletal muscle insulin resistance is the primary defect in type 2 diabetes. Diab Care 32(Suppl 2):S157–S163

Delack JB, Stogdale L (1983) Glycosylated hemoglobin measurement in dogs and cats: implications for its utility in diabetic monitoring. Can Vet J 24:308–311

De Melo IS, dos Santos AF, Bueno NB (2018) Curcumin or combined curcuminoids are effective in lowering the fasting blood glucose concentrations of individuals with dysglycemia: systematic review and meta-analysis of randomized controlled trials. Pharmacol Res 128:137–144

Deore SL, Khadabadi SS, Daulatkar VD et al (2008) Evaluation of hypoglycemic and antidiabetic activity of *Butea monosperma*. Pharmacogn Mag 4(13):134–138

Devi VD, Urooj A (2008a) Hypoglycemic potential of *Morus indica* L and *Costus igneus* Nak: a preliminary study. Indian J Exp Biol 46:614–616

Devi VD, Urooj A (2008b) Nutrient profile and antioxidant components of *Costus specious* Sm. and *Costus igneus* Nak. Indian J Nat Prod 1:116–118

Dhasarathan P, Theriappan P (2011) Evaluation of anti-diabetic activity of *Strychnos potatorum* in alloxan induced diabetic rats. J Med Sci 2 (2):670–674

Dheer R, Bhatnagar P (2010) A study of the antidiabetic activity of *Barleria prionitis* Linn. Indian J Pharmacol 42:70–73

Divi SM, Bellamkonda R, Dasireddy SK (2012) Evaluation of antidiabetic and antihyperlipidemic potential of aqueous extract of *Moringa oleifera* in fructose feed insulin resistant and STZ induced diabetic Wistar rats: a comparative study. Asian J Pharm Clin Res 5 (1):67–72

Dong SF, Hong Y, Liu M et al (2011) Berberine attenuates cardiac dysfunction in hyperglycemic and hypercholesterolemic rats. Eur J Pharmacol 660:368–374

Dong H, Wang N, Zgao L, Lu F (2012) Berberine in the treatment of type 2 diabetes mellitus: a systemic review and meta-analysis. Evid Based Complement Altern Med 2012:591654

Drummond E, Flynn S, Whelan H et al (2018) Casein hydrolysate with glycemic control properties: evidence from cells, animal models, and humans. J Agric Food Chem 66:4352–4363

Edoga CO, Njoku OO, Amadi EN et al (2013) Blood sugar lowering effect of *Moringa oleifera* Lam. in albino rats. Int J Sci Technol 3:88–90

Elavarasi S, Saravanan K (2012) Ethnobotanical study of plants used to treat diabetes by tribal people of Kolli Hills, Namakkal District, Tamilnadu, Southern India. Int J Pharm Tech Res 4:404–411

Etuk EU (2010) Animals models for studying diabetes mellitus. Agric Biol J North Am 1(2):130–134

Etuk EU, Muhammed BJ (2010) Evidence based analysis of chemical method of induction of diabetes mellitus in experimental rats. Int J Pharm Sci 1(2):139–142

Feinman RD, Pogozelski WK, Astrup A et al (2015) Dietary carbohydrate restriction as the first approach in diabetes management: critical review and evidence base. Nutrition 31(1):1–13

Fleck A, Gupta RC, Goad JT et al (2014) Anti-arthritic efficacy and safety of chrminex®3+ (trivalent chromium, *Phyllanthus emblica* extract, and shilajit) in moderately arthritic dogs. J Vet Sci Anim Husb 1(4):401

Fujii A, Kobayashi S, Kuboyama N et al (1990) Augmentation of wound healing by Royal jelly (RJ) in streptozotocin-diabetic rats. Jpn J Pharmacol 53:331–337

Garg RC (2016) Fenugreek: multiple health benefits. In: Gupta RC (ed) Nutraceuticals: efficacy, safety and toxicity. Academic/Elsevier, Amsterdam, pp 599–617

Geetha G, Kalavalarasariel GP, Sankar V (2011) Anti-diabetic effect of *Achyranthes rubrofusca* leaf extracts on alloxan induced diabetic rats. Pak J Pharm Sci 24:193–199

Georgewill OA, Georgewill UO, Nwankwoala RNP (2010) Anti-inflammatory effects of *Moringa oleifera* Lam. extract in rats. Asian Pac J Trop Med 3(2):133–135

Geuns JMC, Buyse J, Vankeirsbilck A et al (2007) Metabolism of stevioside by healthy subjects. Exp Biol Med 232:164–173

Ginsberg BH (2009) Factors affecting blood glucose monitoring: sources of errors in measurement. J Diab Sci Technol 3(4):903–913

Gopalakrishnan L, Doriya K, Kumar DS (2016) *Moringa oleifera*: a review on nutritive importance and its medicinal application. Food Sci Hum Welln 5:49–56

Gregersen S, Jeppesen PB, Holst JJ et al (2004) Anti-hyperglycemic effects of stevioside in type 2 diabetic subjects. Metabolism 53:73–76

Gupta R, Mathur M, Bajaj VK et al (2012) Evaluation of antidiabetic and antioxidant activity of *Moringa oleifera* in experimental diabetes. J Diabetes 4(2):164–171

Gupta RC, Chang D, Nammi S et al (2017) Interactions between antidiabetic drugs and herbs: an overview of mechanisms of action and clinical implications. Diabetol Metab Syndr 9:59

Gupta RC, Srivastava A, Lall R (2018) Toxicity potential of nutraceuticals. In: Nicolotti O (ed) Computational toxicology methods and protocols. Humana/Springer, New York, pp 367–394

Haeri MR, Limaki HM, White CJB et al (2012) Non-insulin dependent anti-diabetic activity of (2S, 3R, 4S) 4-hydroxyisoleucine of fenugreek (*Trigonella foenum graecum*) in streptozotocin-induced type I diabetic rats. Phytomedicine 19:571–574

Hall K (2017) A review of the carbohydrate-insulin model of obesity. Eur J Clin Nutr 71(5):679. https://doi.org/10.1038/ejcn.2017.21

Hariri M, Haghighatdoost F (2017) Effect of curcumin on anthropometric measures: a systematic review on randomized clinical trials. J Am Coll Nutr. https://doi.org/10.1080/07315724.2017.1392263

Hegde PK, Rao HA, Rao PN (2014) A review on insulin plant (*Costus igneus* Nak). Pharmacogn Rev 8(15):67–72

Hikino H, Kobayashi M, Suzuki Y et al (1989) Mechanisms of hypoglycemic activity of aconitan A, a glycan from Aconitum carmichaelii roots. J Ethnopharmacol 27:295–304

Hossain MS, Alam MB, Asadujjaman M et al (2011) Antihyperglycemic and anti-hyperlipidemic effects of different fractions of *Stevia rebaudiana* leaves in alloxan induced diabetic rats. IJPSR 2:1722–1729

Hsu CH, Cheng AL (2007) Clinical studies with curcumin. Adv Exp Med Biol 595:471–480

Hsu CC, Lin MH, Cheng JT et al (2017) Antihyperglycemic action of diosmin, a citrus flavonoid, is induced through endogenous β-endorphin in type I-like diabetes rats. Clin Exp Pharmacol Physiol 44(5):549–555

Hua Y, Clark S, Ren J et al (2012) Molecular mechanisms of chromium in alleviating insulin resistance. J Nutr Biochem 23:313–319

Huang M, Deng S, Han Q et al (2016) Hypoglycemic activity and the potential mechanism of the flavonoid rich extract from *Sophora tonkinensis* Gagnep in KK-Ay mice. Front Pharmacol 7:288

Imenshahidi M, Hosseinzadeh H (2015) *Berberis vulgaris* and berberine: an update review. Phytother Res 30:1745–1764

Irondi EA, Oboh G, Akindahunsi AA (2016) Antidiabetic effects of *Mangifera indica* kernel flour-supplemented diet in streptozotocin-induced type 2 diabetes in rats. J Food Sci Nutr 4 (6):828–839

Iwai K, Kim MY, Onodera A et al (2006) Alpha-glucosidase inhibitory and antihyperglycemic effects of polyphenols in the fruit of *Viburnum dilatatum* Thunb. J Agric Food Chem 54:4588–4592

Jager R, Lowery RP, Calvanese AV et al (2014) Comparative absorption of curcumin formulations. Nutr J 13:1

Jain PG, Patil SD, Haswani NG et al (2010) Hypolipidemic activity of *Moringa oleifera* Lam., *Moringaceae*, on high fat diet induced hyperlipidemia in albino rats. Rev Bras Farmacogn 20:969–973

Jain D, Bansal MK, Dalvi R et al (2014) Protective effect of diosmin against diabetic neuropathy in experimental rats. J Integr Med 12:35–41

Jaiswal D, Rai PS, Kumar A et al (2009) Effect of *Moringa oleifera* Lam. Leaves aqueous extract therapy on hypoglycemic rats. J Ethnopharmacol 123:392–396

Javeri I, Chand N (2016) Curcumin. In: Gupta RC (ed) Nutraceuticals: efficacy, safety and toxicity. Academic/Elsevier, Amsterdam, pp 435–445

Jayaprasad B, Sharavanan PS, Sivaraj R (2015) Effect of *Chloroxylon swietenia* Dc bark extracts on STZ induced diabetic rats with special attention to its glycoprotein levels. Der Pharmac Lett 7(12):414–418

Jayasri MA, Gunasekaran S, Radha A et al (2008) Antidiabetic effect of *Costus pictus* leaves in normal and streptozotocin-induced diabetic rats. Int J Diabetes Metab 16:117–122

Jeppesen PB, Gregersen S, Rolfsen SE et al (2003) Antihyperglycemic and blood pressure-reducing effects of stevioside in the diabetic Goto-Kakizaki rat. Metabolism 52:372–378

Jiménez-Flores LM, López-Briones S, Macías-Cervantes MH et al (2014) A PPARγ, NF-κB, and AMPK-dependent mechanism may be involved in the beneficial effects of curcumin in the diabetic db/db mice liver. Molecules 19:8289–8302

Joseph B, Jini D (2013) Antidiabetic effects of *Momordica charantia* (bitter melon) and its medicinal potency. Asian Pac J Trop Dis 3 (2):93–102

Kaneto H, Katakami N, Kawamori D et al (2007) Involvement of oxidative stress in the pathogenesis of diabetes. Antioxid Redox Sign 9(3):355–366

Kang S, Tsai LT, Rosen ED et al (2016) Nuclear mechanisms of insulin resistance. Trends Cell Biol 26(5):341–351

Kangralkar V, Shivraj A, Patil D (2010) Oxidative stress and diabetes: a review. Int J Pharm Appl 1:38–45

Kanikkannan N, Ramarao P, Ghosal S (1994) Shilajit-induced potentiation of the hypoglycemic action of insulin and inhibition of streptozotocin induced diabetes in rats. Phytother Res 9:478–481

Kaur G, Kamboj P, Kalia AN et al (2011) Antidiabetic and anti-hypercholesterolemic effects of aerial parts of *Sida cordifolia* Linn on streptozotocin-induced diabetic rats. Indian J Nat Prod Resour 2:428–434

Khan W, Parveen R, Chester K et al (2017) Hypoglycemic potential of aqueous extract of *Moringa oleifera* leaf and *in vivo* GC-MS metabolomics. Front Pharmacol 8:577

Kim JA, Wei Y, Sowers JR et al (2008) Role of mitochondrial dysfunction in insulin resistance. Circ Res 102(4):401–414

Kim S, Shin BC, Lee MS et al (2011) Red ginseng for type 2 diabetes mellitus: a systematic review of randomized controlled trials. Chin J Integr Med 17:937–944

King AJF (2012) The use of animal models in diabetes research. Br J Pharmacol 166:877–894

Kong WJ, Zhang H, Song DQ et al (2009) Berberine reduces insulin resistance through protein kinase C-dependent up-regulation of insulin receptor expression. Metabolism 58:109–119

Kouzuma T, Usami T, Yamakoshi M et al (2002) An enzymatic method for the measurement of glycated albumin in biological samples. Clin Chim Acta 324:61–71

Kumar A, Chopra EK, Mukherjee M et al (2015) Current knowledge and pharmacological profile of berberine: an update. Eur J Pharmacol 761:288–297

Kumar Gupta S, Kumar B, Srinivasan BP et al (2013) Retinoprotective effects of *Moringa oleifera* via antioxidant, anti-inflammatory, and anti-angiogenic mechanisms in streptozotocin-induced diabetic rats. J Ocul Pharmacol Ther 29:419–426

Lai DM, Tu YK, Liu IM et al (2006) Mediation of beta-endorphin by ginsenoside Rh2 to lower plasma glucose in streptozotocin-induced diabetic rats. Planta Med 72:9–13

Lailerd N, Saengsirisuwan V, Slonigar JA (2004) Effect of stevioside on glucose transport activity in rat muscle. Metabolism 53:101–107

Lama A, Pirozzi C, Mollica MP et al (2017) Polyphenol-rich virgin olive oil reduces insulin resistance and liver inflammation and improves mitochondrial dysfunction in high-fat diet fed rats. Mol Nutr Food Res 61(3)

Lan J, Zhao Y, Dong F et al (2015) Meta-analysis of the effect and safety of berberine in the treatment of type 2 diabetes mellitus, hyperlipidemia and hypertension. J Ethnopharmacol 161:69–81

Lawley S, Gupta RC, Goad JT et al (2013) Anti-inflammatory and anti-arthritic efficacy and safety of purified shilajit in moderately arthritic dogs. J Vet Sci Anim Husb 1(3):302

Lee CY (2016) Glucagon-like peptide-1 formulation—the present and future development in diabetic treatment. Basic Clin Pharmacol Toxicol 118:173–180

Lee WK, Kao ST, Liu IM et al (2006) Increase of insulin secretion by ginsenoside Rh2 to lower plasma glucose in Wistar rats. Clin Exp Pharmacol Physiol 33(1–2):27–32

Lee WK, Kao ST, Liu IM et al (2007) Ginsenoside Rh2 is one of the active principles of *Panax ginseng* root to improve insulin sensitivity in fructose-rich chow-fed rats. Horm Met Res 39:347–354

Lemus-Mondaca R, Vega-Galvez A, Zura-Bravo L et al (2012) *Stevia rebaudiana* Bertoni, source of a high-potency natural sweetener: a comprehensive review on the biochemical, nutritional and functional aspects. J Food Chem 132:1121–1132

Lenzen S (2008) The mechanisms of alloxan- and streptozotocin-induced diabetes. Diabetology 51:216–226

Li X-Y (2007) Efficacy and safety of berberine in the treatment of diabetes with dyslipidemia. US ClinicalTrials.gov

Liu IM, Cheng JT (2011) Mediation of endogenous beta-endorphin in the plasma glucose-lowering actin of herbal products observed in type 1-like diabetic rats. Evid Based Complement Alternat Med 2011:987876

Liu A, Lou H, Zhao L et al (2006) Validated LC/MS/MS assay for curcumin and tetrahydrocurcumin in rats plasma and application to pharmacokinetic study of phospholipid complex of curcumin. J Pharm Biomed Anal 40(3):720–727

Liu Q, Li W, Nagata K et al (2018) Isolation, structural elucidation, and liquid chromatography-mass spectrometry analysis of steroidal glycosides from *Polygonatum odoratum*. J Agric Food Chem 66 (2):521–531

Ma X, Yang W, Laaksonen O et al (2017) Role of flavonols and proanthocyanidins in the sensory quality of Sea buckthorn (*Hippophae rhamnoides* L.) berries. J Agric Food Chem 65:9871–9879

Mahomed IM, Ojewole JA (2003) Hypoglycemic effect of *Hypoxis hemerocallidea* corm (African potato) aqueous extract in rats. Methods Find 25(8):617

Manolova Y, Deneva V, Antonov L et al (2014) The effect of the water on the curcumin tautomerism: a quantitative approach. Spectroch Acta Part A Mol Biomol Spectrosc 132:815–820

Matsuzawa-Nagata N, Takamura T, Ando H et al (2008) Increased oxidative stress precedes the onset of high-fat diet-induced insulin resistance and obesity. Metabolism 57(8):1071–1077

Menchetti L, Canali C, Castellini C et al (2018) The different effects of linseed and fish oil supplemented diets on insulin sensitivity of rabbit does during pregnancy. Res Vet Sci 118:126–133

Menon VP, Sudheer AR (2007) Antioxidant and anti-inflammatory properties of curcumin. Adv Exp Med Biol 595:105–125

Metzler M, Pfeiffer E, Schulz SI et al (2013) Curcumin uptake and metabolism. BioFactors (Oxford, England) 39(1):14–20

Mishra Y, Khan MSY, Zafar R et al (1990) Hypoglycemic activity of leaves of *Lagerstroemia speciosa* (L) Pers. Indian J Pharmacol 22:174–176

Miura T, Itoh Y, Iwamoto N et al (2004) Suppressive activity of the fruit of Momordica charantia with exercise on blood glucose in type 2 diabetes mice. Biol Pharm Bull 27:248–250

Muhammad DRA, Dewettinck K (2017) Cinnamon and its derivatives as potential ingredient in functional food—a review. Int J Food Prop 20(D2):S2237–S2263

Nagalievska M, Sabadashka M, Hachkova H et al (2018) *Galega officinalis* extract regulate the diabetes mellitus related violations of proliferation, functions and apoptosis of leukocytes. BMC Complement Altern Med 18:4

Nair SS, Kavrekar V, Mishra A (2013) Evaluation of *in vitro* anti-diabetic activity of selected plant extracts. Int J Pharm Sci Invent 2:12–19

Natural Medicines Comprehensive Database (2014) Ginseng. Panax. Retrieved January 25, 2015 from http://www.nlm.nih.gov/medilineplus/druginfo/natural/1000.html

Naveen J, Baskaran V (2018) Antidiabetic plant-derived nutraceuticals: a critical review. Eur J Nutr 57:1275–1299

Nelson KM, Dahlin JL, Bisson J et al (2017) The essential medicinal chemistry of curcumin: miniperspective. J Med Chem 60 (5):1620–1637

Nichol AD, Holle MJ, An R (2018) Glycemic impact of non-nutritive sweeteners: a systematic review and meta-analysis of randomized controlled trials. Eur J Clin Nutr 72:796–804

Njanje I, Bagla VP, Beseni BK et al (2017) Defatting of acetone leaf extract of *Acacia karroo* (Hayne) enhances its hypoglycemic potential. BMC Complement Altern Med 17:482

Ojewole JA (2006) Antinociceptive, antiinflammatory and antidiabetic properties of *Hypoxis hemerocallidea* (*Hypoxidaceae*) corn (African potato) aqueous extract in mice and rats. J Ethnopharmacol 103:126–134

Ojewole JA, Adewole SO, Olayiwola G (2006) Hypoglycemic and hypotensive effects of *Momordica charantia* Linn (Cucurbitaceae) whole-plant aqueous extract in rats. Cardiovasc J South Afr 17:227–232

Okoli CO, Obidike IC, Ezike AC et al (2011) Studies on the possible mechanisms of antidiabetic activity of extract of aerial parts of *Phyllanthus niruri*. Pharm Biol 49(3):248–255

Pang B, Zhao LH, Zhou Q et al (2015) Application of berberine on treating type 2 diabetes mellitus. Int J Endocrinol 2015:905749

Pari L, Srinivasan S (2010) Antihyperglycemic effect of diosmin on hepatic key enzymes of carbohydrate metabolism in streptozotocin-nicotinamide-induced diabetic rats. Biomed Pharmacother 64:477–481

Parveen K, Khan R, Siddiqui WA (2011) Antidiabetic effects afforded by *Terminalia arjuna* in high fat-fed and streptozotocin-induced type 2 diabetic rats. Int J Diab Metab 19:23–33

Patel DK, Prasad SK, Kumar R et al (2012) An overview on antidiabetic medicinal plants having insulin mimetic property. Asian Pac J Trop Biomed 2(4):320–330

Perez-Gutierrez RM, Mota Flores JM (2010) Attenuation of hyperglycemia and hyperlipidemia in streptozotocin diabetic rats by

chloroform extract of fruits of Ferocactus latispinus and Ferocactus histrix. BOL Latinoam Caribe Plant Med Aromat 9:475–484
Prabhu KS, Lobo R, Shirwaikar A (2008) Antidiabetic properties of the alcoholic extract of *Sphaeranthus indicus* in streptozotocin-nicotinamide diabetic rats. J Pharmac Pharmacol 60:909–916
Prakash O, Kumar R, Mishra A et al (2009) *Artocarpus heterophyllus* (Jackfruit): an overview. Pharmacogn Rev 3(6):353–358
Purpura M, Lowery RP, Wilson JM et al (2018) Analysis of different innovative formulations of curcumin for improved relative oral bioavailability in human subjects. Eur J Nutr 57:929–938
Rafieian-Kopaei M, Nasri H (2014) The ameliorative effect of *Zingiber officinale* in diabetic nephropathy. Iran Red Crescent Med J 16:1–2
Ragasa C, Ng VAS, Park JH et al (2014) Chemical constituents of *Artocarpus altilis* and *Artocarpus odoratissimus*. Res J Pharm Biol Chem Sci 5(4):1081–1087
Rahmani AH, Al Shabrmi FM, Aly SM (2014) Active ingredients of ginger as potential candidates in the prevention and treatment of diseases via modulation of biological activities. Int J Pathophysiol Pharmacol 6(2):125–136
Rains JL, Jain SK (2011) Oxidative stress, insulin signaling, and diabetes. Free Radic Biol Med 50:567–575
Rao SS, Najam R (2016) Efficacy of combination herbal product (*Curcuma longa* and *Eugenia jambolana*) used for diabetes mellitus. Pak J Pharm Sci 29(1):201–204
Rather LJ, Ul-Islam S, Mohammad F (2015) *Acacia nilotica* (L.): a review of its traditional uses, phytochemistry, and pharmacology. Sustain Chem Pharm 2:12–30
Raza H, Prabu SK, John A et al (2011) Impaired mitochondrial respiratory functions and oxidative stress in streptozotocin-induced diabetic rats. Int J Mol Sci 12(5):3133–3147
Reed MJ, Meszaros K, Entes LJ et al (2000) A new rat model of type 2 diabetes: the fat-fed, streptozotocin-treated rat. Metabolism 49:1390–1394
Reher G, Slijepcevic M, Kraus L (1991) Hypoglycemic activity of triterpenes and tannins from *Sarcopoterium spinosum* and two *Sanguisorba* species. Planta Med 57:A57–A58
Ribes G, Sauvaire Y, Da Costa C et al (1986) Antidiabetic effects of subfractions from fenugreek seeds in diabetic dogs. Proc Soc Exp Biol Med 182:159–166
Roufogalis BD (2014) *Zingiber officinale* (Ginger): a future outlook on it's potential in prevention and treatment of diabetes and prediabetic states. New J Sci 2014:1–5
Rouse M, Younes A, Egan JM (2014) Resveratrol and curcumin enhance pancreatic β-cell function by inhibiting phosphodiesterase activity. J Endocrinol 223:107–117
Ryan GJ, Wanko NS, Redman AR et al (2003) Chromium as adjunctive treatment for type 2 diabetes. Ann Pharmacother 37:876–885
Sabino M, Capomaccio S, Cappelli K et al (2018) Oregano dietary supplementation modifies the liver transcriptome profile in broilers: RNASeq analysis. Res Vet Sci 117:85–91
Saleh OM, Awad NS, Soliman MM et al (2016) Insulin-mimetic activity of stevioside on diabetic rats: biochemical, molecular and histopathological study. Afr J Tradit Complement Altern Med 13 (2):156–163
Sasaki H, Sunagawa Y, Takahashi K et al (2011) Innovative preparation of curcumin for improved oral bioavailability. Biol Pharm Bull 34 (5):660–665
Sathiyabama RG, Gandhi GR, Denadai M et al (2018) Evidence of insulin-dependent signaling mechanisms produced by *Citrus sinensis* (L.) osbeck fruit peal in an insulin resistant diabetic animal model. Food Chem Toxicol Part B 116:86–99
Sauvaire Y, Petit P, Broca C et al (1998) 4-Hydroxyisoleucine: a novel amino acid potentiator of insulin secretion. Diabetes 47:206–2010
Selvan VT, Manikandan L, Senthil KGP et al (2008) Antidiabetic and antioxidant effect of methanol extract of *Artanema sesamoides* in streptozotocin-induced diabetic rats. Int J Appl Res Nat Prod 1:25–33
Sharman EH, Bondy SC (2016) Melatonin: a safe nutraceutical and clinical agent. In: Gupta RC (ed) Nutraceuticals: efficacy, safety and toxicity. Academic/Elsevier, Amsterdam, pp 501–509
Shen Y, Fukushima M, Ito Y et al (2010) Verification of the antidiabetic effects of Cinnamon (*Cinnamomum zeylanicum*) using insulin-uncontrolled type 1 diabetic rats and cultured adipocytes. Biosci Biotechnol Biochem 74(12):2418–2425
Shetty AJ, Parampalli SM, Bhandarkar R et al (2010) Effect of insulin plant (*Costus igneus*) leaves on blood glucose levels in diabetic patients: a cross sectional study. J Clin Diagn Res 4:2617–2621
Shieh JM, Wu HT, Chung KC et al (2009) Melatonin ameliorates high-fat diet-induced diabetes and stimulates glycogen synthesis via a PKzeta-Akt-GSK3beta pathway in hepatic cells. Pineal Res 47 (4):339–344
Shieh J, Cheng KC, Chung HH et al (2011) Plasma glucose lowering mechanisms of catalpol, an active principle from roots of *Rehmannia glutinosa*, in streptozotocin-induced diabetic rats. J Agric Food Chem 59(8):3747–3753
Shirwaikar A, Rajendran K, Punitha ISR (2005) Antidiabetic activity of alcoholic stem extract of *Coscinium fenestratum* in streptozotocin-nicotinamide induced type 2 diabetic rats. J Ethnopharmacol 97 (2):369–374
Shivanna N, Naika M, Khanum F et al (2013) Antioxidant, anti-diabetic and renal protective properties of *Stevia rebaudiana*. J Diabet Complications 27:103–113
Singh NS, Vats P, Suri S et al (2001) Effect of an antidiabetic extract of *Catharanthus roseus* on enzymic activities in streptozotocin induced diabetic rats. J Ethnopharmacol 76:269–271
Singh V, Singh SP, Singh SM et al (2015) Combined potentiating action of phytochemical(s) from *Cinnamomum tamala* and *Aloe vera* for their anti-diabetic and insulinomimetic effect using *in vivo* rat and *in vitro* NIH/3T3 cell culture system. Appl Biochem Biotechnol 175:2542–2563
Sivapalan SR (2015) Phytochemical study on medicinal plant-*Sida cordifolia* Linn. Int J Multidiscipl Res Dev 2(1):200–204
Soliman MM, Attia HF, El-Shazly SA et al (2012) Biomedical effects of cinnamon extract on obesity and diabetes relevance in Wistar rats. Am J Biochem Mol Biol 2:133–145
Soliman MM, Ahmed MM, El-Shazly SA (2013) Cinnamon extract regulates gene expression of lipid and carbohydrate metabolism in streptozotocin induced diabetic Wistar rats. Am J Biochem Mol Biol 9:172–182
Stohs SJ, Hartman MJ (2015) Review of the safety and efficacy of *Moringa oleifera*. Phytother Res 29:796–804
Stoyanova S, Genus J, Heideg E et al (2011) The food additives insulin and stevioside counteract oxidative stress. Int J Food Sci Nutr 62:207–214
SubashBabu P, Prabuseenivasan S, Ignacimuthu S (2007) Cinnamaldehyde: a potential antidiabetic agent. Phytomedicine 14:15–22
Szkudelski T (2001) The mechanism of alloxan and streptozotocin action in β cells of the rat pancreas. Physiol Res 50:180–185
Tanko Y, Yerima M, Mahdi MA et al (2008) Hypoglycemic activity of methanolic stem bark of *Adansonnia digitata* extract on blood glucose levels of streptozotocin-induced diabetic Wistar rats. Int J Appl Res Nat Prod 1(2):32–36
Tanko Y, Mohammed A, Musa KY et al (2012) Evaluation of ethanolic leaf extract of *Mucuna pruriens* on blood glucose levels in alloxan-induced diabetic rats. Asian J Med Sci 4(1):23–28
Tanquilut NC, Tanquilut MRC, Estacio MAC et al (2009) Hypoglycemic effect of *Lagerstroemia speciosa* (L.) Pers. on alloxan-induced diabetic mice. J Med Plants Res 3(12):1066–1071

Tomoda M, Shimada K, Konno C et al (1985) Structure of panaxan B. A hypoglycemic glycan of *Panax ginseng* roots. Phytochemistry 24:2431–2433

Vetrani C, Sestilli F, Vitale M et al (2018) Metabolic response to amylose-rich wheat-based rusks in overweight individuals. Eur J Clin Nutr 72:904–912

Viana GS, Medeiros AC, Lacerda AM et al (2004) Hypoglycemic and antilipidemic effects of the aqueous extract of *Cissus sicyoides*. BMC Pharmacol 4:9

Villarruel-López A, López-de la Mora DA, Vásquez-Paulino OD et al (2018) Effect of *Moringa oleifera* consumption on diabetic rats. BMC Complement Altern Med 18:127

Volek JS, Fernandez ML, Feinman RD et al (2008) Dietary carbohydrate restriction induces a unique metabolic state positively affecting atherogenic dyslipidemia, fatty acid portioning, and metabolic syndrome. Progr Lipid Res 47(5):307–318

Vongsak B, Sithisarn P, Mangmool S et al (2013) Maximizing total phenolics, total flavonoids contents and antioxidant activity of *Moringa oleifera* leaf extract by the appropriate extraction method. Indian Crop Prod 44:566–571

Wadood A, Wadood N, Shah SA (1989) Effects of *Acacia arabica* and *Caralluma edulis* on blood sugar levels of normal and alloxan diabetic rabbits. J Pak Med Assoc 39(8):208–212

Wang RH, Kim HS, Xiao C et al (2011) Hepatic Sirt1 deficiency in mice impairs mTorc2/Akt signaling and results in hyperglycemia, oxidative damage, and insulin resistance. J Clin Invest 121 (11):4477–4490

Wang H, Fowler MI, Messenger DJ et al (2018) Homoisoflavonoids are potent glucose transporter 2 (GLUT2) inhibitors: a potential mechanism for the glucose-lowering properties of *Polygonatum odoratum*. J Agric Food Chem 66:3137–3145

Westman EC, Feinman RD, Mavropoulos JC et al (2007) Low-carbohydrate nutrition and metabolism. Am J Clin Nutr 86 (2):276–284

Whiteman EL, Cho H, Birnbaum MJ (2002) Role of Akt/protein kinase B in metabolism. ABBV Trends Endocrinol Metab 13(10):444–451

WHO (2016) Global report on diabetes. http://www.who.int/iris/bitstream/10665

Worawalai W, Sompornpisut P, Wacharasindhu S et al (2018) Quercitol: from a taxonomic marker of the genus *Quercus* to a versatile chiral building block of antidiabetic agents. J Agric Food Chem 66:5741–5745

Wu W, Geng H, Liu Z et al (2014) Effect of curcumin on rats/mice with diabetic nephropathy: a systematic review and meta-analysis of randomized controlled trials. J Tradit Chin Med 34:419–429

Wu X-Z, Xie HQ, Long XH et al (2017) Chemical constituents of *Catharanthus roseus*. J Chin Pharm Sci 52(8):631–636

Wyk H, Davis R, Davies J (2016) A critical review of low-carbohydrate diets in people with type 2 diabetes. Diabet Med 33(2):148–157

Xia X, Yan J, Shen Y et al (2011) Berberine improves glucose metabolism in diabetic rats by inhibition of hepatic gluconeogenesis. PLoS One 6:e16556

Xiang YZ, Shang HC, Gao XM et al (2008) A comparison of the ancient use of ginseng in traditional Chinese medicine with modern pharmacological experiments and clinical trials. Phytother Res 22:851–858

Xu Z, Wang X, Zhou M et al (2008) The antidiabetic activity of total lignan from *Fructus arctii* against alloxan-induced diabetes in mice and rats. Phytother Res 22:97–101

Yallapu MM, Nagesh PK, Jaggi M et al (2015) Therapeutic applications of curcumin nanoformulations. AAPS J 17(6):1341–1356

Yan J, Wang C, Jin Y et al (2018) Catalpol ameliorates hepatic insulin resistance in type 2 diabetes through acting on AMPK/NOX4/P13K/AKT pathway. Pharmacol Res 130:466–480

Yanarday R, Colak H (1998) Effect of chard (*Beta vulgaris* L. var cicla) on blood glucose levels in normal and alloxan-induced diabetic rabbits. Pharm Pharmacol Commun 4:309–311

Yang MS, Wu MY (2016) Chinese ginseng. In: Gupta RC (ed) Nutraceuticals: efficacy, safety and toxicity. Academic/Elsevier, Amsterdam, pp 693–705

Yassa HD, Tohamy AD (2014) Extract of *Moringa oleifera* leaves ameliorates streptozotocin-induced diabetes mellitus in adult rats. Acta Histochem 116:844–854

Yin J, Xing H, Ye J (2008) Efficacy of berberine in patients with type 2 diabetes mellitus. Metabolism 57:712–717

Zhang M, Chen L (2012) Berberine in type 2 diabetes therapy: a new perspective for an old antidiarrheal drug? Acta Pharm Sin B 2 (4):379–386

Zhang H, Kong WJ, Shan YQ et al (2010a) Protein kinase D activation stimulates the transcription of the insulin receptor gene. Mol Cell Endocrinol 330:25–32

Zhang H, Wei J, Xue R et al (2010b) Berberine lowers blood glucose in type 2 diabetes mellitus patients through increasing insulin receptor expression. Metabolism 59:285–292

Zhang W, Zhao J, Wang J et al (2010c) Hypoglycemic effect of aqueous extract of sea buckthorn (*Hippophae rhamnoides* L.) seed residues in streptozotocin-induced diabetic rats. Phytother Res 24:228–232

Zheng J, Cheng J, Zheng S et al (2018) Curcumin, a polyphenolic curcuminoid with its protective effects and molecular mechanisms in diabetes and diabetic cardiomyopathy. Front Pharmacol 9:472

Zhou J, Xu G, Ma S et al (2015) Catalpol ameliorates high-fat diet-induced insulin resistance and adipose tissue inflammation by suppressing the JNK and NF-kappaB pathways. Biochem Biophys Res Commun 467(4):853–858

Zia T, Hasnain SN, Hasan SK et al (2001) Evaluation of the oral hypoglycemic effect of *Trigonella foenum-graecum* in normal mice. J Ethnopharmacol 75:191–195

Nutraceuticals for Wound Healing: A Special Focus on *Chromolaena odorata* as Guardian of Health with Broad Spectrum of Biological Activities

Mohamed Ali-Seyed and Kavitha Vijayaraghavan

Abstract

Nutraceuticals are any material, either whole or in part, that may be considered a hybrid of nutrition and pharmaceuticals. This special characteristic, aside from providing good health, leads to treatment of wounds and prevention of certain diseases. Nutraceuticals have gained significant attention due to their presumed safety and promising nutritional and therapeutic outcomes. An important aspect of the use of traditional medicinal remedies in the treatment of burns and wounds is the potential to improve healing and reduce downtime in order to ease the financial burden on the affected patients and their families. Although several medicinal plants and their pharmaceutical preparations/formulations are available for wound care and management, it remains necessary to search for efficacious treatments, as certain current formulations cause adverse effects or lack efficacy. Several herbal medicines have displayed marked activity in the management of wounds, and various natural compounds/extracts have verified in vivo wound healing potential; therefore they are considered as potential drugs of natural origin. *C. odorata* is considered a tropical weed. It exhibits numerous relevant medicinal properties on an appreciable scale and is known in some parts of the world as a traditional medicine used to treat various ailments. Besides the above, volatile oils of the plant are considered a possible source of a nutraceutical to be used for clinical purposes. To understand its precise role as nature's gift for healing wounds and its contribution to affordable health care, this plant must be scientifically assessed based on the available literature. This is crucial to its potential future drug design, development, and application for the treatment of wounds in both animal and human medicine and cost-effective health care.

Keywords

C. odorata · Bioactive principles · Invasive weed · Natural products · Wound healing nutraceuticals

1 Introduction

Wounds are physical injuries that can be classified as open or closed based on the underlying cause (Eming et al. 2002) and on the basis of the physiology of wound healing. Wounds are popularly categorized by their level of chronicity as either acute or chronic (Strodtbeck 2001). An acute wound is an injury to the tissue that normally proceeds through an orderly and timely reparative process resulting in sustained restoration of anatomic and functional integrity. Acute wounds follow trauma or inflammation, are caused by external damage to intact skin, and usually heal within 6 weeks. Surgical wounds, bites, burns, minor cuts and abrasions, and more severe traumatic wounds such as lacerations, crushes, or gunshot injuries are examples of acute wounds. Acute wounds are generally caused by cuts or surgical incisions and complete the wound healing process within the expected time frame (Menke et al. 2008).

Wounds that fail to progress through the normal stages of healing lead to a state of pathologic inflammation. These wounds require either a prolonged time to heal or recur frequently. The most common frequent causes of chronic wounds are local infection, hypoxia, trauma, foreign bodies, and systemic problems such as diabetes mellitus, malnutrition, immunodeficiency, or medications (Menke et al. 2008). Chronic wounds, in addition to failing to heal after 6 weeks, have characteristic pathological associations due to underlying endogenous mechanisms associated with a predisposing condition that ultimately compromises the integrity of dermal

M. Ali-Seyed (✉)
Faculty of Medicine, Department of Clinical Biochemistry, University of Tabuk, Tabuk, Saudi Arabia

K. Vijayaraghavan
Department of Chemical Engineering, Agni College of Technology, Chennai, Tamil Nadu, India

R. C. Gupta et al. (eds.), *Nutraceuticals in Veterinary Medicine*, https://doi.org/10.1007/978-3-030-04624-8_36

and epidermal tissue, inhibiting or delaying healing (Krishnan 2006). Pressure ulcers, venous leg ulcers, and diabetic foot ulcers are examples of chronic wounds (de la Torre and Chambers 2008). These wounds are visible evidence of an underlying condition such as extended pressure on the tissues, compromised tissue perfusion as a consequence of impaired arterial supply (peripheral vascular disease) or impaired venous drainage (venous hypertension) and metabolic diseases such as diabetes mellitus (DM), or even poor nutrition.

2 Pathophysiology of Wound and Wound Healing

It is well known that there is no definite method for classifying wounds. However, there are many different types of wounds ranging from mild to severe to potentially fatal, and some of these are classified as follows:

(a) Wounds can be referred to by their anatomical site, e.g., abdominal or axillary wound.
(b) Based on the underlying cause of the wound, this type of wound is called open or closed. In this class, bleeding is clearly visible as blood escapes from the body. It is further classified as incised, laceration, abrasion or superficial, puncture, gunshot, or penetration wound (Schultz 1999).
(c) Wounds with or without tissue loss (e.g., surgery) or burns (Poul Holm et al. 1974).

The wound healing process has been studied for decades. The proper healing of wounds is essential for the restoration of disrupted anatomical integrity and disturbed function of the affected area (Edlich et al. 2005). Healing is a complex and intricate process initiated in response to an injury that serves to restore the function and integrity of the damaged tissues (Wietecha and DiPietro 2013). In developing countries, such as Africa and Asia, injury to the skin is common, and a general lack of medical resources often results in a chronic state of healing (Starley et al. 1999). Chronic wounds, in particular, are a major concern for both patients and clinicians, and they affect a large number of patients, leading to a serious reduction in their quality of life. Impaired and aberrant wound healing imposes a huge financial burden in the developed world and is an insurmountable problem in the undeveloped one (Wound Forecast to 2021).

Current estimates indicate that nearly six million people suffer from chronic wounds worldwide (Wound Forecast to 2021; WHO 2014). In the USA alone, combat-related and other traumatic wounds lead to over 300,000 hospitalizations annually. The restoration of the affected area in wound injury survivors is the main aim of quality rehabilitation. Surgical wounds account for the vast majority of skin injuries. Approximately 80% of these wounds use some form of closure product such as sutures, staples, and tapes, while other wound management strategies use hemostatic products, fabric bandages, and surgical dressings (Belmont et al. 2010).

Wound healing is a normal biological process in the human body as well as in animals and is achieved through four precisely and highly programmed phases: (1) hemostasis, (2) inflammation, (3) proliferation, and (4) remodeling (Burford et al. 2007). Each phase is characterized by the infiltration of specific cell types into the wound site (Redd et al. 2004), all of which interact and communicate via chemical signals to optimize repair. Wound healing processes are well-organized biochemical and cellular events that lead to the growth and regeneration of wounded tissue. It involves the activity of an intricate network of blood cells, cytokines, and growth factors which ultimately leads to the restoration of normal conditions in the injured skin or tissue (Clark 1991). Upon onset of the inflammatory response, fibroblasts begin to proliferate and migrate into the wound area. Collagen and fibronectin are subsequently deposited in the wound bed, serving as a temporary matrix on which epithelial cells can migrate (Rørth 2007). Throughout the process, cells often migrate in groups.

While understanding how cells move together in tissue repair is important, this process is also fundamentally relevant to other complex events such as morphogenesis and tumor metastasis (Friedl 2004). There are two mechanisms of collective migration that have been identified (Martin 1997). First, lamellipodial crawling involves active migration of cells at the wound edge, mediated primarily through cell-extracellular matrix (ECM) interactions in the wound area (Trepat et al. 2009). This process is commonly observed in adult wound healing and is well-studied in in vitro wound healing models (Bindschadler and McGrath 2007). The second mechanism is known as the purse string model, and it is the primary mode of cell movement in fetal wound healing (Bullard et al. 2003). Following injury, an actomyosin cable assembles around the periphery of the wound and contracts to close the wound by transmitting tension through intercellular junctions (Tamada et al. 2007). While the lamellipodial crawling mechanism results in rapid wound closure, the purse string mechanism leads to scarless wound healing.

The aim of wound care is to promote wound healing in the shortest time possible, with minimal pain, discomfort, and scarring to the patient, and this must occur in a physiologic environment conducive to tissue repair and regeneration (Bowler et al. 2001). Wound healing processes are known to be influenced by many factors including infections, nutritional status, drugs and hormones, type and sites of wound, and wasting diseases like diabetes. The healing process may also be hampered by the presence of oxygen free

radicals or microbial infection. Due to such a poor hygienic status, wound infection is still one of the most common diseases in developing countries (Hussein et al. 2011). Besides the above, multiple local disturbances and systemic disease can result in impaired wound healing (Eming et al. 2002). The resulting prolonged inflammation and toxic microenvironment are the main reasons for a transition from the acute to chronic state (Amadeu et al. 2004).

3 Wound Healing: A New Multi-spectrum Twenty-First-Century Perspective

Despite the tremendous advances in the pharmaceutical industry, the search for more effective and lower cost therapeutic approaches for wound healing remains a challenge for modern medicine due to the chronic nature of therapy and related side effects (Ghosh and Gaba 2013). Many new approaches, such as gene therapy (Petrie et al. 2003) and tissue-engineered skin, have met with limited success. In the search for new therapeutic options, plants and their metabolites are a great source of novel biomolecules. The application of plant-based medicines provides, in principle, a cost-effective therapy. A major criticism of herbal medicines, however, is that they are not subjected to the rigors of their pharmaceutical counterparts (Barreto et al. 2014). However, over the last 20 years, in vitro tests developed to investigate wound healing have exploited all of these processes as targets for enhancing its management. The different phases of the wound healing process overlap, and an ideal plant-based remedy should affect at least two of the different processes before it can be considered to have some scientific support for its traditional use (Burford et al. 2007).

Plants serve as potential resources for the traditional and modern systems of medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates, and chemical entities (Soni and Singhai 2013). They also serve as possible resources for the treatment of various human diseases because of their potential efficacy and proven safety for animal and human use (Hashemi and Davoodi 2011). In folk medicine, herbal plants have been used widely in facilitating wound healing with a high degree of success (Kumar et al. 2007). Some of these plants either possess pro-wound healing activities or exhibit antimicrobial and other related properties which are beneficial in overall wound care (Dawn et al. 2008). This has inspired much research aimed at validating claims and discovering mechanisms which possibly explain the potential of these herbs on wound repair processes. Some examples of wound healing plants (Table 1) include korphad, *Aloe vera* (Maenthaisong et al. 2007); Madeira vines, *Anredera diffusa* (Moura-Letts et al. 2006); jungle geranium, *Ixora coccinea* (Upadhyay et al. 2014); Indian mulberry, *Morinda pubescens* (Mathivanan et al. 2006); simple-leaf chaste tree, *Vitex trifolia*; and peacock chaste tree, *Vitex altissima* (Manjunatha et al. 2007).

4 Models of Wound and Wound Healing

In recent years, it has been well justified that the incorporation of both in vivo and in vitro models are imminent for investigators in all scientific discipline especially in wound care research. This is not surprising because any bioactive principle or new product developed by researchers or pharmacological industries needs both basic and translational research to verify, reproduce, and acquire data as the result of this model experimentation may lead to both preclinical and clinical trial outcomes for improved wound healing. In general, these models are usually adopted in a stepwise order that starts with in silico and in vitro assays and in vivo animal models followed by advanced clinical evaluations. The most commonly used models are briefed below by Wilhelm et al. (2017).

4.1 In Vitro Assays

In vitro assays are laboratory-based tests, which provide abundant opportunities for observing the outcome of test materials/healing agents/plant extracts/drugs on particular or various cell types (e.g., fibroblasts and keratinocytes) and help determine which concentrations of the agent may be effective in vivo. In vitro assays bring results rapidly which help the investigator not only costs but also time. In addition to providing valuable results in a short span of time, they hold a reasonable humane appeal since they usually do not involve the use of animals or humans (Perez and Davis 2008). Another noteworthy characteristic of in vitro testing is the ability to screen multiple agents or samples simultaneously. Besides the above, these tests will help in screening of antimicrobial resistance of pathogenic organisms but also aid to find inhibitory concentrations.

4.2 In Vitro Models of Wound Healing

In vitro assays are playing an important role in finding out the killing efficiency of numerous healing agents against many pathogenic microorganisms and their roles in augmenting the wound healing process (Perez and Davis 2008). This will help investigative the effect of the wound healing agents use in an animal model(s). These tests enable finding of test agents' presence in blood, wound fluid, and others like immune cells and enzyme proteases, which can be influenced by the activity of test agents. However, in vitro assays are not

Table 1 Nutraceuticals/plant extracts with potential for wound healing

Nutraceutical/plant extract	Active ingredient	Effect	References
Acalypha indica	Flavonoids, saponins, sterols, polyphenols, triterpenoids	Wound healing	Reddy et al. (2002)
Aloe vera	β-Sitosterol	Angiogenesis	Moon et al. (1999)
Butea monosperma	Flavonoids, tannins, saponins, glycosides, steroids, and triterpenoids	Wound healing	Murti and Kumar (2012), Bopage et al. (2018)
Camellia sinensis (green tea)	Epigallocatechin-3-gallate	Anti-inflammatory	Li et al. (2016)
Centaurea sadleriana Janka	Flavonoids	Wound healing	Csupor et al. (2010)
Centella asiatica	Asiaticoside	Wound healing	Shukla et al. (1999)
Ceylon cinnamon	(E)-Cinnamaldehyde	Wound healing	Farahpour and Habibi (2012)
Chromolaena odorata	Alkaloids, flavonoids, saponins, phenolics, anthraquinones, tannins, glycosides, stigmasterol, scutellarin, tetramethyl ether (scu), chromomoric acid C-1	Antioxidative, lipid peroxidative inhibitory, anti-inflammatory, increased collagen synthesis, wound healing	Oludare et al. (2000), Phan et al. (2001a, b), Vijayraghavan et al. (2017a, b)
Copaifera langsdorffii	Oleoresin, diterpenes, sesquiterpenes, copaivic acid	Antioxidative, anti-inflammatory, antimicrobial, wound healing	Paiva et al. (2002)
Curcuma longa	Curcumin/curcuminoids	Antioxidative, anti-inflammatory, antimicrobial, wound healing	Chereddy et al. (2013), Mohanty and Sahoo (2017)
Euphorbia hirta Linn.	Alkaloids, flavonoids, glycosides, saponins, tannins	Anti-inflammatory	Tuhin et al. (2017)
Ficus racemosa L.	Lupeol acetate, β-sitosterol	Antibacterial, antifungal, enhanced cell migration, wound healing	Murti and Kumar (2012), Bopage et al. (2018)
Heliotropium indicum	Pyrrolizidine alkaloids, indicine-N-oxide, tannins, saponins, heliotrine	Wound healing	Reddy et al. (2002)
Plagiochasma appendiculatum Lehm.	Saponins, flavonoids, and sesquiterpenes	Antimicrobial, antioxidative, wound healing	Singh et al. (2006)
Plumbago zeylanica	Terpenoids and alkaloids	Wound healing	Reddy et al. (2002)
Royal jelly	Royal jelly proteins	Wound healing	Fujii et al. (1990)
Saba florida Benth	Flavonoids, tannins, and other secondary metabolites	Wound healing	Omale and Isaac (2010)
Wrightia tinctoria	Triterpenoids, steroids, and saponins	Wound healing	Veerapur et al. (2004)
Phyllanthus	Phyllanthin, hypophyllanthin	Wound healing	Yadav et al. (2017)
Jungle geranium	Triterpenoids (lupeol), aromatic acrid oils, tannins, saponins, flavonoids (rutin, formononetin, β-sitosterol)	Wound healing	Upadhyay et al. (2014)
Indian mulberry, *Morinda pubescens*	Phenols, alkaloids, triterpenoids, steroids, carboxylic acids	Wound healing	Mathivanan et al. (2006)
Ixora coccinea	Alkaloids, glycosides, steroids, fixed oils, terpenes, tannins, and flavonoids	Wound healing	Upadhyay et al. (2014)
Peacock chaste tree	Volatile oil, resin, terpenes, flavonoids, glucosides, alkaloids	Wound healing	
Vitex trifolia; *Vitex altissima*	Volatile oil, resin, terpenes, flavonoids, glucosides, alkaloids	Wound healing	

capable of mimicking or reproducing conditions such as natural phases of wound healing, immune responses, or disease like diabetes.

In vitro assays have been recognized for their role in integrating variety of cells and tissues. A mostly used in vitro technique for studying wound healing is the scratch assay, in which a confluent monolayer of cells scratched to form an artificial gap, and cells are observed as they migrate and proliferate to close the gap (Ling et al. 2007). It is comparatively straightforward assay to assess the effect of therapeutic compounds, endogenous biochemical, and genetic alterations on the behavior of the monolayer (Stamm et al. 2016). However, these experiments are time consuming equal to in vivo equivalents and are often considered unrepresentative of healing processes in humans and can have complex levels of experiment based experimental variation (Ansell et al. 2012; Johnston et al. 2016; Pampaloni et al. 2007).

On the other hand, time-lapse microscopy of scratch assays represents an appropriate test to examine cellular process outcomes, which may be used as the base of further studies (Pampaloni et al. 2007). However, in reality cells in in vivo exist in a three-dimensional physiological environment that is not mimicked by monolayers, because the in vivo environment is entirely different from the plastic on which they are routinely cultured (Stamm et al. 2016). However, this shortcoming may be overcome by using advanced 3D culture techniques. Recent commercially available skin substitutes enable and mimic the structure of human skin (Groeber et al. 2011) most of which consist of separate layers comprising dermal and epidermal components. The dermis is simulated using a hydrogel, for example, collagen, populated with dermal fibroblasts, over which layers of keratinocytes are cultured (Stamm et al. 2016).

Since 3D skin substitutes are more complex in nature than monolayer cells, the effects of immune cells and blood vessels are still excluded. Following wounding with a scalpel or punch biopsy, time-lapse microscopy, histological analysis, or PCR may be conducted to investigate closure rates, cell and tissue morphology, and gene expression. These skin substitutes may also use to assess the toxicology or mode of action of a range of topical treatments; however they are limited in their ability to assess responses to novel dressings or other delivery modes (Groeber et al. 2011). Basic cellular processes, such as the secretion of biochemicals that regulate wound healing, may also be elucidated using this experimental approach (Maarof et al. 2016); however, the ability to culture skin substitutes over a long period of time may hinder their use in studying the later advanced phases of cutaneous healing (Ansell et al. 2012). However, there are some new techniques that have been developed to overcome this limitation: ex vivo culturing of human skin samples has been shown to permit experiments to be conducted over longer periods of time, for example, in the study of keloid disease (Bagabir et al. 2012).

4.3 In Vivo Models

In recent years, subjecting of animal models in wound healing investigations has tremendously increased as it coincides with the number of new products introduced yearly. Interestingly, the number of animal wound healing models has almost doubled every 10 years. However, numerous types of in vivo models are available, and each has some unique benefits and disadvantages.

4.4 Small Animals as Wound Healing Models

Numerous previous studies have extensively established appropriate animal models for mammalian wound care investigations, and most of them are successful in overcoming some of the logistical difficulties associated with clinical trials. The animal model system includes not only higher animals but also involve *Drosophila melanogaster* and zebra fish to investigate barrier repair in epithelial surfaces, providing important insights into the processes involved (Razzell et al. 2011; Richardson et al. 2013).

Despite varied options and many animal species available for studying human wound healing process, rodents are being the mostly accepted and generally employed animals with the majority of experiments conducted in mice (Galiano et al. 2004; Ansell et al. 2012; Dunn et al. 2013). In many ways, they are beneficial for wound care investigation for various reasons as these small model animals are less expensive, easy to obtain and handle, and require less space. Besides the above, these animals often have multiple offspring, which develop quickly permitting investigations to proceed through numerous generations. Not only these animals commonly have accelerated modes of healing compared to humans; thus experiment duration takes for days, compared to weeks or months required for human experiments (Nunan et al. 2014). Moreover, few species of small mammals can be altered genetically easily and provide a wound model closer to human disease conditions. In addition, other advantages of involving these small animals in experiments using microbes like bacteria or virus infection for developing infectious disease models (Grose and Werner 2004; Scheid et al. 2000).

4.5 Higher Animals as Wound Healing Models

Although rat, mouse, rabbit, and guinea pig wound models exist and in practice, porcine skin is structurally and biochemically more similar to humans (Sullivan et al. 2001; Seaton et al. 2015) than other mammals used for estimating wound healing therapies (Watson and Moore, 1990; Summereld and Ricklin 2015). However, pigs are less frequently employed than rodents in studies of cutaneous healing, because of reasons such as high cost, lack of genetic tools, and difficulty handling them. Besides the above, other model systems are available to study the mechanistic aspects of healing process, for example, the rabbit ear chamber and the Algire chamber study used for visualizing vascularization and determining angiogenesis (Nunan et al. 2014).

4.6 Human Models of Wound Healing

Despite having the availability of numerous animal models for various experiments, their anatomical and physiological differences distinctly affect the outcome of healing mechanisms. In order to avoid species-specific misinformation, direct human involvement or human sample

requirement is needed to dissect out the actual wound healing mechanisms in detail. To support this, much new molecular and novel technological advancements (Ho et al. 2017) are available in our hand, which enable us to investigate even tiny pinch of human skin tissue samples making it possible to decipher wound healing mechanisms in situ in humans.

It is obvious to expect that improved human-based model systems definitely serve a better choice to reveal the actual acute wound healing process as pathology and physiology of healing which occur among the patient are better correlated and identical; however, this study requires more human volunteers to generate several types of wound models. To support this claim, a review of such models is recently presented by Wilhelm et al. (2017) which are highlighted below:

As per Gao et al. (2013), partial- or full-thickness wounds can be used to recreate more accurate clinical situation where tissue damage occurs in conjunction with tissue loss. This can be done by either excising the tissue or obtaining a punch biopsy to generate these types of wounds. Wounds of this nature normally heal by reepithelialization, dermal reconstitution, and wound contraction permitting us to analyze different phases of the healing or of the entire healing process.

Partial Thickness

According to Xu et al. (2012), a partial-thickness injury is limited to the epidermis and superficial dermis, with no damage to the dermal blood vessels. Healing occurs by regeneration of other tissues. To demonstrate this few standard methods are available including tape stripping.

A report by Lademann et al. (2009) described the term "tape stripping" as the simplest partial-thickness injury of the skin involves removal of stratum corneum with adhesive tape. In this model of wounding, the epidermal compartment is generally left intact. However, the report cautioned that due to removal of stratum corneum layers, the permeability of the skin is temporarily compromised, but this can be measured using transepidermal water loss analysis.

Abrasive Wound Model

A slightly more invasive wound model was established by Wilhelm et al. (2017). In this report authors claimed that they induce uniform abrasions and can be used to evaluate the healing properties of a variety of wound care products (Wigger-Alberti et al. 2009). This model consists of inflicting standardized, superficial abrasions by repeatedly abrading skin with a surgical brush until the first signs of uniform glistening and punctuate bleeding are also observed.

Blister Model

This model was originally designed to measure drug concentrations in various parts of the skin (Kiistala 1968) but later engaged for wound healing investigations as well. A blister is formed as a result of the separation of the epidermis and dermis, at the basal membrane between the lamina lucida and the lamina densa (Chávez et al. 2011).

Full-Thickness Wound Models

Previous studies (Brown et al. 2002; Sørensen et al. 2010) have suggested based on their result outcomes that this model requires complete removal of the epidermis and dermis to the depth of fascial planes or subcutaneous fat and disrupts dermal blood vessels. This can be done using a number of devices to inflict a lesion in a standardized fashion (e.g., punch biopsy, scalpel, dermatome, and laser). Importantly, punch biopsy wounds are widely used in wound healing research both in animal models and human volunteer studies. Hence, both animal and clinical studies have been successfully used to elucidate the overall mechanisms and outcomes of wound healing, whereas it can be difficult to analyze individual mechanisms and cellular processes using in vitro assays.

5 Formulations of Nutraceuticals and Their Delivery Systems for Wound Healing

Nutraceuticals have gained more attention in recent years due to their recognized safety and promising nutritional and therapeutic outcomes. Although it may be easy for an innovator or maker to develop a pharmaceutical (tablets, powders, capsules, suppositories, etc.) which contains a bioactive food, however, it is relatively challenging to obtain a satisfactory bioavailability for such nutraceuticals (Gaurav et al. 2012; Johnson and Wang 2015). Bioavailability is often jeopardized by the following factors such as low solubility, stability, and/or permeability of the bioactive component. Advanced development of targeted delivery using nanotechnology for pharmaceutical applications has opened a new path for stability, solubility, and/or permeability enhancement of some of the challenging nutraceuticals (Yallapu et al. 2011, 2013).

Concurrently, nutraceuticals are also receiving increasing consumer acceptance, generating demand for new product innovations based on superior delivery systems. Most current delivery systems for nutraceutical products are based on direct application or ingestion (Gaurav et al. 2012). These oral delivery systems pose issues relative to their unacceptable odor and taste as well as degradation of the nutraceutical itself during its transport from the digestive system to the site of desired action. Direct delivery system like topical application circumvents some of these issues due to their application near or at the site of affliction (Hassan 2012).

In recent years, there have been significant advances with biomaterial delivery systems to satisfy the clinical need, especially in wound healing. However, there are some limitations that still exist with regard to therapies involving proteins, genes, and small molecule drugs. Once these problems are addressed, these delivery systems can effectively enable their full therapeutic benefit (Boateng et al. 2008; Koria 2012). In the context of wound healing, a delivery system should fulfill the following for its cargo: (a) maintain bioactivity through protection from proteolysis in the wound bed, (b) localize bioavailability by preventing rapid dilution in wound fluid and systemic uptake and distribution, and (c) facilitate release or presentation within the wound at a physiologically relevant rate and duration. If these goals are achieved, a successful delivery system will also minimize the dosage and application frequency necessary for efficacy. A detailed review by Johnson and Wang (2015) summarized and highlighted the recent advances in drug delivery systems for wound healing applications and also identified five recently developed vehicle types such as (1) hydrogels, (2) scaffolds, (3) particles, (4) complexes, and (5) coacervates and emphasized their advantages and wound healing efficacies without any side effects.

Hydrogels are one of the most highly utilized delivery vehicles. They are versatile, able to form from nearly any water-soluble polymer. They also feature a number of tunable parameters such as porosity, swelling ratio, and cross-link density to offer some control over release rate. A final advantage is that to an extent, they may mimic the mechanical properties of granulation tissue and maintain a moist wound environment (Boateng et al. 2008; Drury and Mooney 2003).

Scaffold is a general term, but in this section, we will specifically discuss porous scaffolds which are implanted rather than injected or polymerized in situ as is possible with hydrogels. This is usually not a drawback for wound healing applications as most wounds are superficial and relatively easy to access for manipulation. In fact, a scaffold may be the best choice when good mechanical properties or a long degradation time are desired. Their 3D structure also encourages cell infiltration and enables strategic patterning of stimuli to precisely direct tissue regeneration (Drury and Mooney 2003). Natural materials are often utilized in scaffolds similar to hydrogels, though they need not necessarily be hydrophilic.

Particles. Nano- and microparticles are another highly studied type of drug delivery system which holds certain advantages over hydrogels and scaffolds. With respect to wound healing applications, particles may by injected by a fine-gauge needle into the healthy tissue surrounding the wound so as not to complicate healing within the wound bed. They may also be tuned through numerous parameters to have complex release profiles unobtainable with gels and scaffolds (Soppimath et al. 2001; Kumari et al. 2010).

Complexes and conjugates. A fourth type of delivery system comprises complexes and conjugates. Conjugates are typically formed by chemical bonds, while complexes are typically formed by physical interactions. Both approaches seek to sequester soluble drugs in order to stabilize and extend their half-life or to provide targeting and facilitate their interactions with cell receptors. Polyethylene glycol (PEG) was attached to fibroblast growth factor-1 (FGF-1) to improve its thermal and structural stability in vivo (Huang et al. 2011). Another very interesting approach involves engineering ECM mimetics, considering its significance in orchestrating the regenerative process (Lutolf and Hubbell 2005; Uebersax et al. 2009; Schultz and Wysocki 2009).

Coacervates are an interesting new class of drug delivery vehicles developed only recently for controlled release of proteins and small molecule drugs. Coacervates are nanometer-sized liquid droplets, held together and apart from their environment by hydrophobic forces (Overbeek and Voorn 1957).

6 Nutraceuticals/Phytochemical Extracts from *C. odorata* for Wound Healing

Although many wound healing plants are reported and serve as important sources for a variety of nutraceuticals/extracts/phytochemicals (listed in Table 1), it is very difficult to discuss all of these in one review or book chapter. Hence, the aim of the present chapter is to provide up-to-date information about the properties of *C. odorata* (Fig. 1a–c), one of the potential wound healing plant that belongs to Compositae or Asteraceae family of flowering plants that is being investigated for its diverse pharmacological activity. The Compositae family is also rich in terpenoids (essential oil), which can be used for food flavoring or liqueur flavors. Volatile oils of the plant are considered a possible source of nutraceuticals for clinical purposes. Phenolic substances are also important in the preparation of modern medicines (Heywood et al. 1977).

C. odorata (L.) King and Robinson (formerly known as *Eupatorium odoratum* L.) belongs to the kingdom Plantae (Table 2). The genus *Chromolaena* includes 1200 species of small herbs, shrubs, or under shrubs distributed chiefly in the USA, with a few in Europe, Asia, and tropical Africa (GISD 2006). It is an ornamental plant considered to be one of the top 100 most invasive environmental weeds of wastelands, roadsides, and other exposed areas in many parts of the world (Chakraborty et al. 2011). *C. odorata* has numerous common vernacular various countries, which are listed in Table 3.

Though *C. odorata* has been known for its negative impact as an invasive weed, the potential medicinal uses are enormous (Vaisakh and Pandey 2013). Many parts of the *C. odorata* plant are used in the indigenous system of

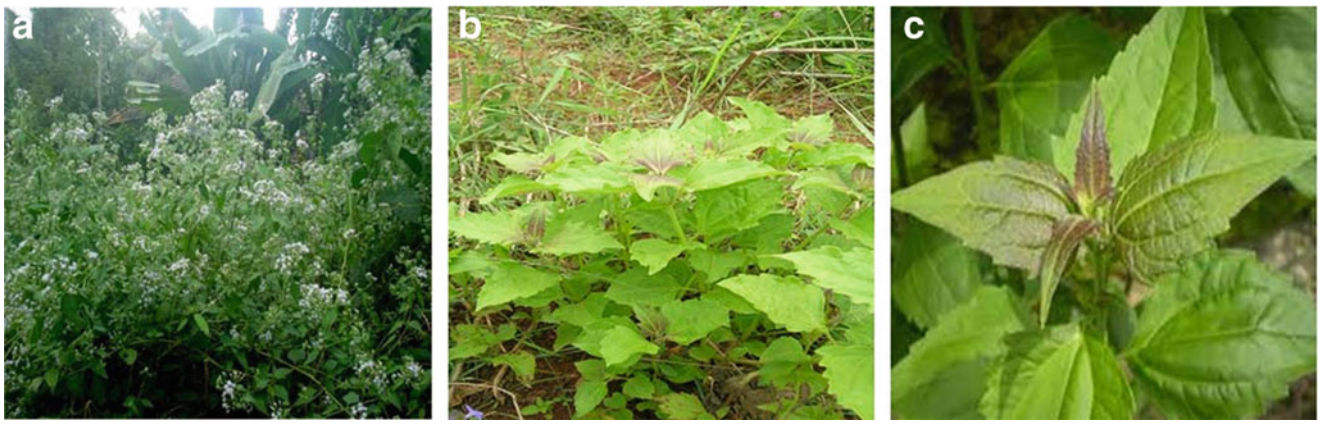


Fig. 1 (**a–c**) Images of *C. odorata*

Table 2 General description, distribution, and pharmacological uses of *C. odorata*

General description (Chakraborty et al. 2011)		*C. odorata* synonyms	Distribution	Pharmacological uses
Kingdom	Plantae—plants	*Eupatorium affine* Hook & Arn. *Eupatorium brachiatum* Wikstrom *Eupatorium clematitis* DC *Eupatorium conyzoides* M. Vahl *Eupatorium divergens* Less. *Eupatorium floribundum* Kunth *Eupatorium graciliflorum* DC. *Eupatorium odoratum* L. *Eupatorium sabeanum* Buckley *Eupatorium stigmatosum* Meyen & Walp. *Osmia conyzoides* (Vahl) Sch.-Bip. *Osmia divergens* (Less.) Schultz-Bip. *Osmia floribunda* (Kunth) Schultz-Bip. *Osmia graciliflora* (DC) Sch. Bip. *Osmia odorata* (L.) Schultz-Bip.	North America, from Florida and Texas to Mexico and the Caribbean, tropical Asia, West Africa, and parts of Australia	For the treatment of cuts, wounds, amenorrhea, amygdalitis, bite (leech) in human; cataplasm; catarrh; cold; decongestant; diabetes; diarrhea; fever; gargle; hemostat; hoarseness; inflammation; laryngitis; brutus; leptospirosis; pertussis; rheumatism; vermifuge; analgesic; antipyretic; antimicrobial; antimalarial (Vijayaraghavan et al. 2013) Biogas production (Jagadeesh et al. 1990)
Subkingdom	Tracheobionta—vascular plants			
Super division	Spermatophyta—seed plants			
Division	Magnoliophyta—flowering plants			
Class	Magnoliopsida—dicotyledons			
Subclass	Asteridae			
Order	Asterales			
Family	Asteraceae—Aster family (sunflower family)			
Genus	Chromolaena DC.—thoroughwort			
Species	*Chromolaena odorata* (L.) King & H. Rob.—Jack in the bush Common names: Siam weed, Christmas bush, and common floss flower			
C. odorata General impacts	It forms dense stands preventing establishment of other species, both due to competition and allelopathic effects. When dry, it becomes a fuel, which may promote wild bushfires (PIER 2001). It causes skin complaints and asthma in allergy-prone people. It is a major weed in plantations and croplands, including plantations of rubber, oil palm, forestry, and coffee plants			

medicine. In fact, traditional healers in some parts of Africa explore the plant as a source of medicine in curing different ailments, which are listed in Table 4. The plant is also known for its medicinal properties especially in the treatment of wounds (Phan et al. 2001a, b).

Phytochemicals or bioactive principles are naturally occurring plant substances/chemicals synthesized during secondary metabolism and are called primary and secondary metabolites (Brielmann et al. 2006). Specific phytochemicals have been identified as being responsible for particular pharmacological actions and medicinal properties of these plants. While the primary metabolites play essential roles in the life of the plants, secondary metabolites are often referred to as phytochemicals and are generally used in defense mechanisms against enemies such as herbivorous animals, viruses, parasites, and bacteria, of which 12,000 have been isolated and are preferred as alternative therapeutic options to synthetic drugs (Lai 2004). With time, plants containing these phytochemicals have been shown to serve as sources of medicine for humans and animals (Omokhua et al. 2016) because they may possess biological activities including antioxidant, antibacterial, antifungal, antipyretic, anthelmintic, antispasmodic, anti-inflammatory, and wound healing effects. Phytochemicals can also be used as antiseptics, fragrances, dyes, insecticides, stimulants, and poisons (Zulak et al. 2006).

Table 3 Common vernacular for *C. odorata* in various countries

Country	Common names
English (UK)	Siam weed, triffid weed, bitter bush, Jack in the bush, Christmas bush, baby tea
African	Sekou toure, acheampong, jabinde, matapa, mighbe
Chamorro	Masigsig, masigsig
Cameroon	Bokassa
Chinese:	Fei ji cao
Central Africa	Bokassa
Cote d'Ivoire	Sekou toure
Chuukese	Otuot
Democratic Republic of the Congo	*Matapa mbala* (the invader), *Lantana ngouabi*
Filipino	Agano, huluhagonoi
French	Fleurit-Noël, herbe du Laos
German	Siam kraut
Ghana	Acheampong weed
Guam	Kesengesil
India	Gandhi gulabi, communist weed, sam-solokh, tongal-lati, Sam-rhabi, bagh dhoka, tivra gandha, pokok kapal terbang, rumput jepun, rumput Siam, ropani, seekhrasarpi
Indonesia	Kumpai jepang (Japanese grass), rumput gol kar (business party grass), kirinyu, independent shrub
Kosraean	Mahsrihsrihk
Laos	Herbe du Laos, French weed
Malaysia	Siam weed
Myanmar	Bizat, tawbizat, curse of Ceylon, Kal-bun, Kombat-nong-rim, Rel-hlow, Campur grass
Nepal	Banmara (killer of the forest)
Palauan	Kesengesil, ngesngesil
Philippines	Devil weed, gonoy hagonoy, triffid weed, paraffin weed
Pohnpeian	Masigsig, masikisik, wisolmat, wisolmat en rehnwel
Spanish	Santa Maria
South Africa	Armstrong weed, kingsweed, triffid weed, paraffin weed
Thailand	Saab sua, yah sua mop
Trinidad	Christmas weed
Vietnam	Co hoi, communist weed

Phytochemical extraction involves the separation of the medicinally active fraction from the plant using selective solvents. These include classes of preparations known as decoctions, infusions, fluid extracts, tinctures, pilular extracts, or powdered extracts. Phytochemicals found in most medicinal plants include alkaloids, terpenes, and glycosides (tannins, saponins, anthraquinones, flavonoids, etc.) (Trease and Evans 2002). The herbal extract thus obtained may be ready for use as a medicinal agent as such, or it may be further processed to be incorporated in any dosage form. An extract may be further processed through various techniques of fractionation to isolate individual chemical entities to be used as modern drugs (Handa 2008). There are many processes patented throughout the world for the extraction of plant ingredients.

C. odorata possess many secondary metabolites such as terpenes, alkaloids, and glycosides. Polyphenols like flavonoids and tannins are abundant in the Asteraceae family. Many of these phytochemicals possess various pharmacological activities which are very useful to human welfare (Panda et al. 2010). Phytochemical studies on the extracts of *C. odorata* from various parts of the plant (Fig. 2) have indicated the presence of tannins, terpenoids, cardiac glycosides, saponins, anthraquinones, phenols, and alkaloids (Anyasor et al. 2011; Vijayaraghavan et al. 2013). About 44 different compounds have been isolated from *C. odorata* extracts using GC-MS (Raman et al. 2012). Because of the presence of these phytochemicals, the plant is said to have anthelmintic (Panda et al. 2010), antioxidant (Raman et al. 2012; Vijayaraghavan et al. 2013), analgesic, anti-inflammatory, antipyretic, antispasmodic (Owoyele and Soladoye 2006; Chakraborty et al. 2011), antimicrobial (Chomnawang 2005), antimalarial, and antioxidant properties as well as wound healing potential (Anyasor et al. 2011). Eupolin, a product from *C. odorata* leaves for soft tissue burns and wounds has been licensed for use in Vietnam (Raina et al. 2008).

Previous investigation of the leaves and stems of *C. odorata* revealed the presence of essential oils, steroids triterpenes, and flavonoids (Suksamran et al. 2004). Flowers of this plant species have been subjected to investigation for essential oils, fats, alkaloids, and flavonoids (Odunbaku et al. 2008). The reported isolated chemical constituents from various extracts of *C. odorata* are shown in Table 5.

6.1 Chemical Constituents of *C. odorata*

C. odorata has been the subject of chemical analyses in several research studies (Zhang et al. 2012; Heiss et al. 2014). The chemical groups that have been identified in this plant are monoterpenes, sesquiterpene hydrocarbons, triterpenes/steroids, alkaloids, and flavonoids. The leaves of this plant have been found to be a rich source of flavonoids including quercetin, sinensetin, sakuranetin, padmatin, kaempferol, and salvigenin, which were isolated and identified. The leaves of *C. odorata* have the highest amount of allelochemicals (Afolabi et al. 2007) isolated from a plant. A study in Vietnam showed that the aqueous extract of the leaf contained flavonoids (salvigenin, sakuranetin, isosakuranetin, kaempferide, betulenol, 2-5-7-3 tetra-o-methyl quercetagetin, tamarixetin, two chalcones, and odoratin and its alcoholic compound), essential oils (geyren, bornyl acetate, and beta eubeden), saponin triterpenoids, tannins, organic acids, and numerous trace substances (Zhang et al. 2012). Another study by Heiss et al. (2014)

Table 4 Description of traditional usage of different parts of the *C. odorata* plant for curing ailments and wounds

Category of use	Description of traditional usage	References
Coughs and colds	The plant is squeezed in water, and the extract is taken to cure colds and coughs	Timbilla et al. (2003)
Skin diseases	The leaf is squeezed in water to bathe	Morton (1981)
Wounds and antiseptic	The leaves are squeezed, and the juice is directly applied to the wound	Inya-Agha et al. (1987)
Dysentery	The leaves are squeezed and taken as a tonic	Gill (1992)
Headache	The leaves are squeezed and taken as a tonic	
Toothache	The leaves are squeezed, and the juice is applied to the aching part	
Malaria fever	A decoction of the leaves with *Azadirachta indica* is prepared, and the water is taken	Idu and Onyibe (2007)
Antiseptic	The juice of the leaves, sometimes mixed with water, is used to stop bleeding	Gill (1992)
Stomach problems	Fresh leaves are squeezed in water, and the juice is taken as a tonic	Idu and Onyibe (2007)
Antiseptic and hemostatic	Fresh juice from the leaves is used to arrest bleeding in fresh cuts and nosebleeds	Phan et al. (2001a), Idu and Onyibe (2007)
Diarrhea	The leaves are squeezed with water, and the decoction is taken as a tonic	Amatya and Tuladhar (2011), Bhargava et al. (2011)
Skin eruption	The fresh leaves are squeezed, and the juice is applied to affected areas of the skin	

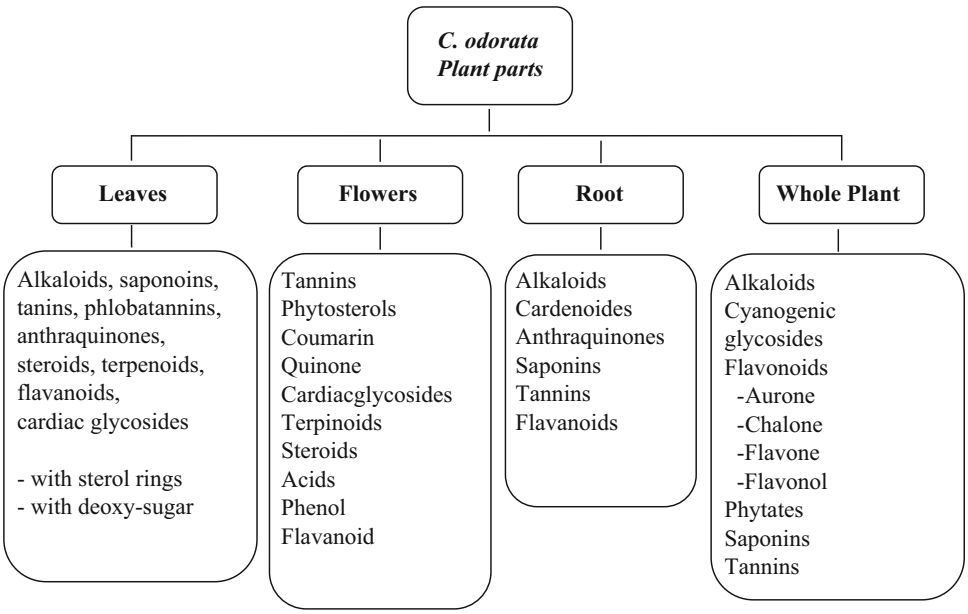


Fig. 2 Nutraceuticals/plant extracts of *C. odorata* with the potential for wound healing

showed that the crude ethanol extract of *C. odorata* contains phenolic acids (protocatechuic, p-hydroxybenzoic, p-coumaric, ferulic, and vanillic acids) and complex mixtures of lipophilic flavonoid aglycones (flavanones, flavonols, flavones, and chalcones). To date, studies on *C. odorata* have resulted in the isolation of 17 compounds such as 5aα,6,9,9aβ,10-pentahydro-10β-hydroxy-7-methylanthra[1,2-d][1,3]dioxol-5-one, 1,2-methylenedioxy-6-methylanthraquinone, 3-hydroxy-1,2,4-trimethoxy-6-methylanthraquinone, 3-hydroxy-1,2-dimethoxy-6-methylanthraquinone, and 7-methoxy-7-epi-medioresinol, as well as 12 known compounds including odoratin, 3β-acetyloleanolic acid, ursolic acid, ombuin, 4,2′-dihydroxy-4′,5′,6′-trimethoxychalcone, (−)-pinoresinol, austrocortinin, tianshic acid, cleomiscosin D, (−)-medioresinol, (−)-syringaresinol, and cleomiscosin A (Zhang et al. 2012). Figure 3 shows the structure and composition of some of the important bioactive principles in *C. odorata* such as stigmasterol (**1**), scutellarin, tetramethyl ether (scu, **2**), flavonoids (**3a–3l**) (Pandith et al. 2013a, b), and the phytoprostane compound chromomoric acid C-1 (Heiss et al. 2014).

6.2 Pharmacological Activities of *C. odorata* Leaf Extracts

In Africa, Thailand, and Vietnam, the juice of the leaf is used as a hemostatic on wounds (Phan et al. 2001a) and as an anti-inflammatory, and also decoction of the flowers is used as

Table 5 Phytochemical extracts and their chemical constituents from various parts of *C. odorata* plant

Parts of plant	Chemical constituents	References
Flower (aqueous extract)	3,5,4′-Trihydroxy-7-methoxyflavanone 5,7,3′′′′-Trihydroxy-5′-methoxyflavanone 3,5,7-Trihydroxy-4′-methoxyflavanone	Odunbaku et al. (2008)
Flower	Akuranetin Persicogenin 5,6,7,4′-Tetramethoxyflavanone 4′-Hydroxy-5,6,7-trimethoxyflavanone 2′-Hydroxy-4,4′,5′,6′-tetramethoxychalcone 4,2′-Dihydroxy-4′,5′,6′-trimethoxyflavanone Acacetin Luteolin	Suksamran et al. (2004), Pisutthanan et al. (2005)
5,7-Dihydroxy-6-4′-dimethoxyflavanone (latest)		
Whole plant—aboveground parts (dichloromethane extract)	2′-Hydroxy-3,4,4′,5′,6′-pentamethoxy-chalcone 2′,4-Dihydroxy-4′,5′,6′-trimethoxychalcone Scutellarein tetramethyl ether Sinensetin 2′-Hydroxy-4,4′,5′,6′-tetramethoxychalcone	Ling et al. (2007)
Whole plant (ethanol and methanol extract)	Aromadendrin 4′-methyl ether Eriodictyol 7,4′-dimethyl ether Naringenin 4′-methyl ether Taxifolin 4′-methyl ether; taxifolin 7-methyl ether Quercetin 7,4′-dimethyl ether Kaempferol 4′-dimethyl ether Quercetin 3-*O*-rutinoside Quercetin 4′-methyl ether Quercetin 7-methyl ether	Suksamran et al. (2004), Ling et al. (2007)
Leave (ethanol extract)	Tamarixetin Trihydroxymonomethoxyflavanone Pentaethoxyflavanone Dihydroxytrimethoxychalcone Eupatilin 5,6,7,4′-Tetramethoxyflavanone 5-Hdroxy6,7,3′,4′-tetramethoxyflavone Kaempferide Protocatechuic acid *p*-Coumaric acid *p*-Hydroxybenzoic acid Ferulic acid Vanillic acid Sinensetin Rhamsetin Tetrahydroxymonomethoxyflavanone	Phan et al. (2001b)

tonic, antipyretic, and heart tonic (Bunyapraphatsara and Chokechajiaroenprom 2000). The leaf extracts have been used for the treatment of leech bite, soft tissue wounds, burnt wounds, skin infection, and dento-alveolitis (Phan et al. 2001b; Owoyele et al. 2008). Figure 4 listed various pharmacological activities of leaf extracts of *C. odorata*. Other uses of the leaf extracts include treatment of diabetes (leaf infusion) and skin rashes (crushed leaves) (Obute and Adubor 2007). A formulation prepared from the aqueous extract of the leaves of *C. odorata* has been licensed for clinical use in Vietnam to treat skin irritations (Ling et al. 2007). More support for the medicinal use of the leaves of *C. odorata* includes a study which showed that methanol extract of the leaves had anti-inflammatory, antipyretic, and antispasmodic properties in mice (Taiwo et al. 2000). Aqueous extract of the leaf of *C. odorata* showed anti-inflammatory activity in rat (Vijayaraghavan et al. 2017a, b). Besides the above, extracts of leaves of *C. odorata* have been found to encourage proliferation of fibroblasts and delay the visible signs of skin aging such as wrinkles and hyperpigmentation. This knowledge was used to formulate a cosmetic composition containing extracts of *C. odorata* for the prevention of normal skin conditions due to chrono-aging or photoaging (Donovan 2007).

6.3 Plant-Derived Agents and Their Wound Healing Mechanism

The use of medicinal plants in health care is a common practice. Numerous medicinal plants have been reported to possess wound healing activity and to be useful in the

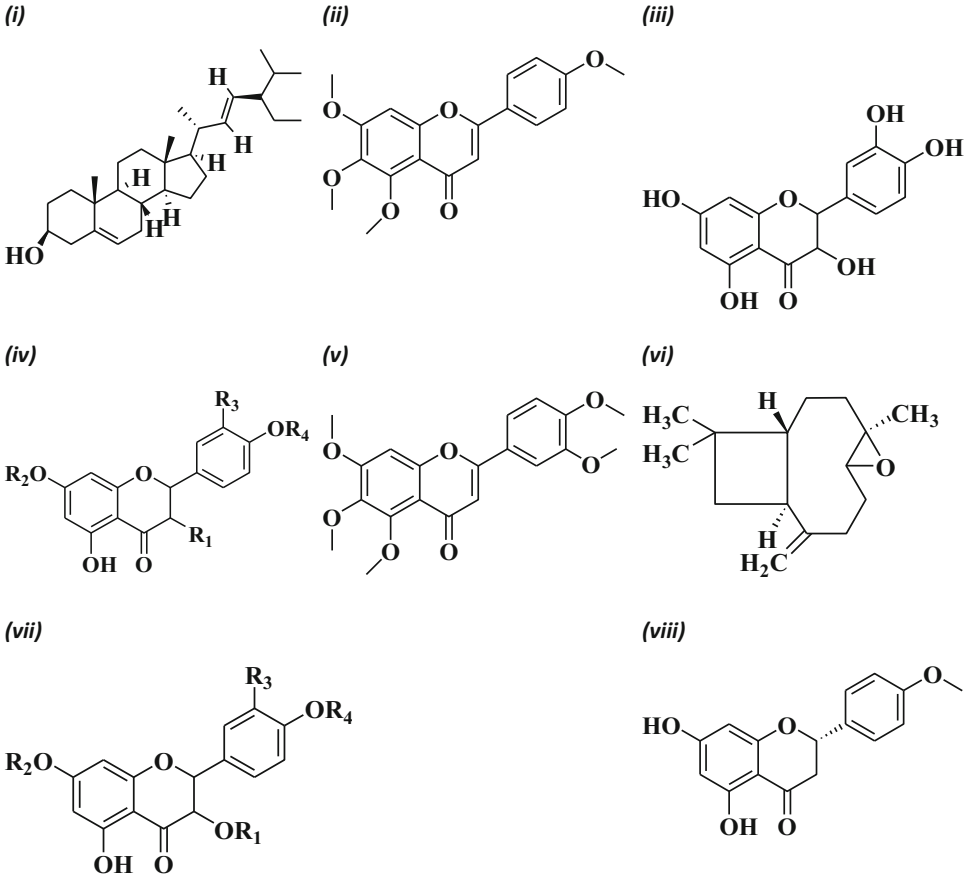


Fig. 3 Chemical structures of bioactive principles of *C. odorata*. (i) Stigmasterol. (ii) Scutellarein tetramethyl ether (Scu). (iii) Quercetin. (iv) Aromadendrin 4′-methyl ether: $R_1 = OH$; R_2, $R_3 = H$; $R_4 = CH_3$. (iv) Eriodictyol 7,4′-dimethyl ether: $R_1 = H$; R_2, $R_4 = CH_3$; $R_3 = OH$. (iv) Naringenin 4′-methyl ether: R_1, R_2, $R_3 = H$; $R_4 = CH_3$. (iv) Taxifolin 4′-methyl ether: R_1, $R_3 = OH$; $R_2 = H$; $R_4 = CH_3$. (iv) Taxifolin 7′-methyl ether: R_1, $R_3 = OH$; $R_2 = CH_3$; $R_4 = H$. (v) Sinensetin. (vi) Caryophyllene oxide. (vii) Quercetin 7,4′-dimethyl ether: R_1, $R_3 = H$; R_2, $R_4 = CH_3$. (vii) Kaempferol 4′-methyl ether: R_1, R_2, $R_3 = H$; $R_4 = CH_3$. (vii) Quercetin 3-*O*-rutinoside: R_1 = glu (6-1)rham; R_2, $R_4 = H$; $R_3 = OH$. (vii) Kaempferol 3-*O*-rutinoside: R_1 = glu (6-1)rham; R_2, R_3, $R_4 = H$. (vii) Quercetin 4′-methyl ether: R_1, $R_2 = H$; $R_3 = OH$; $R_4 = CH_3$. (vii) Quercetin 7′-methyl ether: R_1, $R_4 = H$; $R_2 = CH_3$; $R_3 = OH$. (viii) Isosakuranetin

treatment of wounds (Soni and Singhai 2013). To support this claim, a study (Manju et al. 2013) found that almost 31% of these plants have been used to treat wounds, 29% have been used for cuts, and 10% for burns, whereas 22% have been used for cuts and wounds. Some of these plants have been scientifically screened for evaluation of their wound healing activity in different pharmacological models and patients, but the potential of most remains unexplored. In a few cases, active chemical constituents were identified (Biswas and Mukherjee 2003). In addition, aromatic plants have a long history of use in treating wounds. The essential oils obtained from the various parts of the plants are very effective in treating small to medium wounds, skin abrasions, excoriations, skin infections, and other topical health problems provided an appropriate concentration of the essential oil is used (Kerr 2002).

Many biological experiments as well as pharmaceutical validation studies (Sirinthipaporn and Jiraungkoorskul 2017; Stanley et al. 2017) have revealed that various herbal extracts promote faster wound healing than standard reference control and non-medicated groups. A number of secondary metabolites, nutraceuticals, and active compounds isolated from plants have been demonstrated as active principles responsible for facilitating wound healing in animal models (Chaudhari and Mengi 2006). Furthermore, some of the most important examples include tannins from *Terminalia arjuna* (Chaudhari and Mengi 2006); oleanolic acid from *Anredera diffusa* (Moura-Letts et al. 2006); polysaccharides from *Opuntia ficus-indica* (Trombetta et al. 2005); gentiopicroside, sweroside, and swertiamarin from *Gentiana lutea* (Ozturk et al. 2006); shikonin derivatives (deoxyshikonin, acetyl shikonin, 3-hydroxy-isovaleryl shikonin, and 5,8-odimethyl acetyl shikonin) from *Onosma argentatum* (Ozgen et al. 2006); asiaticoside, asiatic acid, and madecassic acid from *Centella asiatica* (Hong et al. 2005); quercetin, isorhamnetin, and kaempferol from *Hippophae rhamnoides* (Fu et al. 2005); curcumin from *Curcuma longa* (Jagetia and Rajanikant 2004); oleoresin from *Copaifera langsdorffii* (Paiva et al. 2002); proanthocyanidins and resveratrol from grapes (Khanna et al. 2002); acylated iridoid glycosides from *Scrophularia nodosa* (Stevenson et al. 2002); phenolic acids (protocatechuic, p-hydroxybenzoic, p-coumaric, ferulic, and vanillic acids) from *C. odorata* (Phan et al. 2001a, b); glycoprotein fraction of *Aloe vera* (Choi et al. 2001); (+)-epi-alpha-bisabolol from *Peperomia galioides* (Villegas et al. 2001); fukinolic and cimicifugic acids from *Cimicifuga* spp. (Kusano et al. 2001); and xyloglucan from *Tamarindus indica* (Burgalassi et al. 2000).

It is important to note that wound healing is complicated and involves a number of processes, including inflammation,

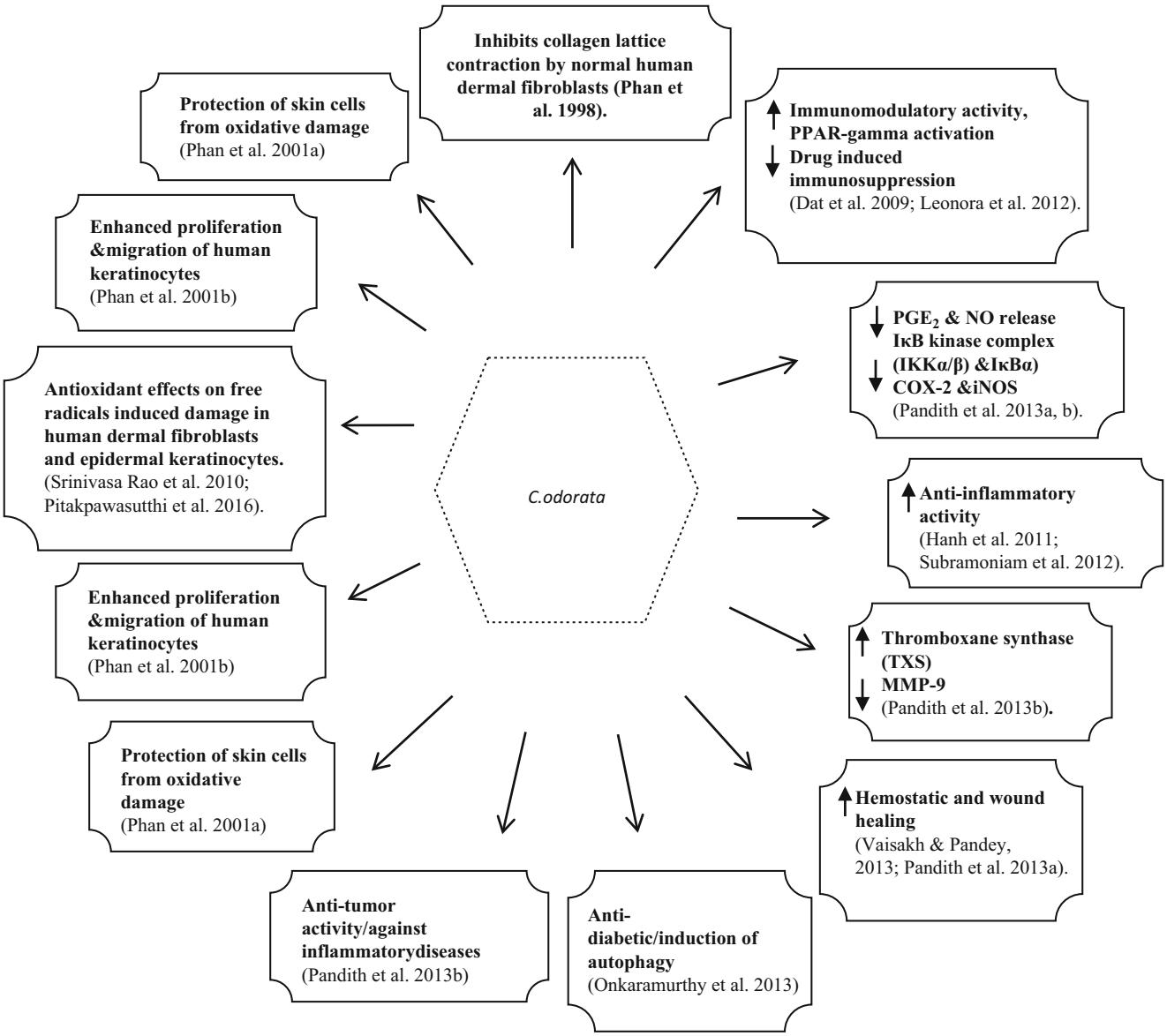


Fig. 4 Various pharmacological activities of leaf extracts of *C. odorata*

epithelization, antioxidant defense, biochemical changes (hydroxyproline), granulation, neovascularization, and wound contraction (Joshi et al. 2013). Medicinal plants heal wounds via several mechanisms such as upregulation of vascular endothelial growth factor (VEGF) and transforming growth factor beta 1 (TGF-β1), activation of nuclear factor-κ appa B (NF-κB), activation of interleukin-8 (IL-8), increased expression of iNOS and alpha-1 type 1 collagen, and antioxidant activity (Mirmalek et al. 2016; Shen et al. 2017). It is well known that VEGF is responsible for angiogenesis. These growth factors act on their respective receptors present in keratinocytes and macrophages and carry out their important functions during wound healing. Insufficient vascularization is the common feature of the chronic and non-healing wound. In diabetic animal models, delayed wound healing has been demonstrated in which poor vascularization is the factor responsible for delayed wound closure, epithelialization, and granuloma tissue formation (Stallmeyer et al. 2001). TGF-β is a major factor in the recent research of wound healing. It acts via intracellular serine/threonine kinase receptors to the intracellular mediators (SMAD) pathway which regulates cell proliferation (Klass et al. 2009). TGF-β causes migration of leukocytes into the injured tissue. As a result, monocytes transform into macrophages and clear the area of debris and itself to release TGF-β and other growth factors, which in turn help in the formation of granulation tissue (Behm et al. 2012).

The phosphatidylinositol 3-kinase (PI3K) pathway plays a crucial role in wound healing by rendering proliferation of cells. Activation of PI3K pathway causes phosphorylation of

Akt (serine/threonine-specific protein kinase) at serine 473 residues. This signaling pathway has been revealed to be essential for directional migration of corneal and skin epithelial cells in response to wound or injury (Zhao et al. 2006). Medicinal plants are reported to induce wound healing via the phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K) pathway. For example, *C. officinalis* tincture encourages wound healing through activation of the PI3K pathway (Dinda et al. 2015). Aqueous extract of Korean red ginseng also kindles in vivo and in vitro angiogenesis through activation of the PI3K/Akt pathways (Kim et al. 2007).

NF-κB activation is noticed in both immune and nonimmune cells affected by chronic inflammation. Activation of NF-κB increases the expression of proinflammatory mediators which organize and sustain the inflammatory processes that cause tissue damage. However, there are many reports that inhibition of NF-κB may cause harmful effects to the organisms and may sometimes cause inflammatory disease. The beneficial effect of NF-κB in epithelial cells has been reported. NF-κB signaling has a major role in the maintenance of immune homeostasis in epithelial cells (Wullaert et al. 2011). It has been demonstrated that plant extract can promote wound healing by NF-κB activation. The n-hexane extract of *C. officinalis* has been reported to increase the activity of transcription factor NF-κB in human immortalized keratinocytes and dermal fibroblast cells (Nicolaus et al. 2017).

Interleukin-8 (IL-8) is a proinflammatory cytokine. Keratinocytes are rich in interleukin-8. The effect of recombinant interleukin-8 on migration and adhesion of HaCaT keratinocytes has been demonstrated. It was found that IL-8 increased the migration and adhesion of HaCaT keratinocytes. Interestingly it was also observed that inhibition of phospholipase C-γ (PLC-γ) completely eradicated the migration of HaCaT keratinocytes. Thus, IL-8 directs migration via PLC-γ pathway. Medicinal plant extracts also endorse wound healing by activation of IL-8. The n-hexane extract of *C. officinalis* was found to increase the activity of IL-8 in human immortalized keratinocytes (Nicolaus et al. 2017).

Nitric oxide (NO) is a small gaseous radical (Pang et al. 2018). Synthesis of NO has been reported during the proliferative phase after wound formation (Vijayaraghavan et al. 2017a, b). Increased inducible nitric oxide synthase (iNOS) expression release NO regulates collagen formation, cell proliferation, and wound contraction (Witte and Barbul 2002). Moreover, iNOS regulates keratinocyte proliferation (Frank et al. 1998). The polysaccharide-rich extract of *C. ferrea* has been to increase the expression of iNOS (Pereira et al. 2016).

Alpha-1 (α-1) type 1 collagen is encoded by Col 1α (I) gene. This gene contributes to wound healing by the production of pro alpha-1 (I) chain which is a component of type 1 collagen. This pro alpha-1 (I) chain combines with another pro alpha-1 (I) chain and also with a pro alpha-2 (I) chain to produce a molecule of type 1 pro-collagen, which undergoes processing and rearrangement to produce type 1 collagen fibers. It has been demonstrated that topical application of ointment of *D. elata* showed upregulation of alpha-1 type 1 collagen encoded by Col 1α (I) gene contributes to wound healing.

Rho family GTPase like Rac-1, Rho-A, and Cdc-42 plays a pivotal role in fibroblast cell proliferation and migration (D'Souza et al. 2011). Cell cycle regulators such as cyclins and cyclin-dependent kinase 1 and 2 are involved in cytoskeleton formation in fibroblasts (Yoshizaki et al. 2004). *C. tamurana* has been reported to increase the migration of mammalian cells toward the wounded area through activation of Rac-1, Rho-A, and Cdc-42 m-RNA and Cdk 1 and 2 genes.

Much evidence suggests that wounds experience oxidative stress due to increased activity of neutrophils and resultant oxidants and myeloperoxidase (MPO; a peroxidase enzyme) activity. Increased activity of neutrophil resultant oxidants and MPO activity causes tissue damage in the chronic wound (Song et al. 2008). Generation of reactive oxygen species (ROS) results in cell toxicity via oxidative stress in chronic wounds and delay wound healing (Mikhal'chik et al. 2006). The antioxidant activity of medicinal plants is due to the presence of various phytochemicals (Pawar et al. 2007). To support this claim, it has been reported that topical application of bioadhesive gel of ethanolic extract of *L. macrophylla* (5% w/v) increased the activity of catalase, superoxide dismutase, and glutathione and decreased MPO activity (Joshi et al. 2016). Thus, medicinal plants potentiate wound healing by multiple mechanisms.

6.4 *C. odorata* as Nature's Wound Healer

In recent years, chemical research has increased interest in the medicinal effects of *C. odorata* as this plant is used as an antibacterial, antispasmodic, antiprotozoal, antitrypanosomal, antifungal, antihypertensive, anti-inflammatory, astringent, diuretic, hepatotropic (Hanh et al. 2011), immunomodulatory (Taleb-Contini et al. 2006), and anticancer agent (Harun et al. 2012; Kouamé et al. 2013). Traditionally, fresh leaves or a decoction of *C. odorata* has been used throughout Vietnam and other parts of the world for many years as well as in other tropical countries for the treatment of leech bites, soft tissue wounds, burns, skin infections, rashes, diabetes, and dentoalveolitis and as an insect repellent (Vaisakh and Pandey 2013). A poultice of the leaves is traditionally applied to cuts or wounds to stop bleeding and promote healing (Wang et al.

2014). A product made from *Chromolaena* spp. named eupolin has already been licensed for use in Vietnam for the treatment of soft tissue burns and wounds (Ayyanar and Ignacimuthu 2005; Raina et al. 2008). The fresh leaves and extract of *C. odorata* are a traditional herbal treatment in some developing countries for burns, soft tissue wounds, and skin infections (Sirinthipaporn and Jiraungkoorskul 2017; Stanley et al. 2017).

C. odorata has been the subject of chemical analyses in several research studies (Zhang et al. 2012; Heiss et al. 2014). Determination of the phytochemical profile of plants helps with understanding the class of compounds present which are responsible for the wound healing process (Karodi et al. 2009; Sirinthipaporn and Jiraungkoorskul 2017; Stanley et al. 2017). The chemical groups that have been identified in this plant are monoterpenes, sesquiterpene hydrocarbons, triterpenes/steroids, alkaloids, and flavonoids. The leaves of this plant have been found to be a rich source of flavonoids including quercetin, sinensetin, sakuranetin, padmatin, kaempferol, and salvigenin, which were isolated and identified. The leaves of *C. odorata* have the highest amount of allelochemicals (Afolabi et al. 2007) isolated from a plant. A study in Vietnam showed that the aqueous extract of the leaf contained flavonoids (salvigenin, sakuranetin, isosakuranetin, kaempferide, betulenol, 2-5-7-3 tetra-o-methyl quercetagetin, tamarixetin, two chalcones, and odoratin and its alcoholic compound), essential oils (geyren, bornyl acetate, and beta eubeden), saponin triterpenoids, tannins, organic acids, and numerous trace substances (Zhang et al. 2012). Another study by Heiss et al. (2014) showed that the crude ethanol extract of *C. odorata* contains phenolic acids (protocatechuic, p-hydroxybenzoic, p-coumaric, ferulic, and vanillic acids) and complex mixtures of lipophilic flavonoid aglycones (flavanones, flavonols, flavones, and chalcones). To date, studies on *C. odorata* have resulted in the isolation of 17 compounds such as 5aα,6,9,9aβ,10-pentahydro-10β-hydroxy-7-methylanthra [1,2-d][1,3]dioxol-5-one, 1,2-methylenedioxy-6-methylanthraquinone, 3-hydroxy-1,2,4-trimethoxy-6-methylanthraquinone, 3-hydroxy-1,2-dimethoxy-6-methylanthraquinone, and 7-methoxy-7-epi-medioresinol, as well as 12 known compounds including odoratin, 3β-acetyloleanolic acid, ursolic acid, ombuin, 4,2′-dihydroxy-4′,5′,6′-trimethoxychalcone, (−)-pinoresinol, austrocortinin, tianshic acid, cleomiscosin D, (−)-medioresinol, (−)-syringaresinol, and cleomiscosin A (Zhang et al. 2012).

One major application of *C. odorata* in traditional medicine is in the treatment of wounds. It is essential that any treatment of a wound must include the following (Badoe et al. 2000): (a) stopping excessive loss of blood without damaging blood supply to the wound site, (b) preventing or clearing residual bacterial infection, and (c) encouraging formation of fibroblasts at the wound site. Iwu (1993) showed that with its astringent properties, the leaf of *C. odorata* is able to arrest bleeding at wound sites. Furthermore, a previous study has shown that the leaf extracts of *C. odorata* are capable of preventing the growth and even causing the death of bacteria that could infect wounds. Moreover, Donovan (2007) showed that the leaf extracts of *C. odorata* do encourage proliferation of fibroblasts. This takes care of the third condition given for treatment of wounds (Badoe et al. 2000). Consequently, the leaf of *C. odorata* has all the properties required for effective treatment of a wound. Its uses in traditional medicine to treat wounds are therefore scientifically justified.

In addition, flavonoids have been shown to exhibit their actions through their effects on membrane permeability and by inhibiting membrane-bound enzymes such as ATPase and phospholipase A2 (Li et al. 1983). Flavonoids serve as health-promoting compounds due to the presence of anion radicals (Hausteen 1983). Flavonoids significantly inhibit lysosomal enzyme secretion and arachidonic acid release from membranes by inhibiting lipoxygenase, cyclooxygenase, and phospholipase A2 (Kuponiy and Ibibia 2013). The inhibition of arachidonic acid release in the inflamed cells would provide less arachidonic substrate for the lipoxygenase and cyclooxygenase pathways. This, however, leads to a lesser quantity of endoperoxides, prostaglandins, prostacyline, and thromboxane, as well as hydroperoxy, hydroxyl eicosatrienoic acids, and leukotrienes (Garbor 1986). Such an effect confirms the decrease in histamine which is known to act in the first stage of the inflammatory process (Farquar 1996). However, some flavonoids behave as a powerful protective agent against inflammatory disorders. They reduce edema formation and inhibit the synthesis of prostaglandin E_2, prostaglandin F_2, and thromboxane B2 (Kuponiy and Ibibia 2013). Hence, the anti-inflammatory action of the selected plant drug might be attributed to the presence of flavonoids as reported in the earlier works.

Phenol was also present in a moderate quantity in *C. odorata*. Phenols have been found to be of great importance as they protect the human body from oxidative stress (Robards et al. 1999). The presence of these phenolic compounds in this plant might have contributed to the antioxidant and antimicrobial properties as reported by Baravkar et al. (2008). Phenols are responsible for blocking specific enzymes that cause inflammatory disorders. They also modify the prostaglandin pathways and thereby protect platelets from clumping (Duke 1992). Tannins were also present both in aqueous and alcohol extracts. Tannins are useful as a dietary supplement for the maintenance of health due to their antioxidant, antimicrobial, and anti-inflammatory properties (Santos-Buelga and Scalbert 2000). Tannins are known to be useful in treating inflamed or ulcerated tissues (Motar et al. 1985).

Besides the above, both in vitro wound assay and in vivo studies have demonstrated that the leaf extracts of *C. odorata*

possibly enhance the proliferation of fibroblasts, endothelial cells, and keratinocytes; stimulate keratinocyte migration; upregulate the keratinocyte-induced production of extracellular matrix proteins and basement membrane components including collagen VII, anchoring fibrils, and fibrillin microfibrils; and inhibit collagen lattice contraction by fibroblasts (Kusano et al. 2001; Subramoniam et al. 2012).

Despite the natural cascades of the healing process, healing progression may be delayed by an infection mediated via various mechanisms, like decreased blood supply, which can promote impaired leukocyte function, prolong inflammatory and debridement phases, and produce proteolytic enzymes. Therefore, infection is the major impediment to the wound healing process, and antibacterial compounds play a major role (Annan and Houghton 2008; Kahkeshani et al. 2013; Kumari et al. 2013). Medicinal plants with their antioxidant, anti-inflammatory, and antimicrobial activities have an extensive potential for the management and treatment of wounds (Trabucchi et al. 1986; Trombetta et al. 2006; Akinmoladun et al. 2010).

Inflammation is a response to any tissue injury in the body caused by infection, trauma, chemicals, heat, or unrecognized particles (Kim et al. 2007). Inflammation, which constitutes a part of the acute response, results in a coordinated influx of neutrophils to the wound site which, through their characteristic "respiratory burst" activity, produce free radicals (Pan et al. 2008). Topical applications of compounds with free-radical-scavenging properties in patients have shown to considerably improve wound healing and protect tissues from oxidative damage (Pisutthanan et al. 2005).

Proinflammatory cytokines like cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) play a critical role in inflammation (Hanh et al. 2011). In addition, proinflammatory mediators such as prostaglandin E_2 (PGE_2) and nitric oxide (NO) enhance the expression of proinflammatory cytokines, including tumor necrosis factor (TNF)-α and interleukin (IL)-1β. Previous studies have demonstrated that *C. odorata* has anti-inflammatory activity both in vitro and in vivo (Karodi et al. 2009; Park et al. 2012). The scutellarein tetramethyl ether (Scu) (4′,5,6,7-tetramethoxyflavone), isosakuranetin, and stigmasterol have also been reported to possess anti-inflammatory activity (Csupor et al. 2010).

Based on available literature, *C. odorata* would be a useful pharmaceutical ingredient for the management of various wounds including the chronic ones. However, further studies must be conducted in various wound models, and research at the cellular and molecular levels are required to identify specific mechanism that could induce healing in those wounds. Wound healing is a very complex, multifactor sequence of events involving several cellular and biochemical processes. The various processes of wound healing will help to regenerate and reconstruct the disrupted anatomical continuity and functional status of the skin. Initially wound healing involves an acute inflammatory phase followed by the synthesis of collagen and other extra cellular macromolecules, which are later removed to form a scar (Mukherjee 2002). The use of a single model for a wound healing study is inadequate, and no reference standard exists that can collectively represent the various phases of wound healing. For example, drugs, which influence one phase, may not necessarily influence another.

The available reports highlight the fact that the inclusion of antioxidant and antipathogenic microbial rich extract or fractions of *C. odorata* as a potential healing agent would also benefit both animal and human health. The data obtained from various studies are useful for future research aiming to further identify the specific bioactive principles other than Scu and stigmasterol (Pandith et al. 2013a; Vijayaraghavan et al. 2017a, b) that are indeed responsible for the healing efficacy of *C. odorata*. Figure 5 depicts the possible mechanism of action and other pharmacological roles of bioactive compounds of the plant extracts.

Overall, several herbal medicines have shown marked activity in the management of wounds, and therefore, *C. odorata* can be considered as an alternative treatment source. Furthermore, various natural compounds with verified in vivo wound healing potential can be considered potential natural drugs. The novelty with this plant includes the new opening in the field of antimicrobial, antioxidant, and especially wound healing potential of *C. odorata*, based on the available literature, which are crucial to its potential future drug design, development, and application for the treatment of wounds in biological systems. In summary, the present review concludes that *C. odorata* is a promising wound healing agent in treating various ailments as a veterinary or alternative medicine in animals and humans, respectively, in the future after further preclinical and clinical trials.

7 Concluding Remarks and Future Directions

Many healthy subjects and patients are taking potentially bioactive products for the prevention and treatment of multiple conditions, including wounds and infections. This forms the basis of a worldwide, multimillion dollar major commercial industry. While the scientific validity of the use of a number of these products is lacking, in the past few years much effort has been made to provide solid knowledge of the mechanisms underlying the beneficial effects of nutraceuticals. Scientifically rigorous research is warranted in order to identify novel compounds to be used alone or in combination with standard drugs in wound healing. Also, future research should be aimed at further increasing the efficacy of a promising nutraceutical, trying to use it as a chemical template for combinatorial synthesis. Finally,

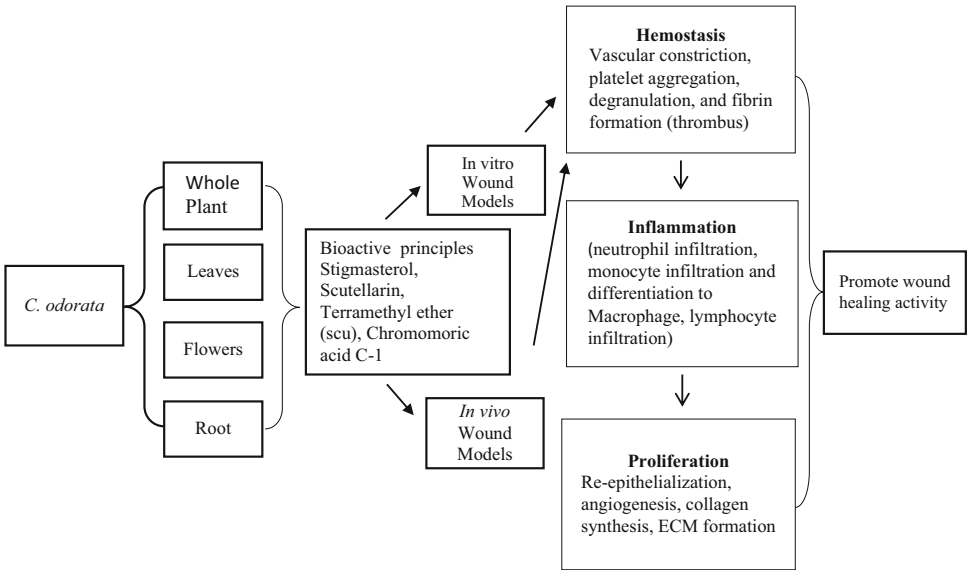


Fig. 5 Possible mechanism of action of bioactive compounds from various parts of the plant extracts of *C. odorata*

researchers in this area should focus on understanding of the molecular action of each nutraceutical and test the possible synergistic effects with other nutraceuticals and/or derivatives, food components, or conventional drugs. However, one must keep in mind that just because isolated compounds start from a natural food, they are not necessarily safe and natural, and therefore, strict quality control and regulatory issues are mandatory. The judicious application of nutraceuticals is based on the objective assessment of the clinical evidence and subjective characterization of the risk, merits, economic prices, and probable drug interactions. Future clinical studies are necessary to determine whether these compounds will be as interesting as preventive or therapeutic agents as they are in preclinical studies.

Competing Interests Authors have declared that no competing interests exist.

References

Afolabi C, Akinmoladun EO, Dan-Ologe IA (2007) Phytochemical constituents and antioxidant properties of extracts from the leaves of *Chromolaena odorata*. Sci Res Essays 2(6):191–194

Akinmoladun AC, Obuotor EM, Farombi EO (2010) Evaluation of antioxidant and free radical scavenging capacities of some Nigerian indigenous medicinal plants. J Med Food 13:444–451

Amadeu TP, Braune AS, Porto LC et al (2004) Fibrillin I and elastin are differentially expressed in hypertrophic scars and keloids. Wound Repair Regen 12(2):169–174

Amatya S, Tuladhar SM (2011) In vitro antioxidant activity of extracts from *Eupatorium odoratum* L. Res J Med Plant 5:79–84

Annan K, Houghton PJ (2008) Antibacterial, antioxidant and fibroblast growth stimulation of aqueous extracts of *Ficus asperifolia* Miq. and *Gossypium arboreum* L., wound-healing plants of Ghana. J Ethnopharmacol 119:141–144

Ansell DM, Holden KA, Hardman MJ (2012) Animal models of wound repair: are they cutting it? Exp Dermatol 21(8):581–585

Anyasor GN, Aina DA, Olushola M et al (2011) Phytochemical constituents, proximate analysis, antioxidants, anti-bacterial and wound healing properties of leaf extracts of *Chromolaena odorata*. Ann Biol Res 2:441–451

Ayyanar M, Ignacimuthu S (2005) Ethnomedicinal plants used by the tribals of Tirunelveli hills to treat poisonous bites and skin diseases of Indian. Ind J Tradit Knowl 4(3):229–236

Badoe EA, Archampong EO, da Rocha-Afodu JT (2000) Principles and practice of surgery: including pathology in the tropics, 3rd edn. Ghana Publishing, Accra, Ghana, pp 53–64

Bagabir R, Byers RJ, Chaudhry IH, Müller W, Paus R, Bayat A (2012) Site-specific immunophenotyping of keloid disease demonstrates immune upregulation and the presence of lymphoid aggregates. Br J Dermatol 167(5):1053–1066

Baravkar AA, Kale RN, Patil RN et al (2008) Pharmaceutical and biological evaluation of formulated cream of methanolic extract of *Acacia nilotica* leaves. Res J Pharm Technol 1(4):481–483

Barreto RS, Albuquerque-Júnior RL, Araújo AA et al (2014) A systematic review of the wound-healing effects of monoterpenes and iridoid derivatives. Molecules 19:846–862

Behm B, Babilas P, Landthaler M et al (2012) Cytokines, chemokines and growth factors in wound healing. J Eur Acad Dermatol Venereol 26:812–820

Belmont P, Schoenfeld AJ, Goodman G (2010) Epidemiology of combat wounds in operation iraqi freedom and operation enduring freedom, Orthopaedic burden of disease. J Surg Orthop Adv 19:2–7

Bhargava D, Sanjay K, Jagadish NS et al (2011) Screening of anti-gonorrhoeal activity of some medicinal plants in Nepal. Int J Pharmacol Biosci 2:203–212

Bindschadler M, McGrath JL (2007) Sheet migration by wounded monolayers as an emergent property of single-cell dynamics. J Cell Sci 120(Pt 5):876–884

Biswas TK, Mukherjee B (2003) Plant medicines of Indian origin for wound healing activity, a review. Int J Lower Extrem Wounds 2:25–39

Boateng JS, Matthews KH, Stevens HN et al (2008) Wound healing dressings and drug delivery systems: a review. J Pharm Sci 97 (8):2892–2923

Bopage NS, Kamal Bandara Gunaherath GM, Jayawardena KH et al (2018) Dual function of active constituents from bark of *Ficus racemosa* L in wound healing. BMC Complement Altern Med 18 (1):29. https://doi.org/10.1186/s12906-018-2089-9

Bowler PG, Duerden BI, Armstrong DG (2001) Wound microbiology and associated approaches to wound management. Clin Microbiol Rev 14:244–269

Brielmann HL, Setzer WN, Kaufman PB et al (2006) Phytochemicals: the chemical components of plants. In: Cseke LJ, Kirakosyan A, Kaufman PB, Warber SL, Duke JA, Brielmann HL (eds) Natural products from plants. Taylor and Francis, Boca Raton, FL

Brown NJ, Smyth EA, Cross SS, Reed MW (2002) Angiogenesis induction and regression in human surgical wounds. Wound Repair Regen 10:245–251

Bullard KM, Longaker MT, Lorenz HP (2003) Fetal wound healing: current biology. World J Surg 27(1):54–61

Bunyapraphatsara N, Chokechajiaroenprom O (2000) Thai medicinal plants, vol 4. Faculty of Pharmacy, Mahidol University and National Center for Genetic Engineering and Biotechnology, Bangkok, pp 622–626

Burford G, Bodeker G, Ryan TJ (2007) Skin and wound care: traditional, complementary and alternative medicine in public health dermatology. In: Bodeker G, Burford G (eds) Traditional, complementary and alternative medicine: policy and public health perspectives. Imperial College Press, London

Burgalassi S, Raimondi L, Pirisino R et al (2000) Effect of xyloglucan (tamarind seed polysaccharide) on conjuctival cell adhesion to laminin and corneal epithelium wound healing. Eur J Ophthalmol 10:71–76

Chakraborty AK, Sujit R, Umesh KP (2011) *Chromolaena odorata*, an overview. J Pharm Res 43:573–576

Chaudhari M, Mengi S (2006) Evaluation of phytoconstituents of *Terminalia arjuna* for wound healing activity in rats. Phytother Res 20(9):799–805

Chávez EJJ, Martínez BD, González VMA, Trinidad ME, Alancaster CN, Vázquez RAL (2011) Microneedles: a valuable physical enhancer to increase transdermal drug delivery. J Clin Pharmacol 51:964–977

Chereddy KK, Coco R, Memvanga PB et al (2013) Combined effect of PLGA and curcumin on wound healing activity. J Controlled Releas 171(2):208–215

Choi SW, Son BW, Son YS et al (2001) The wound-healing effect of a glycoprotein fraction isolated from aloe vera. Br J Dermatol 145 (4):535–545

Chomnawang MT (2005) Antimicrobial effects of Thai medicinal plants against acne-inducing bacteria. J Ethnopharmacol 101:330–333

Clark RAF (1991) Cutaneous wound repair. In: Goldsmith LA (ed) Biochemistry and physiology of the skin. Oxford University Press, London, pp 576–601

Csupor D, Blazso G, Balogh A, Hohmann J (2010) The traditional Hungarian medicinal plant *Centaurea sadleriana* Janka accelerates wound healing in rats. J Ethnopharmacol 127:193–195

D'Souza KM, Malhotra R, Philip JL et al (2011) G protein-coupled receptor kinase-2 is a novel regulator of collagen synthesis in adult human cardiac fibroblasts. J Biol Chem 286:15507–15516

Dawn TA, Jialin W, Zhihong J, Hubiao C, Guanghua L, Zhongzhen Z (2008) Ethnobotanical study of medicinal plants used by Hakka in Guangdong, China. J Ethnopharmacol 117:41–50

de la Torre JI, Chambers JA (2008) Wound healing, chronic wounds. Emedicine

Dinda M, Dasgupta U, Singh N et al (2015) PI3K-mediated proliferation of fibroblasts by *Calendula officinalis* tincture: implication in wound healing. Phytother Res 29:607–616

Donovan RM (2007) Skin treatment based on the use of *Chromolaena odorata*. http://www.freepatentsonline.com/EP1367988.html

Drury JL, Mooney DJ (2003) Hydrogels for tissue engineering: scaffold design variables and applications. Biomaterials 24(24):4337–4351

Duke J (1992) Handook of biological active phytochemical and their activities. CRC Press, Boca Raton, FL, pp 99–131

Dunn L, Prosser HC, Tan JT et al (2013) Murine model of wound healing. J Vis Exp 28:e50265

Edlich RF, Winters KL, Britt LD et al (2005) Difficult wounds: an update. J Long Term Eff Med Implants 15:289–302

Eming SA, Smoler H, Krieg T (2002) Treatment of chronic wounds wound: state of the art and future concepts. Cells Tissues Organs 172 (2):105–117

Farahpour MR, Habibi M (2012) Evaluation of the wound healing activity of an ethanolic extract of *Ceylon cinnamon* in mice. Vet Med Czech 57(1):53–57

Farquar JN (1996) Plant sterols: their biological effects in human, handbook of lipids in human nutrition. CRC Press, Boca Rotan, FL, pp 101–105

Frank S, Madlener M, Pfeilschifter J et al (1998) Induction of inducible nitric oxide synthase and its corresponding tetrahydrobiopterin-cofactor-synthesizing enzyme GTPcyclohydrolase I during cutaneous wound repair. J Invest Dermatol 111:1058–1064

Friedl P (2004) Prespecification and plasticity: shifting mechanisms of cell migration. Curr Opin Cell Biol 16:14–23

Fu SC, Hui CW, Li LC et al (2005) Total flavones of *Hippophae rhamnoides* promotes early restoration of ultimate stress of healing patellar tendon in a rat model. Med Eng Phys 27(4):313–321

Fujii A, Kobayashi S, Kuboyama N et al (1990) Augmentation of wound healing by Royal jelly (RJ) in streptozotocin-diabetic rats. Jpn J Pharmacol 53:331–337

Galiano RD, Michaels J, Dobryansky M et al (2004) Quantitative and reproducible murine model of excisional wound healing. Wound Repair Regen 12:485–492

Gao Y, Wang X, Chen S et al (2013) Acute skin barrier disruption with repeated tape stripping: an in vivo model for damage skin barrier. Skin Res Technol 19:162–168

Garbor M (1986) Anti-inflammatory and anti-allergic properties of flavonoids in biology and medicine, biochemical, pharmacological and structural activity relationship. In: Harborne JB, Bertz A (eds) Progress in clinical and biological research. Alan R. Liss, New York, pp 471–480

Gaurav T, Ruchi T, Birendra S, Bhati L, Pandey S, Pandey P, Saurabh KB (2012) Drug delivery systems: an updated review. Int J Pharm Investig 2(1):2–11

Ghosh PK, Gaba A (2013) Phyto-extracts in wound healing. J Pharm Pharm Sci 16:760–820

Gill LS (1992) Ethnomedical uses of plants in Nigeria. University of Benin Press, Benin City, Nigeria

GISD (2006) Global invasive species database. Online data sheet. *Chromolaena odorata* (herb). http://www.iucngisd.org/gisd/species.php?sc=47

Groeber F, Holeiter M, Hampel M, Hinderer S, Schenke-Layland K (2011) Skin tissue engineering in vivo and in vitro applications. Adv Drug Deliv Rev 63(4):352–366

Grose R, Werner S (2004) Wound-healing studies in transgenic and knockout mice. Mol Biotechnol 28(2):147–166

Handa SS (2008) An overview of extraction techniques for medicinal and aromatic plants, in extraction technologies for medicinal and aromatic plants. In: Handa SS, SPS K, Longo G, Rakesh DD (eds) Extraction technologies for medicinal and aromatic plants. ICS-UNIDO, Trieste, Italy, pp 21–52

Hanh TT, Hang DT, Van Minh C et al (2011) Anti-inflammatory effects of fatty acids isolated from *Chromolaena odorata*. Asian Pac J Trop Med 4(10):760–763

Harun FB, Syed Sahil Jamalullail SM, Yin KB et al (2012) Autophagic cell death is induced by acetone and ethyl acetate extracts from *Eupatorium odoratum* in vitro: effects on MCF-7 and vero cell lines. Sci World J 2:439–479

Hashemi SR, Davoodi H (2011) Herbal plants and their derivatives as growth and health promoters in animal nutrition. Vet Res Commun 35:169–180

Hassan R (2012) Overview on drug delivery system. Pharm Anal Acta 3:10

Hausteen B (1983) Flavonoids, a class of natural products of high pharmacological potency. Biochem Pharmacol 32:1141–1148

Heiss EH, Tran TV, Zimmermann K et al (2014) Identification of chromomoric acid C-I as an Nrf2 activator in *Chromolaena odorata*. J Nat Prod 77:503–508

Heywood VH, Harborne JB, Turner BL (1977) Biology and chemistry of the compositae, vol 1. Academic Press, London

Ho J, Walsh C, Yue D, Dardik A, Umber C (2017) Current advancements and strategies in tissue engineering for wound healing: a comprehensive review. Adv Wound Care 6(6):2017. https://doi.org/10.1089/wound.2016.0723

Hong SS, Kim JH, Li H et al (2005) Advanced formulation and pharmacological activity of hydrogel of the titrated extract of *C. asiatics*. Arch Pharm Res 28(4):502–508

Huang Z, Lu M, Zhu G et al (2011) Acceleration of diabetic-wound healing with PEGylated rhaFGF in healing-impaired streptozocin diabetic rats. Wound Repair Regen 19(5):633–644

Hussein J, Mavalankar DV, Sharma S et al (2011) A review of health system infection control measures in developing countries, What can be learned to reduce maternal mortality. Glob Health 7:14

Idu M, Onyibe HI (2007) Medicinal plants of Edo state, Nigeria. Res J Med Plants 1:32–41

Inya-Agha SI, Oguntimein BO, Sofowora A et al (1987) Phytochemical and antibacterial studies on the essential oil of *Eupatorium odoratum*. Int J Crude Drug Res 25:49–52

Iwu MM (1993) Handbook of African medicinal plants, 1st edn. CRC Press, Boca Raton, FL, p 464. ISBN-10: 084934266X

Jagadeesh KS, Geeta GS, Reddy TKR (1990) Biogas production by anaerobic digestion of Eupatorium odoratum L. Biol Wastes 33:67

Jagetia GC, Rajanikant GK (2004) Role of curcumin, a naturally occurring phenolic compound of turmeric in accelerating the repair of excision wound, in mice whole-body exposed to various doses of γ-radiation. J Surg Res 120(1):127–138

Johnson NR, Wang Y (2015) Drug delivery systems for wound healing. Curr Pharm Biotechnol 16(7):621–629

Johnston ST, Ross JV, Binder BJ, Sean McElwain D, Haridas P, Simpson MJ (2016) Quantifying the effect of experimental design choices for in vitro scratch assays. J Theor Biol 400:19–31

Joshi A, Sengar N, Prasad SK et al (2013) Wound-healing potential of the root extract of *Albizzia lebbeck*. Planta Med 79(9):737–743

Joshi A, Joshi VK, Pandey D et al (2016) Systematic investigation of ethanolic extract from *Leea macrophylla*: implications in wound healing. J Ethnopharmacol 191:95–106

Kahkeshani N, Farahanikia B, Mahdaviani P et al (2013) Antioxidant and burn healing potential of *Galium odoratum* extracts. Res Pharm Sci 8:197–203

Karodi R, Jadhav M, Rub R et al (2009) Evaluation of the wound healing activity of a crude extract of *Rubia cordifolia* L. (Indian madder) in mice. Int J Appl Res Nat Prod 2(2):12–18

Kerr J (2002) The use of essential oils in healing wounds. Int J Aroma Ther 12:202–206

Khanna S, Venojarvi M, Roy S et al (2002) Dermal wound healing properties of redox-active grape seed proanthocyanidins. Free Radic Biol Med 33(8):1089–1096

Kiistala U (1968) Suction blister device for separation of viable epidermis from dermis. J Invest Dermatol 50:129–137

Kim YM, Namkoong S, Yun YG et al (2007) Water extract of Korean red ginseng stimulates angiogenesis by activating the PI3K/Akt dependent ERK1/2 and eNOS pathways in human umbilical vein endothelial cells. Biol Pharm Bull 30:1674–1679

Klass BR, Grobbelaar AO, Rolfe KJ (2009) Transforming growth factor beta 1 signaling, wound healing and repair: a multifunctional cytokine with clinical implications for wound repair, a delicate balance. Postgrad Med J 85:9–14

Koria P (2012) Delivery of growth factors for tissue regeneration and wound healing. BioDrugs 26(3):163–175

Kouamé PB, Jacques C, Bedi G et al (2013) Phytochemicals isolated from leaves of *Chromolaena odorata*: impact on viability and clonogenicity of cancer cell lines. Phytother Res 27(6):835–840

Krishnan P (2006) The scientific study of herbal wound healing therapies: current state of play. Curr Anaesth Crit Care 17(1):21–27

Kumar B, Vijayakumar M, Govindarajan R et al (2007) Ethnopharmacological approaches to wound healing – exploring medicinal plants of India. J Ethnopharmacol 114(2):103–113

Kumari A, Yadav SK, Yadav SC (2010) Biodegradable polymeric nanoparticles based drug delivery systems. Colloid Surg B 75 (1):1–18

Kumari P, Yadav P, Verma PR et al (2013) A review on wound healing properties of Indian medicinal plants. Indian J Fundam Appl Life Sci 3:220–232

Kuponiy E, Ibibia T (2013) A review on antimicrobial and other beneficial effects of flavonoids. Int J Pharm Sci Rev Res 21(1):20–33

Kusano A, Seyama Y, Nagai M et al (2001) Effects of fukinolic acid and cimicifugic acids from *Cimicifuga* species on collagenolytic activity. Biol Pharm Bull 24(10):1198–1201

Lademann J, Jacobi U, Surber C, Weigmann HY, Fluhr JW (2009) The tape stripping procedure – evaluation of some critical parameters. Eur J Pharm Biopharm 72:317–323

Lai PK (2004) Antimicrobial and chemopreventive properties of herbs and spices. Curr Med Chem 1(11):1451–1460

Li H, Wang Z, Liu Y (1983) Review in the studies on tannins activity of cancer prevention and anticancer. Zhong Yao Cai 26(6):444–448

Li M, Xu J, Shi T (2016) Epigallocatechin-3-gallate augments therapeutic effects of mesenchymal stem cells in skin wound healing. Clin Exp Pharmacol Physiol 43:1115–1124

Ling SK, Abdul Rashih A, Salbiah M et al (2007) Extraction and simultaneous detection of flavonoids in the leaves of *Chromolaena odorata* by RP-HPLC with DAD. In: Nik Zanariah NM, Sarifah KA, Nor Azman H (eds) Highlights of FRIM's IRPA projects 2006: identifying potential commercial collaborations project evaluation meeting. 14–15 Dec 2006. Forest Research Institute Malaysia, pp 32–37

Lutolf MP, Hubbell JA (2005) Synthetic biomaterials as instructive extracellular microenvironments for morphogenesis in tissue engineering. Nat Biotechnol 23(1):47–55

Maarof M, Law JX, Chowdhury SR, Khairoji KA, Saim AB, Idrus RBH (2016) Secretion of wound healing mediators by single and bi-layer skin substitutes. Cytotechnology 68(5):1873–1884

Maenthaisong R, Chaiyakunapruk N, Niruntraporn S et al (2007) The efficacy of Aloe vera used for burn wound healing: a systematic review. Burns 33:713–718

Manju RS, Shailendra S, Vyas A et al (2013) Innovative approaches in wound healing: trajectory and advances. Artific Cells Nanomed Biotechnol 41:202–212

Manjunatha BK, Vidya SM, Krishna V et al (2007) Comparative evaluation of wound healing potency of *Vitex trifolia* L and *Vitex altissima* L. Phytother Res 21:457–461

Martin P (1997) Wound healing-aiming for perfect skin regeneration. Science 276(5309):122–126

Mathivanan N, Surendiran G, Srinivasan K et al (2006) Morinda pubescens JE Smith (Morinda tinctoria Roxb) fruit extract accelerates wound healing in rats. J Med Food 9:591–593

Menke MN, Menke NB, Boardman CH et al (2008) Biologic therapeutics and molecular profiling to optimize wound healing. Gynecol Oncol 111(2):S87–S91

Mikhal'chik EV, Anurov MV, Titkova SM (2006) Activity of antioxidant enzymes in the skin during surgical wounds. Bull Exp Biol Med 142:667–669

Mirmalek S, Parsa T, Parsa Y et al (2016) The wound healing effect of *Iris forentina* on full thickness excisional skin wounds: a histomorphometrical study. Bangla J Pharmacol 11:86–90

Mohanty C, Sahoo SK (2017) Curcumin and its topical formulations for wound healing applications. Drug Discov Today 22(10):1582–1592

Moon EJ, Lee YM, Lee H et al (1999) A novel angiogenic factor derived from *Aloe vera* gel: β-sitosterol, a plant sterol. Angiogenesis 3:117–123

Morton JF (1981) Atlas of medicinal plants of Middle America, vol 2. Charles C. Thomas, Springfield, IL

Motar ML, Thomas RB, Fillo JM (1985) Effect of Anarcardium occidental stem bark extracts on in vitro inflammatory models. J Ethnopharmacol 95(2-3):139–142

Moura-Letts G, Villegas LF, Marçalo A et al (2006) In vivo wound-healing activity of oleanolic acid derived from the acid hydrolysis of *Anredera diffusa*. J Nat Prod 69:978–979

Mukherjee PW (2002) Quality control of herbal drugs: an approach to evaluation of botanicals. Business Horizons, New Delhi

Murti K, Kumar U (2012) Enhancement of wound healing with roots of *Ficus racemosa* L in albino rats. Asian Pac J Trop Biomed 2:276–280

Nicolaus C, Junghanns S, Hartmann A et al (2017) In vitro studies to evaluate the wound healing properties of *Calendula officinalis* extracts. J Ethnopharmacol 196:94–103

Nunan R, Harding KG, Martin P (2014) Clinical challenges of chronic wounds: searching for an optimal animal model to recapitulate their complexity. Dis Model Mech 7(11):1205–1213

Obute GC, Adubor GO (2007) Chemicals detected in plants used for folk medicine in South Eastern Nigeria. Ethnobotan Leaflets 11:173–194

Odunbaku OA, Ilusanya OA, Akasoro KS (2008) Antibacterial activity of ethanolic leaf extract of *Ficus exasperata* on *Escherichia coli* and *Staphylococcus albus*. Sci Res Essay 3:562–564

Oludare TB, Olumayokun OA, Olufunmilola SO et al (2000) Anti-inflammatory, antipyretic and antispasmodic properties of *Chromolaena odorata*. Pharm Biol 38:367–370

Omale J, Isaac AV (2010) Excision and incision wound healing potential of *Saba florida* (Benth) leaf extract in *Rattus norvegicus*. Int J Pharma Biomed Res 1:101

Omokhua AG, McGaw LJ, Finnie JF, Van Staden J (2016) Chromolaena odorata (L.) R.M. King & H. Rob. (Asteraceae) in sub-Saharan Africa: a synthesis and review of its medicinal potential. J Ethnopharmacol 183:112–122

Overbeek JTG, Voorn MJ (1957) Phase separation in polyelectrolyte solutions. Theory of complex coacervation. J Cell Comp Physiol 49 (S1):7–26

Owoyele BV, Soladoye AO (2006) Anti-inflammatory and analgesic activities of ethanolic extract of *Chromolaena odorata* leaves. Chronic Comm Disease 18:397–406

Owoyele BV, Oguntoye SO, Dare K, Ogunbiyi BA, Aruboula EA, Soladoye AO (2008) Analgesic, anti-inflammatory and antipyretic activities from flavonoid fractions of *Chromolaena odorata*. J Med Plant Res 2:219–225

Ozgen U, Ikbal M, Hacimuftuoglu A et al (2006) Fibroblast growth stimulation by extracts and compounds of *Onosma argentatum* roots. J Ethnopharmacol 104(1–2):100–103

Ozturk N, Korkmaz S, Ozturk Y et al (2006) Effects of gentiopicroside, sweroside and swertiamarine, secoiridoids from Gentian (*Gentiana lutea sp. symphyandra*), on cultured chicken embryonic fibroblasts. Planta Med 72(4):289–294

Pacific Island Ecosystems at Risk (PIER) (2001) Invasive plant species: *Chromolaena odorata* Sw. Asteraceae. http://www.hear.org/pier/sotor.htm. p 2

Paiva LA, de Alencar Cunha KM, Santos FA et al (2002) Investigation on the wound healing activity of oleo-resin from *Copaifera langsdorffi* in rats. Phytother Res 16:737–739

Pampaloni F, Reynaud EG, Stelzer EHK (2007) The third dimension bridges the gap between cell culture and live tissue. Nat Rev Mol Cell Biol 8(10):839–845

Pan CH, Kim ES, Jung SH et al (2008) Tectorigenin inhibits IFN-γ/LPS-induced inflammatory responses in murine macrophage RAW 264.7 cells. Arch Pharm Res 31:1447–1456

Panda D, Dash KS, Dash KG (2010) Qualitative phytochemical analysis and investigation of anthelmintic and wound healing potentials of various extracts of *Chromolaena odorata* (L.) collected from the locality of Mohuda Village, Berhampur (South Orissa). Int J Pharm Sci Rev Res 1:122–126

Pandith H, Zhang X, Thongpraditchote S et al (2013a) Effect of Siam weed extract and its bioactive component scutellarein tetramethyl ether on anti-inflammatory activity through NF-κB pathway. J Ethnopharmacol 147:434–441

Pandith H, Zhang X, Liggett J et al (2013b) Hemostatic and wound healing properties of *Chromolaena odorata* leaf extract. ISRN Dermatol 2013:168269. https://doi.org/10.1155/2013/168269

Pang KL, Vijayaraghavan K, Al Sayed B, Seyed MA (2018) Betulinic acid-induced expression of nicotinamide adenine dinucleotide phosphate-diaphorase in the immune organs of mice: a possible role of nitric oxide in immunomodulation. Mol Med Rep 17 (2):3035–3041

Park JW, Kwon OK, Jang H et al (2012) A leaf methanolic extract of Wercklea insignis attenuates the lipopolysaccharide-induced inflammatory response by blocking the NF-κB signaling pathway in RAW 264.7 macrophages. Inflammation 35:321–331

Pawar RS, Khan SI, Khan IA (2007) Glycosides of 20-deoxy derivatives of jujubogenin and pseudojujubogenin from *Bacopa monniera*. Planta Med 73:380–383

Pereira LDP, Mario RLM, Brizeno LAC et al (2016) Modulator effect of a polysaccharide-rich extract from *Caesalpinia ferrea* stem barks in rat cutaneous wound healing: role of TNF-α, IL-1β, NO, TGF-β. J Ethnopharmacol 187:213–223

Perez R, Davis SC (2008) Relevance of animal models for wound healing. Wounds 20(1):3–8

Petrie NC, Yao F, Eriksson E (2003) Gene therapy in wound healing. Surg Clin N Am 83:597–616

Phan TT, Wang L, See P et al (2001a) Phenolic compounds of *Chromolaena odorata* protect cultured skin cells from oxidative damage: implication for cutaneous wound healing. Biol Pharm Bull 24:1373–1379

Phan TT, Hughes MA, Cherry GW (2001b) Effects of an aqueous extract from the leaves of *Chromolaena odorata* (Eupolin) on the proliferation of human keratinocytes and on their migration in an in vitro model of reepithelialization. Wound Repair Regen 9:305–313

Pisutthanan N, Liawruangrath S, Bremner J et al (2005) Chemical constituents and biological activities of *Chromolaena odorata*. J Sci Fac Chiang Mai Univ 32:139–148

Poul Holm P, Fenstad AM, Lars Folke EA (1974) DNA, RNA and protein synthesis in healing wounds in young and old mice. Mech Ageing Dev 3:173–185

Raina R, Prawez S, Verma PK et al (2008) Medicinal plants and their role in wound healing. Outline Vet J 3:1–7

Raman VB, Samuel LA, Saradhi PM et al (2012) Antibacterial, antioxidant activity and GC-MS analysis of *Eupatorium odoratum*. Asian J Pharm Clin Res 5:99–106

Razzell W, Wood W, Martin P (2011) Swatting flies: modelling wound healing and inammation in Drosophila. Dis Model Mech 4(5):2011

Redd MJ, Cooper L, Wood W et al (2004) Wound healing and inflammation: embryos reveal the way to perfect repair. Philos Trans R Soc B 359:777–784

Reddy S, Rao P, Reddy MS (2002) Wound healing effects of *Heliohopium indicum*, *Plumbgo zeylanicum* and *Acalypha indica* in rats. J Ethnopharmacol 79:249–251

Richardson R, Slanchev K, Kraus C, Knyphausen P, Eming S, Hammerschmidt M (2013) Adult zebrafish as a model system for cutaneous wound-healing research. J Invest Dermatol 133 (6):1655–1665

Robards K, Prenzler PD, Tucke G et al (1999) Phenolic compounds and their role in oxidative processes in fruits. Food Chem 66:401–436

Rørth P (2007) Collective guidance of collective cell migration. Trends Cell Biol 17:575–579

Santos-Buelga C, Scalbert A (2000) Proanthocyanidins and tannin-like compounds: nature, occurrence dietary intake and effects on nutrition and health. J Sci Food Agr 80(7):1094–1117

Scheid A, Meuli M, Gassmann M, Wenger RH (2000) Genetically modified mouse models in studies on cutaneous wound healing. Exp Physiol 85(6):687–704

Schultz GS (1999) Molecular regulation of wound healing. In: Bryant RA (ed) Acute and chronic wounds nursing management, 2nd edn. WB Saunders, St. Louis, MO, pp 413–429

Schultz GS, Wysocki A (2009) Interactions between extracellular matrix and growth factors in wound healing. Wound Repair Regen 17 (2):153–162

Seaton M, Hocking A, Gibran NS (2015) Porcine models of cutaneous wound healing. ILAR J 56:127–138

Shen HM, Chen C, Jiang JY et al (2017) The N-butyl alcohol extract from *Hibiscus rosasinensis* L. flowers enhances healing potential on rat excisional wounds. J Ethnopharmacol 198:291–301

Shukla A, Rasik M, Jain GK et al (1999) *In vitro* and *in vivo* wound healing activity of asiaticoside isolated from *Centella asiatica*. J Ethnopharmacol 65:1–11

Singh M, Govindarajan R, Nath V et al (2006) Antimicrobial, wound healing and antioxidant activity of *Plagiochasma appendiculatum* Lehm. et Lind. J Ethnopharmacol 107(1):67–72

Sirinthipaporn A, Jiraungkoorskul W (2017) Wound healing property review of Siam Weed, *Chromolaena odorata*. Pharmacogn Rev 11 (21):35–38

Song HS, Park TW, Sohn UD et al (2008) The effect of caffeic acid on wound healing in skinincised mice. Korean J Physiol Pharmacol 12:343–347

Soni H, Singhai AK (2013) A recent update of botanicals for wound healing activity. Int Res J Pharm 3(7):1–7

Soppimath KS, Aminabhavi TM, Kulkarni AR et al (2001) Biodegradable polymeric nanoparticles as drug delivery devices. J Control Release 70(1–2):1–20

Sørensen LT, Toft BG, Rygaard J, Ladelund S, Paddon M, James T, Taylor R, Gottrup F (2010) Effect of smoking, smoking cessation, and nicotine patch on wound dimension, vitamin C, and systemic markers of collagen metabolism. Surgery 148:982–990

Stallmeyer B, Pfeilschifter J, Frank S (2001) Systemically and topically supplemented leptin fails to reconstitute a normal angiogenic response during skin repair in diabetic ob/ob mice. Diabetologia 44:471–479

Stamm A, Reimers K, Strauß S, Vogt P, Scheper T, Pepelanova I (2016) In vitro wound healing assays state of the art. BioNanoMaterials 17 (1–2):79–87

Stanley A, Imeobong JI, Enosakhare AA et al (2017) Effect of ethanolic extract of *Chromolaena odorata* on the kidneys and intestines of healthy albino rats. Integr Med Res 6(3):292–299

Starley IF, Mohammed P, Schneider G et al (1999) The treatment of paediatric bums using topical paoava. Burns 25(7):636–639

Stevenson PC, Simmonds MS, Sampson J et al (2002) Wound healing activity of acylated iridoid glycosides from *Scrophularia nodosa*. Phytother Res 16(1):33–35

Strodtbeck F (2001) Physiology of wound healing. Newborn Infant Nurs Rev 11:43–52

Subramoniam A, Asha VV, Nair SA et al (2012) Chlorophyll revisited: anti-inflammatory activities of chlorophyll a and inhibition of expression of TNF-α gene by the same. Inflammation 35(3):959–966

Suksamran A, Chotipong A, Suavansri T et al (2004) Antimycobacterial activity and cytotoxicity of flavanoids from the leaves of *Chromolaena odorata*. Arch Pharm Res 5:507–511

Sullivan TP, Eaglstein WH, Davis SC et al (2001) The pig as a model for human wound healing. Wound Repair Regen 9:66–76

Summereld A, Ricklin ME (2015) The immunology of the porcine skin and its value as a model for human skin. Mol Immunol 66(1):14–21

Taiwo OB, Olajide OA, Soyanwo OO et al (2000) Antiinflammatory, antipyretic and antispasmodic properties of *Chromolaena odorata*. Pharm Biol 38(5):367–370

Taleb-Contini SH, Kanashiro A, Kabeya LM et al (2006) Immunomodulatory effects of methoxylated flavonoids from two *Chromolaena* species: structure-activity relationships. Phytother Res 20 (7):573–575

Tamada M, Perez TD, Nelson WJ et al (2007) Two distinct modes of myosin assembly and dynamics during epithelial wound closure. J Cell Biol 176:27–33

Timbilla JA, Zachariades C, Braimah H (2003) Biological control and management of the alien invasive shrub *Chromolaena odorata* in Africa. In: Neuenschwander P, Borgemeister C, Langewald J (eds) Biological control in IPM Systems in Africa. CABI Publishing, Wallingford, UK

Trabucchi E, Preis-Baruffaldi F, Baratti C et al (1986) Topical treatment of experimental skin lesions in rats: macroscopic, microscopic and scanning electron-microscopic evaluation of the healing process. Int J Tissue React 8:533–544

Trease GE, Evans WC (2002) Pharmacognosy, 15th edn. Saunders, London

Trepat X, Michael RW, Thomas EA et al (2009) Physical forces during collective cell migration. Nat Phys 5:426–430

Trombetta D, Castelli F, Sarpietro MG et al (2005) Mechanisms of antibacterial action of three monoterpenes. Antimicrob Agents Chemother 49:2474–2478

Trombetta D, Puglia C, Perri D et al (2006) Effect of polysaccharides from *Opunta ficus-indica* (L.) cladodes on the healing of dermal wounds in the rat. Phytomedicine 13:289–294

Tuhin RH, Begum M, Rahman S et al (2017) Wound healing effect of *Euphorbia hirta* Linn. (*Euphorbiaceae*) in alloxan induced diabetic rats. BMC Complement Altern Med 17:423

Uebersax L, Merkle HP, Meinel L (2009) Biopolymer-based growth factor delivery for tissue repair: from natural concepts to engineered systems. Tissue Eng Part B Rev 15(3):263–289

Upadhyay A, Chattopadhyay P, Goyary D et al (2014) *Ixora coccinea* enhances cutaneous wound healing by upregulating the expression of collagen and basic fibroblast growth factor. ISRN Pharmacol 2014:751824., 9 pages. https://doi.org/10.1155/2014/751824

Vaisakh MN, Pandey A (2013) The invasive weed with healing properties: a review on *Chromolaena odorata*. Int J Pharmaceut Sci Res 3:80–83

Veerapur VP, Palkar MB, Srinivasa H et al (2004) Effect of ethanol extract of *Wrightia tinctoria* bark on wound healing in rats. J Nat Remedies 4(2):155–156

Vijayaraghavan K, Ali MS, Maruthi R (2013) Studies on phytochemical screening and antioxidant activity of *Chromolaena odorata* and *Annona squamosa*. Int J Innov Res Sci Engg Tech 2:7315–7321

Vijayaraghavan K, Rajkumar J, Bukhari SN et al (2017a) *Chromolaena odorata*: a neglected weed with a wide spectrum of pharmacological activities (Review). Mol Med Rep 15(3):1007–1016

Vijayaraghavan K, Rajkumar J, Seyed MA (2017b) Efficacy of *Chromolaena odorata* leaf extracts for the healing of rat excision wounds. Vet Med 62:565–578

Villegas LF, Marçalo A, Martin J et al (2001) (+)-epi-Alpha-bisabolol [correction of bisbolol] is the wound-healing principle of Peperomia galioides: investigation of the in vivo wound-healing activity of related terpenoids. J Nat Prod 64(10):1357–1359

Wang L, Waltenberger B, Pferschy-Wenzig EM et al (2014) Natural product agonists of peroxisome proliferator-activated receptor gamma (PPARγ): a review. Biochem Pharmacol 92(1):73–89

Watson SAJ, Moore GPM (1990) Postnatal development of the hair cycle in the domestic pig. J Anat 170:1–9

WHO Report (2014). http://www.who.int/mediacentre/factsheets/fs365/en/

Wietecha MS, Dipietro LA (2013) Therapeutic approaches to the regulation of wound angiogenesis. Adv Wound Care 2:81–86

Wigger-Alberti W, Kuhlmann M, Ekanayake S, Wilhelm D (2009) Using anovel wound model to investigate the healing properties of products for superficial wounds. J Wound Care 18:123–131

Wilhelm KP, Wilhelm D, Bielfeldt S (2017) Models of wound healing: an emphasis on clinical studies. Skin Res Technol 23(1):3–12

Witte MB, Barbul A (2002) Role of nitric oxide in wound repair. Am J Surg 183:406–412

Wound Management, Forecast to 2021 (2013, March) Established and emerging products, technologies and markets in the Americas, Europe, Asia/Pacific and rest of world. http://woundcare-today.com/news/world-at-glance/projected-global-wound-prevalence-by-wound-types

Wullaert A, Bonnet MC, Pasparakis M (2011) NF-κB in the regulation of epithelial homeostasis and inflammation. Cell Res 21:146–158

Xu W, Jong Hong S, Jia S, Zhao Y, Galiano RD, Mustoe TA (2012) Application of a partial-thickness human ex vivo skin culture model in cutaneous wound healing study. Lab Investig 92(4):584–599

Yadav SS, Singh MK, Singh PK et al (2017) Traditional knowledge to clinical trials: a review on therapeutic actions of Emblica officinalis. Biomed Pharmacother 93:1292–1302

Yallapu MM, Jaggi M, Chauhan SC (2011) Design and engineering of nanogels for cancer treatment. Drug Discov Today 16 (9–10):457–463

Yallapu MM, Jaggi M, Chauhan SC (2013) Curcumin nanomedicine: a road to cancer therapeutics. Curr Pharm Des 19(11):1994–2010

Yoshizaki H, Ohba Y, Parrini MC et al (2004) Cell type-specific regulation of RhoA activity during cytokinesis. J Biol Chem 279:44756–44762

Zhang M-L, Irwin D, Li X-N et al (2012) PPARγ agonist from *Chromolaena odorata*. J Nat Prod 75:2076–2081

Zhao M, Song B, Pu J et al (2006) Electrical signals control wound healing through phosphatidylinositol-3-OH kinase-gamma and PTEN. Nature 442:457–460

Zulak KG, Liscombe DK, Ashihara H et al (2006) Alkaloids. In: Crozier A, Clifford MN, Ashihara H (eds) Plant secondary metabolites – occurrence, structure and role in human diet. Blackwell, Oxford

Nutraceuticals in Dermatological Disorders

Moges Woldemeskel

Abstract

Dermatological disorders refer to skin abnormalities that could be induced by various agents including infectious organisms, noninfectious physical agents, and immunological reactions. Various modern medicinal drugs are being used to treat different types of skin disorders. Although some of them are effective, unwanted side effects are often observed. Currently, there is an increased tendency to use traditional medicine including use of nutraceuticals which are in use since ancient times, are effective to treat and manage skin disorders, and cause no apparent side effects. This chapter briefly highlights on the use of some nutraceuticals in the treatment of skin disorders.

Keywords

Veterinary nutraceuticals · Dermatological disorders

1 Introduction

Skin is one of the largest immunologic organs of the body and is affected by both external and internal factors, as well as innate and adaptive immune responses. Dermatological disorders refer to skin abnormalities caused by various agents including infectious organisms, noninfectious or physical agents, and immunological reactions. Skin disorders such as atopic dermatitis, contact dermatitis, urticaria, angioedema, psoriasis, and autoimmune blistering disorders are immune-mediated conditions. Most of these diseases are chronic, inflammatory, and proliferative, in which both genetic and environmental factors play important roles in their development (Fonacier et al. 2010).

M. Woldemeskel (✉)
Tifton Veterinary Diagnostic and Investigational Laboratory, Department of Pathology, College of Veterinary Medicine, University of Georgia, Tifton, GA, USA
e-mail: mwoldem@uga.edu

Skin disorders are often seen in both humans and animals since the skin is the most external organ exposed to various physical, biological, and chemical agents. Due to diverse causes underlying skin (dermatological) disorders, methods used to treat dermatological abnormalities also are diverse. These vary from the use of traditional methods that encompass different natural agents, herbal medicine including nutraceutical products, to the application of modern medicine.

Herbal medicine is gaining popularity both in developing and developed countries due to its low cost and fewer side effects. Plants contain different types of phytoconstituents in the form of either primary metabolite or secondary metabolite, and many drugs used in the modern medicine are mainly derived from the medicinal plants (Patel and Patel 2017). Different types of natural bioactive compounds or foodstuff that provides extra health benefits in addition to basic nutritional values (nutraceuticals) have been in use for several generations to treat skin diseases in humans and animals as part of traditional medicine. For example, *Adenium obesum* (Lav, *Apocynaceae*) is generally used for the treatment of wounds and other skin diseases in Omani ethnic community (Akhtar et al. 2017). In this chapter, a brief overview will be given on the role of nutraceuticals in allergic skin diseases, atopic dermatitis, infectious skin diseases, and skin tumors.

2 Nutraceuticals in Dermatitis

Different types of agents are incriminated as underlying factors in skin disorders. Some skin diseases are associated with microbial infections, others are associated with exposure to irritants such as chemicals in cosmetics, while many others are immunological in origin and result from hypersensitivity responses to environmental allergens (Fonacier et al. 2010).

Since skin is constantly exposed to the external environment, any insult to skin may induce reactive response

R. C. Gupta et al. (eds.), *Nutraceuticals in Veterinary Medicine*, https://doi.org/10.1007/978-3-030-04624-8_37

including various types of dermatitis. Dermatitis is an inflammation of skin caused by infectious and noninfectious agents that may come in contact with skin. For example, atopic dermatitis is a common, recurring chronic inflammatory disease characterized by pruritus and eczema and is commonly associated with hypersensitivity to allergens, more frequently with allergic diseases such as allergic rhinitis and asthma (Kim et al. 2016).

Different types of dermatitis elicited by infectious and noninfectious agents are treated using modern medicine as well as traditional medicine including nutraceuticals. This section will briefly describe effects of some nutraceuticals used in the treatment of different types of dermatitis. *Aloe vera*, a plant which has a long history of popular and traditional use, is one such example. It is used in traditional Indian medicine for various skin diseases and infections. The first case report of the beneficial effects of *Aloe vera* in the treatment of skin and wound healing was published in 1935, with fresh whole-leaf extract reported to provide rapid relief from itching and burning associated with severe radiation-induced dermatitis and skin regeneration. Later on, numerous studies reported the role of topical *Aloe vera* application alone or in combination with others for the management of different skin conditions including wound healing, psoriasis, dermatitis, burn injuries, and surgical wounds. Foster et al. (2011) citing several authors summarized results of a number of clinical trials in which *Aloe vera* is positively indicated in the treatment of skin disorders. For example, a trial of wound healing management after the full-faced dermabrasion of patients with acne vulgaris demonstrated that the saturation of a standard polyethylene wound gel dressing with *Aloe vera* significantly reduced time to reepithelization compared to use of the standard dressing alone. It was reported that polysaccharides, particularly mannose-containing polysaccharides, cellulose, and pectic polysaccharides, comprise the major part of *Aloe vera* gel. Acetylated glucomannan is primarily responsible for the gel's mucilaginous properties and has been found in vitro and in animal studies to modulate immune function through macrophage activation and cytokine production and accelerate wound healing. In a randomized, double-blind, controlled trial of *Aloe vera* or placebo cream in 60 patients with chronic psoriasis, the cure rate of *Aloe vera* was 83%, with no relapses at 1 year of follow-up, compared to only 7% in the placebo group (Foster et al. 2011).

Furthermore, other various plant nutraceuticals are used to treat skin diseases in general. To give some examples, ethnobotanical studies indicate that Rasaut, decoction of *Berberis aristata*, was commonly used to treat skin disorders. Malani tribal communities from Himachal Pradesh, India, also use *Berberis aristata* to cure various skin diseases (Nimisha et al. 2017). In another report (Patel and Patel 2017), it was shown that topical anti-inflammatory activity of bioactivity-guided fractionation of methanolic extract of the leaves of *Santolina insularis* mainly luteolin, which is among the active constituents of the extract, prevented ear edema in croton oil-induced dermatitis in mouse ear more effectively compared to the standard drug indomethacin. The effect of other nutraceuticals on specific categories of skin abnormalities is highlighted in various sections below.

3 Nutraceuticals in Otitis Externa

Otitis externa is an inflammation or infection of skin of external ear canal and ear pinna. Otitis externa can be caused by bacteria, viruses, parasites, and noninfectious agents in animals. Among skin diseases that affect dogs, chronic, recurrent otitis externa poses one of the most frustrating pathologies of daily veterinary clinical practice (Rosser 2004; Pietschmann et al. 2013).

Many studies, in dogs and humans, affirm benefits of nutraceutical administration in the treatment of otitis externa. For example, the effect of a commercial nutraceutical diet composed of fish hydrolyzed proteins, rice carbohydrates, *Melaleuca alternifolia*, *Tilia cordata*, *Allium sativum* L., *Rosa canina* L., zinc, and omega-3/omega-6 (1:0.8 ratio), with anti-inflammatory and antioxidant activities as an adjuvant, was clinically evaluated in the treatment of different ages and breeds of dogs afflicted with chronic otitis externa. The dogs were also pharmacologically treated with a topical product (OTOMAX, Schering-Plough, Kenilworth, NJ, USA) eight drops a day for 7 days. The commercial nutraceutical diet has been proven effective, in combination with drugs, in relieving otitis externa-related symptoms and in reduction of the main symptoms of otitis externa such as external ear canal occlusion, erythema, odor, and mucus when compared to those that received the standard diet (Di Cerbo et al. 2016). In another study, the use of medical grade honey demonstrated overall effectiveness in the management of otitis externa in a population of dogs. The results obtained such as rapid onset of clinical and cytological improvement, positive owner evaluations, in vitro biocidal results, and durability of clinical resolution during follow-up were encouraging (Maruhashi et al. 2016). There also are several nutraceuticals claimed to have effect on various skin lesions but need scientific evaluation to confirm their specific effect on otitis externa.

4 Nutraceuticals in Infection-Associated Dermatitis

Skin diseases are often associated with infectious agents such as bacteria and parasites. Different types of treatments using various antibiotics and physical agents such as irradiations

are used to treat skin diseases. Current treatments for some chronic skin conditions are not effective; for example, microbial drug resistance has hindered the effectiveness of antibiotics in the treatment of chronic wounds, and ultraviolet radiation therapy used to treat psoriasis and atopic dermatitis may induce development of skin cancer (Gasparro 2000). This section will highlight on the use of nutraceuticals in alleviating and treating some infection-associated skin disorders/diseases. For example, a study was made on the effect of an immune-modulating diet in treating canine leishmaniosis in dogs that received no specific prior treatment for the disease. Canine leishmaniosis is a zoonotic disease that affects humans and dogs and is caused by the protozoan parasite *Leishmania infantum*. Several clinical manifestations have been described in leishmaniosis in dogs, and the clinical appearance and evolution of the disease appear to be influenced by complex interactions between the parasite and patient's genetic and immunological profile.

A diet supplement that contained *Ascophyllum nodosum*, *Cucumis melo*, *Carica papaya*, *Aloe vera*, astaxanthin from *Haematococcus pluvialis*, *Curcuma longa*, *Camellia sinensis*, *Punica granatum*, *Piper nigrum*, *Poligonum* spp., *Echinacea purpurea*, *Grifola frondosa*, *Glycine max*, and omega-3 and omega-6 unsaturated fatty acids from fish oil induced an intriguing immune modulation in leishmaniosis-affected dogs undergoing 12 months of treatment with standard pharmacological therapy. The immune-modulating diet appears to regulate the immune response in the affected dogs during the standard pharmacological treatment. The presence of nutraceuticals in the diet correlates with the decrease of Th1 cells and with the increase of regulatory T-cells in sick dogs. It was shown that pharmacological treatment alone was unable to induce long-lasting changes in pro-inflammatory response and to modulate regulatory T-cells in sick dogs, while the combination with immune-modulating diet was associated with a significant restoration of regulatory T-cell level and with the decrease in Th1 inflammatory response. Therefore, the administration of the specific dietary supplement improved the clinical response to the standard treatment in a model of canine leishmaniosis (Cortese et al. 2015) signifying the importance of nutraceutical supplement in the treatment of infection-associated skin diseases.

Other studies also indicate that various plant-based nutraceuticals were also effective against microbial-induced skin diseases. *Nerium oleander* (*Apocynaceae*) and *Aloe vera* are among the widely used herbal remedies that possess numerous activities such as antibacterial, antiviral, antifungal, and antioxidative activities and are used in treating skin diseases. Wound healing effect of *Aloe vera*-based extract of the *Nerium oleander* leaf based on its antioxidant, anti-inflammatory, and DNA repair capacity was investigated and compared with the traditional silver sulfadiazine treatment in Wistar albino rats with skin burn injury that received topical application twice a day for 2 weeks. Thermal injury-induced alterations were significantly reversed by the treatment. The healing effect of the extract was also supported by histological findings, suggesting that *Aloe vera*-based extract of the *Nerium oleander* leaf is a promising remedy for treating skin burn injury (Akgun et al. 2016).

Moreover, reports indicate that among other natural products, honey also has a healing effect on infection-associated skin diseases. Honey is being used since ancient times as a traditional medicine and has recently received attention as a complementary and alternative treatment in modern medicine. It is mainly composed of various sugars, phenolic acids, flavonoids, enzymes, amino acids, proteins, phytochemicals, and other miscellaneous compounds (Ahmed and Othman 2017). Traditionally, it has been used around the world to treat skin disorders associated with microbial infections and immunological reactions. In a traditional Hindu system (Ayurvedic) medicine, for example, honey is used to treat cuts and wounds, eczema, dermatitis, burns, and Fournier's gangrene. McLoonea et al. (2016) citing several authors indicated that the skin healing properties of honey have been largely attributed to its antimicrobial properties; a plethora of in vitro studies have demonstrated antimicrobial activity of honeys from all over the world against dermatologically important microbes. More recent in vitro research suggested that honeys of diverse floral origins can modulate the skin immune system through both immunostimulatory and anti-inflammatory effects (McLoonea et al. 2016).

There is also biologic evidence to support the use of honey in modern wound care. Honey has been shown to have effect against various bacterial species including *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Acinetobacter*, and *Stenotrophomonas*. Its antimicrobial activity has been shown to be directed against even antibacterial-resistant strains such as methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococcus* (Lee et al. 2011). Maruhashi et al. (2016) also reported that success was attained using honey against multidrug-resistant bacteria.

Plant products are also used against parasite-induced skin diseases or dermatitis. In Oman, various preparations of the plant material (bark and leaves) from *Adenium obesum* are used to treat skin diseases in humans. In Kenya, the stems and powder from the barks of *Adenium obesum* are used against skin parasites in camels and cattle. The crude extracts from root and bark are also used to prepare a lotion for the treatment of different skin diseases and to eliminate lice (Akhtar et al. 2017). Effects of such plant-based products on specific infection-associated skin lesions should be confirmed through scientific experiments and well-designed clinical studies.

5 Nutraceuticals in Allergic/Atopic Dermatitis

Allergic dermatitis is a type of dermatitis that develops due to a skin reaction to allergens that come in contact with the skin. It often develops in animals with a hereditary tendency to develop Ig-E-mediated allergic reaction to environmental agents such as house mite dust proteins, plant pollens, mold spores, and various other allergens. It commonly occurs in pets such as dogs and cats and in humans (Gross et al. 2005).

Usually, anti-inflammatory drugs and steroids are used to treat allergy-associated dermatitis. However, due to safety concerns consequent to the side effects of the drugs, use of nutraceuticals and certain food supplements to alleviate the clinical signs and help in the treatment of allergic dermatitis is increasing. As a result, scientific studies on nutraceuticals are also being undertaken in increasing rate. For example, flaxseed (*Linum usitatissimum*) supplementation was evaluated on the skin-test response in atopic horses that displayed allergic response to extracts from *Culicoides* spp. It was observed that supplementation with flaxseed for 42 days reduced the mean skin-test response to the extracts of *Culicoides* spp. with a significant decrease in the area of allergic reaction. In this study it was noted that flaxseeds were able to reduce the skin-test response of atopic horses, alter the fatty acid profile of the hair, reduce inflammation, and did not elicit any negative side effects suggesting a promising benefit of flaxseed to mitigate skin response to *Culicoides* extract elicited atopic dermatitis in horses (O'Neill et al. 2002).

It is worth noting that cutaneous allergic response may occasionally occur associated with food allergy, a clinical syndrome caused by an acute or chronic immunological reaction following food ingestion. Food intolerance, a non-immunological abnormal physiological response toward some specific foods or food additives, may also be observed associated with ingestion of certain food. Due to their often-difficult differentiation, these phenomena are generally included in the generic non-specific adverse food reaction syndrome, which can also involve the cutaneous system and are most often referred to as cutaneous adverse food reactions.

The effect of nutraceutical diet on indoor-housed domestic cats with cutaneous adverse food reactions, which among others included drooling, skin lesions such as back and neck intense itching, and neck eczema and had high amount of oxytetracycline as determined by oxytetracycline enzyme-linked immunosorbent assay (ELISA), was studied. The cats were grouped into those that received a standard feed and those that received a nutraceutical diet composed of a mixed formula of fish hydrolyzed proteins, rice carbohydrates, *Aloe vera*, *Arctium lappa*, *Malva sylvestris*, *Ribes nigrum*, *Allium sativum*, and omega-3 and omega-6 fatty acids at the ratio of 1:3 for 60 days. The nutraceutical diet was found to significantly reduce intensity of pruritus and severity of skin lesion with a significant improvement in the clinical picture of affected cats (Mazzeranghi et al. 2017).

Supplementation with some nutraceuticals is known to augment and support treatment effects of other chemical methods of treatment of skin conditions in humans and animals. For example, in humans, vitamin D supplementation decreased severity of atopic dermatitis and improved the associated symptoms and clinical signs in patients with atopic dermatitis particularly in pediatric patients (Kim et al. 2016).

Similarly supplementation of dogs suffering from atopic dermatitis with zinc methionine was documented to provide benefit in conjunction with treatment with two effective anti-inflammatory medications: glucocorticoids and ciclosporin (cyclosporine), which are known to have certain adverse side effects. The study provided evidence supporting a potential benefit of adjunctive zinc methionine supplementation in dogs with mild to moderate, chronic, nonseasonal canine atopic dermatitis. Dogs receiving glucocorticoids may be more likely to benefit from zinc methionine supplementation. The exact role of zinc and the potential benefits of supplementation in atopic dermatitis in humans are largely unknown. Interestingly, serum zinc levels have been found to be lower in atopic children compared to healthy children (McFadden et al. 2017). Furthermore, Tsuji et al. (2015) citing various studies in animal models indicated that selenium deficiency enhanced type-I allergic response in a mouse model of active cutaneous anaphylaxis indicating the importance and potential significance of selenium in managing cutaneous allergic response.

6 Nutraceuticals in Skin (Cutaneous) Tumors (Neoplasms)

Skin (cutaneous) neoplasms are tumors that develop in skin due to uncontrolled growths of various components of skin and associated connective tissues. Large numbers of skin tumors are recognized in various animal species. In veterinary medicine canines (dogs) are among animal species that often develop skin cancers. For example, Raditic and Bartges (2014) indicated that cancer is an important disease in dogs and accounts for 27% of all deaths in purebred dogs in the United Kingdom.

The incidence of cancer is continually rising (Ranzato et al. 2014), and chemical drugs are often prescribed for the treatment of cancer in humans and animals. However, chemical drugs possess limited therapeutic capacity for the treatment of patients with cancers consequent to the occurrence of resistance (Mao et al. 2018). Furthermore, the currently available cancer therapeutic options are expensive, and none of them are safe (Sayeed and Ameen 2015) due to

serious side effects that often appear in patients treated with chemical drugs (Mao et al. 2018). However, traditional plant-derived medicines or compounds are relatively safe (Sayeed and Ameen 2015). Henceforth, phytomedicines are increasingly gaining importance since they are used more readily and are relatively safe. Nutraceuticals rich in phytochemicals provide a promising source of compounds with chemopreventive effects because they are inexpensive and most of them show no evidence of toxicity (Ranzato et al. 2014).

In addition to inherited factors, diet sources are also reported to be closely involved in the pathogenesis of diverse neuropsychiatric disorders and cancers. Nutraceutical antioxidants, which have a wide distribution in diverse sorts of foods, fruits, vegetables, and minerals, exhibit potent neuroprotection and anticancer properties in vivo and in vitro. Currently, natural products or nutraceuticals also commonly called medical foods are increasingly employed for adjunctive therapy of patients with neuropsychiatric disorders and cancers. Nutraceuticals are reported to exert neuroprotection and suppressed tumor growth possibly through the differential modulations of redox homeostasis (Mao et al. 2018).

In dogs, nutraceuticals including some vitamins are reported to have an effect in reducing risks of certain cancers. There is evidence that growth of canine mast cell tumors is inhibited by vitamin D, and Labrador retriever dogs with low vitamin D levels have an increased risk in developing mast cell tumors (Pucheu-Haston et al. 2015). Furthermore, in Scottish terrier dogs, an inverse relationship was reported between the consumption of vegetables and the risk of developing transitional cell carcinoma of the urinary bladder (Raghavan et al. 2005). A study also showed that a combination of turmeric and rosemary extracts diminished the growth of cancer cells in vitro. Although the bioactive molecules inducing these effects are not definitely identified, it was suggested that the high concentrations of curcuminoids and carnosic acid are likely involved (Levine et al. 2016). Experimental and pharmacological trials have also demonstrated that curcumin as an anti-inflammatory agent is effective in managing and treating skin cancers among others (Fadus et al. 2017).

Retinoids are natural or synthetic derivatives of vitamin A (retinol) that exert profound effects on the growth and differentiation of many cell types both in vivo and in vitro. Retinoid is a term used to describe the entire set of vitamin A-derived compounds, including naturally occurring vitamin A, its metabolites, and synthetic analogs (Zhang and Duvic 2003; de Mello Souza et al. 2010). Retinoids suppress premalignant lesions such as oral leukoplakia, cervical dysplasia, and xeroderma pigmentosum in people and actinic keratosis in dogs (de Mello Souza et al. 2010). Retinoids have long been used alone or in combination with other agents in cutaneous T-cell lymphomas, a heterogeneous group of lymphoproliferative disorders characterized by localization of malignant T-lymphocytes to the skin (Zhang and Duvic 2003). In dogs, retinoids have been used in dermatology as a differentiation agent for cases affected by actinic keratosis, sebaceous adenitis, and benign pilomatrixomas. Few studies have demonstrated the effects of retinoids in canine cancer. Various retinoids derived from retinoic acid have long been used alone or in combination with other agents in cutaneous T-cell lymphomas. A 42% response rate to retinoid treatment has been observed in dogs with cutaneous lymphoma indicating that retinoid and rexinoid receptor-binding drugs may have an impact on the treatment of dogs with cutaneous lymphoma. Moreover, positive results have also been recorded with in vitro canine mast cells and canine osteosarcoma cell lines (de Mello Souza et al. 2010). The advantage of using retinoids as well as the other biological response modifiers is that they can be orally administered, are non-immunosuppressive, may boost immune function by inducing an antitumor response, and may induce apoptosis of the malignant T-cells (Zhang and Duvic 2003). In another study, it was indicated that hydrophobic flavonoids from *Scutellaria baicalensis* and magnolol isolated from *Magnolia officinalis* have also been reported to have antitumor activities in various tumors (Chen et al. 2013; Raditic and Bartges 2014).

7 Concluding Remarks and Future Directions

As part of the body system constantly exposed to external agents, the skin is affected by an array of abnormalities elicited by infectious and noninfectious agents and immune disorders. Nowadays, wide spread use of synthetic cosmetic products of chemical origin applied to the skin has undoubtedly increased the incidence of skin diseases including allergic dermatitis and cutaneous neoplasms. Various applications of modern medicine most of which leave unintended side effects are often prescribed to manage and treat skin diseases. Due to the undesirable side effects of modern medical treatments, there is an increased tendency to use traditional and alternative medicine, mainly nutraceuticals to prevent or minimize the rate of occurrence and also to treat clinical skin diseases. Consequently, many studies and research undertakings on nutraceuticals are progressing with encouraging results indicating the promising effects and potentials that nutraceuticals have in the future for the treatment of various dermatopathies in humans and animals.

References

Ahmed S, Othman NH (2017) The anti-cancer effects of Tualang honey in modulating breast carcinogenesis: an experimental animal study. BMC Complement Altern Med 17:208. https://doi.org/10.1186/s12906-017-1721-4

Akgun SG, Aydemir S, Ozkan N et al (2016) Evaluation of the wound healing potential of *Aloe vera*-based extract of *Nerium oleander*. North Clin Istanb 4(3):205–212

Akhtar MS, Hossain MA, Said SA (2017) Isolation and characterization of antimicrobial compound from the stem-bark of the traditionally used medicinal plant, *Adenium obesum*. J Tradit Complement Med 7:296–300

Chen MC, Lee CF, Huang WH et al (2013) Magnolol suppresses hypoxia-induced angiogenesis via inhibition of HIF-1alpha/VEGF signaling pathway in human bladder cancer cells. Biochem Pharmacol 85:1278–1287

Cortese L, Annunziatella M, Palatucci AT et al (2015) An immune-modulating diet increases the regulatory T-cells and reduces T-helper-1 inflammatory response in Leishmaniosis affected dogs treated with standard therapy. BMC Vet Res 11:295

de Mello Souza CH, Valli VE, Selting KA et al (2010) Immunohistochemical detection of retinoid receptors in tumors from 30 dogs diagnose with cutaneous lymphoma. J Vet Intern Med 24 (5):1112–1117

Di Cerbo A, Centenaro S, Beribè F et al (2016) Clinical evaluation of an anti-inflammatory and antioxidant diet effect in 30 dogs affected by chronic otitis externa: preliminary results. Vet Res Commun 40 (1):29–38

Fadus MC, Lau C, Bikhchandani J, Lynch HT (2017) Curcumin: an age-old anti-inflammatory and anti-neoplastic agent. J Tradit Complement Med 7:339–346

Fonacier LS, Dreskin SC, Leung DYM (2010) Allergic skin diseases. J Allergy Clin Immunol 125(Suppl. 2):S138–S149

Foster M, Hunter D, Samman S (2011) Evaluation of the nutritional and metabolic effects of Aloe vera. In: Benzie IFF, Wachtel-Galor S (eds) Herbal medicine: biomolecular and clinical aspects, 2nd edn. CRC Press/Taylor & Francis, Boca Raton, pp 1–41

Gasparro FP (2000) The role of PUVA in the treatment of psoriasis: photobiology issues related to skin cancer incidence. Am J Clin Dermatol 1(6):337–348

Gross TL, Ihrke PJ, Walder EJ et al (2005) Diseases of the dermis. In: Skin diseases of the dog and cat: clinical and histopathologic diagnosis, 2nd edn. Blackwell Science, Ames, pp 200–206

Kim MJ, Kim S-N, Lee YW et al (2016) Vitamin-D status and efficacy of Vitamin-D supplementation in atopic dermatitis: a systematic review and meta-analysis. Nutrients 8:789. https://doi.org/10.3390/nu8120789

Lee DS, Sinno S, Khachemoune A (2011) Honey and wound healing: an overview. Am J Clin Dermatol 12(3):181–190

Levine CB, Bayle J, Biourge V et al (2016) Effects and synergy of feed ingredients on canine neoplastic cell proliferation. BMC Vet Res 12:159

Mao XY, Jin MZ, Chen JF et al (2018) Live or let die: neuroprotective and anti-cancer effects of nutraceutical antioxidants. Pharmacol Ther 183:137–151

Maruhashi E, Braz BS, Nunes T et al (2016) Efficacy of medical grade honey in the management of canine otitis externa-a pilot study. Vet Dermatol 27:93–e27

Mazzeranghi F, Zanotti C, Di Cerbo A et al (2017) Clinical efficacy of nutraceutical diet for cats with clinical signs of cutaneous adverse food reaction (CAFR). Pol J Vet Sci 20(2):269–276

McFadden RA, Heinrich NA, Haarstad AC et al (2017) A double-blinded, randomized, controlled, crossover evaluation of a zinc methionine supplement as an adjunctive treatment for canine atopic dermatitis. Vet Dermatol 28:569–e138

McLoonea P, Warnockb M, Fyfeb L (2016) Honey: an immunomodulatory agent for disorders of the skin. Food Agric Immunol 27 (3):338–349

Nimisha DAR, Fatima Z et al (2017) Antipsoriatic and anti-inflammatory studies of *Berberis aristata* extract loaded nanovesicular gels. Pharmacogn Mag 13(Suppl 3):S587–S594

O'Neill W, McKee S, Clarke AF (2002) Flaxseed (*Linum usitatissimum*) supplementation associated with reduced skin test lesional area in horses with Culicoides hypersensitivity. Can J Vet Res 66 (4):272–277

Patel K, Patel DK (2017) Medicinal importance, pharmacological activities, and analytical aspects of hispidulin: a concise report. J Tradit Complement Med 7:360–366

Pietschmann S, Meyer M, Voget M et al (2013) The joint in vitro action of polymyxin B and miconazole against pathogens associated with canine otitis externa from three European countries. Vet Dermatol 24:439–445

Pucheu-Haston CM, Santoro D, Bizikova P et al (2015) A review: innate immunity, lipid metabolism and nutrition in canine atopic dermatitis. Vet Dermatol 26(2):104–e28

Raditic DM, Bartges JW (2014) Evidence-based integrative medicine in clinical veterinary oncology. Vet Clin Small Anim 44:831–853

Raghavan M, Knapp DW, Bonney PL et al (2005) Evaluation of the effect of dietary vegetable consumption on reducing risk of transitional cell carcinoma of the urinary bladder in Scottish Terriers. J Am Vet Med Assoc 227:94–100

Ranzato E, Martinotti S, Calabrese CM et al (2014) Role of nutraceuticals in cancer therapy. J Food Res 3(4). https://doi.org/10.5539/jfr.v3n4p18

Rosser EJ Jr (2004) Causes of otitis externa. The veterinary clinics of North America. Vet Clin Small Anim Pract 34:459–468

Sayeed B, Ameen SS (2015) Beta-Sitosterol: a promising but orphan nutraceutical to fight against cancer. Nutr Cancer 67(8):1214–1220

Tsuji PA, Carlson BA, Anderson CB et al (2015) Dietary selenium levels affect selenoprotein expression and support the interferon-γ and IL-6 immune response pathways in mice. Nutrients 7:6529–6549

Zhang C, Duvic M (2003) Retinoids: therapeutic applications and mechanisms of action in cutaneous T-cell lymphoma. Dermatol Ther 16:322–330

Nutraceuticals in Mastitis

Robert W. Coppock

Abstract

Acute and chronic forms of mastitis are the costliest disease in the dairy industry. Resistance of microbial pathogens to antimicrobials approved for clinical use is a significant threat to controlling mastitic pathogens and is a public health issue. In some countries, the cost of antimicrobial drugs reduces their usage, and ethnic remedies are being used. Organic dairies have prioritized the maintenance of mammary health and the use of nutraceuticals to prevent and treat mastitis. In some jurisdictions, dairy animals on organic farms that receive antibiotics are disqualified for life as dairy animals. There is conflicting evidence on the efficacy of nutraceuticals, homeopathy, and traditional medicine in treating mastitis and a lack of standards for evaluation of these remedies. Studies are showing that nutraceuticals can be efficacious. Phytotherapeutics generally are complex chemical mixtures and can be multifaceted in mechanisms of action. Intramammary infusions of probiotics and bacteriocins are being shown to be efficacious, and their mechanisms of action include being an immune stimulant. Immunotherapy with antibodies and immune system components can be efficacious. Intermingled treatments with nutraceuticals and pharmaceuticals can be more efficacious than either treatment alone.

Keywords

Nutraceutical · Mastitis · Cattle · Cow · Goats · Sheep · Antimicrobial · Milk · Udder · Mammary gland · Breast · Ruminants · Somatic cell count · Antibiotics · Alternative therapies · Veterinary · Herbal medicine · Probiotic · Holistic medicine · Homeopathy · Herbalism · Herbal medicine · Oregano oil · Carvacrol · α-pinene · Ginsenoside · Ginseng · Cumin · Onion · Manuka · Linseed oil · Olive oil · Tea tree oil · *Terminalia* · *Allium* · *Bunium* · *Oryza* · *Thymus* · *Triticum* · *Origanum* · *Mentha* · *Salvia* · Atractylodis · Origanum · *Escherichia coli* · *Klebsiella* · *Staphylococcus* · *Streptococcus* · *E. coli* · *Lactococcus* · *Lactobacillus* · *Serratia* · *Corynebacterium* · *Enterococci* · *Prototheca* · *Linum* · *Candida* · Chinese medicine · Knotweed · Safflower · Red sage root · Berberine · Sage · Honey · Bees wax · Calendula oil · Eucalyptus oil · Eugenol · Cinnamon · Thymol · *Eucalyptus* · *Glycyrrhiza* · *Curcuma* · *Cedrus* · *Paedaria* · *Leptospermum* · *Ziziphus* · *Panax* · Angelica · Tinospora · *Gaultheria* · *Glycyrrhiza* · *Pulsatilla* · Teat dips · Teat sealer · Mineral supplement · Vitamin E · Bacteriophages · Bacteriocins · Nisin · Lysostaphin · Dry cow treatments · Essential oils · Phytopharmaceutical · Pigs · Porcine

1 Introduction

Mastitis, inflammation of the mammary gland, is one of the most significant and costly diseases affecting lactating ruminants (Arsenault et al. 2008; Cooper et al. 2016; Aghamohammadi et al. 2018). The mammary glands, also referred to as the udder in some species, are susceptible to infection by a wide variety of microorganisms. Milk in the mammary gland is also an excellent culture media for many microorganisms (Rainard 2017). Infectious mastitis exists in subclinical and clinical forms. Treatment of clinical infections with antibiotics and other therapeutics has increased risk of recurrence especially when a bacteriological cure is not achieved (Gomes and Henriques 2016; Jamali et al. 2018). The worldwide cost to the dairy industry is estimated to be 35 billion US dollars in direct losses, and this loss does not include the loss of valuable genetics due to culling and deaths. Cows with mastitis can also have reduced fertility and metabolic dysfunctions (Ribeiro et al. 2016). The pathogenesis of mastitis has been reviewed (Akers and

R. W. Coppock (✉)
Toxicologist and Assoc Ltd, Vegreville, AB, Canada
e-mail: r.coppock@toxicologist.ca

R. C. Gupta et al. (eds.), *Nutraceuticals in Veterinary Medicine*, https://doi.org/10.1007/978-3-030-04624-8_38

Nickerson 2011; Lainesse et al. 2012; Bhattarai et al. 2018). In summary, the innate immune system is the first defense mechanisms. Pathogenesis of infection is due primarily to ascending infection, inflammation that may block the milk duct drainage system, inflammation of the alveoli with sloughing, and apoptosis of secretory cells with appearance of fibroblasts and poorly differentiated cuboidal epithelium. Acute and chronic infections generally cause some loss of secretory function. There is a reduction in milk production, and this is associated with an increase in milk somatic cell counts (SCC, primarily white blood cells). Biomarkers of mastitis include increase milk SCC, bacteria isolated in secretions from the mammary gland, bacteria in milk identified by colony plate counts, cardinal signs of inflammation in the mammary gland, and proteomic changes in mammary secretions. There is widespread use of antibiotics as dry cow prophylactics and therapeutics for mastitis. For treating mastitis in small ruminants, the preferred antimicrobial chemotherapeutic may not have regulatory approval. There are animal welfare and public health issues associated with mastitic animals in the dairy industry. Antimicrobial resistance is an increasing concern in milk-producing species and the animal-to-animal and zoonotic spread of these organisms (Holmes and Zadoks 2011; Tavakol et al. 2012; Saini et al. 2013). The route of administration of antibiotics is important in development of resistance for penicillins, third-generation cephalosporins, and macrolides with the risk being low for strategic intramammary and intrauterine administration (Nobrega et al. 2018a). A recent Canadian study showed that there is antibiotic resistance in non-aureus staphylococci isolated from milk collected from the mammary gland (Nobrega et al. 2018b). The pattern of antibiotic resistance was found to be species-dependent with *Staphylococcus gallinarum* being resistant to quinupristin/dalfopristin combination at a prevalence of 98%. *S. cohnii* and *S. arlettae* were resistant to erythromycin with a prevalence of 63 and 100%, respectively. The resistance of bacteria from milk to vancomycin, fluoroquinolones, linezolid, and daptomycin was prevalent at <1%. The formation of intramammary biofilms may also be a factor in antimicrobial resistance (Gomes et al. 2016). A public health concern is milk can be an important source of antimicrobial chemotherapeutics and antibiotic-resistant microorganisms entering the human food chain (Kromker and Leimbach 2017). These are important drivers to identify and use alternatives to antibiotics to prevent and treat mastitis in food-producing species. Organic farming and economics can also be an important driver in the regional use of nutraceuticals to prevent and treat mastitis (Mushtaq et al. 2018). There is considerable debate on the bacteria that constitute the normal flora of the mammary gland of ruminants (Rainard 2017). Bacteria isolated from the mammary gland demonstrate in vitro antibacterial activity against mastitic pathogens (Al-Qumber and Tagg 2006). A recent review of the literature on qualitative analysis of treatments for infectious mastitis other than antimicrobials concluded that alternative treatments are essentially ineffective (Francoz et al. 2017). Government regulations for organic farming vary between government jurisdictions. In the USA, a dairy animal producing milk certified as organic that receives an antibiotic treatment is banned for life as a producer of organic milk. Recommending a balanced diet using the least cost ingredients to maximize production has been the hallmark of nutritional recommendations. The use of antioxidants in the peripartum period is gaining attention in the prevention of mastitis linked with oxidative stress (Abuelo et al. 2015). The role of diet in preventing mastitis and other diseases in dairy animals is gaining more attention especially in organic farming. Organic farms have been reported to have a lower occurrence of mastitis, and the credit is given to proactive management, reduced animal stressors, better focus on mammary gland health, and feeding more forages (Hamilton et al. 2006; Ruegg 2009). Compliance with organic farming standards results in better udder health (Honorato et al. 2014).

2 Alternative Therapies

Herbal remedies have been practiced in veterinary and human medicine for more than 60,000 years (Boothe 2004). Classical plant source therapeutics include salicylates, cardiac glycosides, quinine, opium, and morphine. Alternative therapies include phytotherapy (herbalism, herbal medicine), acupuncture, homeopathy and ethnic medicine (Table 1) (Hektoen 2004; Mushtaq et al. 2018). Holistic medicine emphasizes the ability of the body to heal itself and the influence of the external environment and diet on both the cause of disease (health protection) and its important role in healing (restoration). Holistic medicine seeks a natural therapy with minimal unwanted side effects. Veterinary homeopathy is commonly used on farms producing animal-sourced organic products (Loken 2001). Herbal remedies contain a mixture of phytochemicals, and it is generally assumed this cocktail of chemicals target multiple receptors and have complex interactions important for healing processes (Spinella 2002). The current concept in pharmacology is based on specific target receptors and minimizing the number of drugs used to simply pharmacodynamics. This is contrasted to herbal medicines that have multiple lignans that target multiple receptors, a phyto-shotgun approach to impacting multiple targets (Spelman et al. 2006).

2.1 Antimicrobial Activity of Plant Extracts

Ethyl acetate extract of powered *Terminalia chebula* Retz. fruit was tested in vitro as an antimicrobial against mastitic pathogens isolated from dairy cows (Kher et al. 2018). At 500 μg/mL, the *T. chebula* extract was as efficacious as amoxicillin. Extracts of *T. chebula* are also used as an ethnic drug for urinary infections. Plants, growing in the northeast

Table 1 Examples of ethnic phytoremedies for mastitis

Condition (species)	Scientific name	Plant parts	Route of administration	Reference
Mastitis	*Gloriosa superba* L.	Crushed root tea	Oral	Upadhyay et al. (2011)
(Bovine)	*Vitex negundo* Linn.	Root juice	Oral	
(Ruminants)	*Achillea millefolium* L.; *Arctium* spp.; *Salix* spp.	Tea[a] made from aerial parts, burdock root, and willow bark with honey and salt added	Cotton cloth is dipped in warm infusion and put on udder	Lans et al. (2007)
	Teucrium scorodonia L.	Tincture	Infused into udder	
	Galium aparine L.	Infusion (steeping) made from 15 mL of cleavers in 80 mL of water	Oral	
(Bovine, ovine)	*Avena sativa* L.	Germinated *caryopses*	Poultice	Uncini Manganelli et al. (2001)
(Bovine)	*Sambucus nigra* L.	Udders—used as a topical fumigate	Topical	
(Bovine, ovine, porcine)	*Malus sylvestris* L.	May be mixed with other seeds and decoctions	Oral	
Bovine, ovine)	*Vitis vinifera* L.	Fruit, shoots mixed with vinegar	Topical	
(Bovine)	*Foeniculum vulgare* Miller.	Fruits added to hay, decoction of flowers cooked in wine	Oral	
(Bovine, ovine)	*Salix alba*	Added to decoctions of other plants	Oral	
(Ovine)	*Urtica dioica* L.	Added to udder sling	Topical	
(Bovine)	*Allium cepa* L.	200 g crushed with 2–4 flower buds of *Syzygium aromaticum* L. and oil of Brassica napus L.	Oral	Kumar and Bharati (2013)
Mastitis (Ruminants)	*Bunium persicum* Boiss.	Seeds (80 g)	Oral. Ground and mixed with wheat flour	Amber et al. (2018)
	Oryza sativa L.	Seeds (1–2 g)	Oral. Decoction (1–2 glasses)	
	Triticum aestivum L.	Fruit and seeds (500 g)	Oral. Ground and mixed with fodder	
	Allium sativum L.	Bulbs (100 g)	Oral. Ground and mixed with butter	
Prophylactic (Bovine)	*Buxus sempervirens* L.	Leaves	Used as bedding	Uncini Manganelli et al. (2001)
Galactagogue (Bovine)	*Ficus carica* L.	Leaves added to forage	Oral	

[a]0.33 cup (about 80 mL) of each herb; about 700 mL of water used

region of Pakistan and used in ethnoveterinary practices to treat clinical mastitis in cattle and buffalo, were tested in vitro for antimicrobial activity against mastitic pathogens (Amber et al. 2018). The plants studied were *Allium sativum* L., *Bunium persicum* Boiss., *Oryza sativa* L., and *Triticum aestivum* L. (Table 1). The crude extract, alkaloids, flavonoids, and saponins were dissolved in dimethyl sulfoxide and standardized at 50 mg/mL and used for determining antibacterial activity. Dilutions were used to determine the minimum inhibitory concentration. Multidrug-resistant strains of *Escherichia coli*, *Klebsiella pneumoniae*, and *Staphylococcus aureus* were used as test bacteria. The alkaloids of *Allium sativum* had the largest bacteria inhibiting zones (*K. pneumoniae* strains and *S. aureus*) when compared to crude extract, flavonoids, and saponins. The alkaloids and crude extract of *Bunium persicum* showed significantly higher inhibitory activity. The alkaloids from *Allium sativum* and *Bunium persicum* had antimicrobial inhibitions similar to sensitive antibiotics. For the alkaloids, flavonoids, and saponins from *Allium sativum*, the minimum inhibitory concentration range for all multidrug-resistant bacteria studies was 25–50 mg/mL. For *Bunium persicum* this range was 12.5–50 mg/mL, and for *Oryza sativa* and *Triticum aestivum*, the range was 25–50 mg/mL. In the plants studied, alkaloids were in the highest concentration followed by flavonoids, and the lowest level was saponins. The in vitro anti-algal activities against mastitic strains were done on the essential oils from *Thymus vulgaris* L., *Origanum vulgare* L., *Origanum majorana* L., *Mentha* x *piperita* L. (hybrid), and *Allium ursinum* L. against *Prototheca zopfii*. (Grzesiak et al. 2018a).

Marjoram oil from *Origanum majorana* had the highest antialgal activity followed by oregano oil (*Origanum vulgare*). Oregano essential oil contains carvacrol, thymol, and monoterpene hydrocarbons and has antibacterial activity. A proprietary intramammary product[1] containing 0.9 mL of oregano oil in 9.1 mL of a diluent was compared to an intramammary product[2] containing 10 g gentamycin and saline control for the treatment of clinical mastitis (Cho et al. 2015). Treatment was twice a day for 3 days, and oregano was given in single and double doses to two different groups. Study enrollment criteria were clinical mastitis that had not responded in 1 week to previous therapy, milk SCC >200,000 cells/mL, and detection of *S. aureus* and/or *E. coli* in milk. Treatment with gentamycin and oregano decreased clinical signs of mastitis and milk SCC, and *S. aureus* and/or *E. coli* were not detected in milk. In the quarters[3] receiving saline, clinical and laboratory signs of mastitis increased. The pharmacokinetics of carvacrol, thymol, and diallyl disulfide has been studied in dairy cattle (Mason et al. 2017). The approximate plasma half-life ($t_{½}$) of thymol is 1.6 h and carvacrol is 1.5 h, and no levels of diallyl disulfide could be measured in plasma. The tissue $t_{½}$ of thymol was 13.9–31.5 h and 16.9–25 h for carvacrol with the slowest depletion being for liver. Topical and intramammary administration resulted in measurable levels in plasma, kidney, and liver up to 72 h. The efficacy of *Linum usitatissimum* (flax or linseed) oil was studied in vitro and in vivo against mastitic organisms (Kaithwas et al. 2011). The linseed oil was extracted from macerated flax seeds using petroleum ether and had a specific gravity of 0.952 g/mL, 5.5% palmitic acid, 4.7% stearic acid, 19.1% oleic acid, 13.7% linoleic acid, and 57.4% linolenic acid. The antimicrobial activity of linseed oil against *S. aureus* and *Streptococcus agalactiae* was comparable to cefoperazone and exceeded cefoperazone against *Candida albicans*. Cattle selected for intramammary infusions of linseed oil had localized clinical signs of mastitis plus positive milk SCC. Treatment groups were linseed oil infusion, linseed oil infusion plus a proprietary cefoperazone[4] intramammary formulation, or a proprietary cefoperazone intramammary formulation. All treatments were given for 7 days. Milk SCC were not increased by treatments, and the SCC were reduced on study day 7 with the SCC being lower in the milk from cows treated with cefoperazone. Total microbial counts were reduced 87.7% by linseed oil treatment and 93% by linseed oil plus cefoperazone and cefoperazone alone.

[1] Eco-Mast, 10 mL/tube, Daehan New Pharm (Seoul, South Korea)

[2] Daesung Gentamicin Cream, Daesung Microbiological Labs (Seoul, South Korea)

[3] The bovine udder has four distinct mammary glands with each gland draining into one teat.

[4] 25 mg cefoperazone/mL, Mastiwock, Wockhardt Laboratories (Mumbai, India)

2.2 Traditional Chinese Medicine

The treatment of mastitis, in traditional Chinese medicine, is focused on increasing blood flow, reduction of heat, removal of toxic material, and relief of tumefaction and pain (Lu et al. 2008). The formulas used in traditional Chinese prescriptions for dairy cow mastitis generally include ingredients that increase blood flow. To investigate herbal drugs, hemorheology was studied in rabbits using the high molecular weight dextran model. Decoctions were made from giant knotweed rhizome (*Rhizoma Polygoni Cuspidati*), safflower (*Flos Carthami*), red sage root (*Radix Salviae Miltiorrhizae*), and chuanxiong rhizome (*Rhizoma Chuanxiong*). These herbal materials were purchased from a commercial company[5] and are widely used in China to cure "blood stasis." The rabbits in each group were orally administered 1.0 mL of the assigned decoction/kg body weight once a day for 7 days. Red sage root was the most potent in reducing dextran-induced blood stasis in the rabbit high molecular weight dextran-induced blood stasis model and is considered first choice in a recipe for bovine mastitis. *Atractylodis macrocephalae* Koidz. (white atractylodes) has a history of its rhizome being used in traditional Chinese medicine. It has phenolics with antioxidant properties and polysaccharides with immune-stimulating activities (Li et al. 2012; Xu et al. 2015). The concentrated and purified atractylodes polysaccharides were studied as an immune stimulant in dairy cows with an infectious mastitis or cows with SCC >500,000 cells/mL (Xu et al. 2015). Briefly, the purified polysaccharide (89.63%) (RAMP) was suspended in rapeseed oil containing sorbitan monooleate (Span[6] 80) and polyethylene glycol sorbitan monooleate (Tween[7]-80), and the final product contained 8% Tween-80 and 12, 24, and 36 mg of RAMP/mL. The irritancy of the final product was tested in one cow that had a somatic cell count <500,000 cells/mL and negative milk cultures. The mammary lymph node of this cow was injected with 36 mg of RAMP. No clinical signs or laboratory evidence of inflammation was observed. Cows selected for Experiment 1 had milk SCC >500,000 cell/mL, and cows in Experiment 2 had at least one mammary quarter that was positive for mastitic bacteria and milk SCC >500,000 cell/mL. All cows in Experiments 1 and 2 received injections of 0, 12, 24, or 36 mg of RAMP in the area of the supramammary lymph node. For Experiment 1, 3 weeks after treatment, there were temporal declines in milk SCC and milk N-acetyl-β-D-glucosaminidase, but there were no significant differences between treatment and control groups. In Experiment 2, the 32 mg RAMP/mL group, there were significant decreases in milk SCC and milk N-acetyl-β-D-

[5] Dahua Traditional Chinese Medicine Company (Nanjing, Jiangsu Province)

[6] Sigma-Aldrich

[7] Sigma-Aldrich

glucosaminidase. Composite milk sample, when compared to the control group, had significant reduction in milk SCC. There was significant reduction in bacterial infections in the majority of the infected mammary quarters. The isolated bacteria were *S. aureus*, *Streptococcus agalactiae*, *Streptococcus dysgalactiae*, *Streptococcus uberis*, coagulase-negative (-) staphylococci, and other unidentified bacteria. The chemistry of RAMP is rhamnose, arabinose, xylose, mannose, glucose, and galactose with molar ratios of 1.00:2.49:2.07:4.94:11.33:1.35. Berberine is used in traditional Chinese medicine and is reported to have both antibacterial and anti-inflammatory activities. Berberine hydrochloride (berberine) is an isoquinoline-type quandary ammonia alkaloid. It is isolated from Berberidaceae (barberry), Rutaceae (citrus), and Ranunculaceae families. The anti-inflammatory activity of berberine[8] has been studied in a mouse mastitis model (Wang et al. 2018). Briefly, 50 μL of *E. coli* lipopolysaccharide (LPS) solution (0.2 mg LPS/mL) was injected into the milk ducts of lactating mice using the two largest mammary glands of the five ventral pairs. Treatment groups were control (LPS only); three groups receiving intraperitoneal injections of LPS plus 5, 10, and 20 mg berberine/kg body weight, respectively; and a group receiving LPS plus 5 mg dexamethasone (intraperitoneally)/kg body weight. The berberine and dexamethasone were injected 1 h before LPS and 12 h after LPS administration. The effects of berberine were significant for decreasing neutrophil infiltration and decreasing the mRNA expressions and secretion of tissue necrosis factor (TNF)-α, IL-1β, and IL-6 in a dose-dependent manner. Treatment with berberine suppressed LPS-linked toll-like receptor 4 (TLR4) and NF-κB p65 activation and the phosphorylation of I-κB. The histopathology of LPS infusion was inflammatory exudates and cellular exfoliation, and these were decreased by berberine and dexamethasone treatments.

2.3 Treatment with Essential Oils

Sage (*Salvia officinalis*) essential oils were evaluated in sheep as treatment by infusion for chronic mastitis (Alekish et al. 2017). The minimum inhibitory concentration of sage essential oil to *S. aureus* was 120 mg/mL, and the minimum bactericidal concentration was 6.1 mg/mL. The milk SCC were decreased at 24 and 48 h. Oregano oil from *Origanum vulgare* L. was evaluated as a treatment for bovine mastitis associated with *E. coli* and *S. aureus* being shed in milk (Byung-Wook et al. 2015). Clinical mastitis in this study was defined as milk SCC >200,000 cells/mL and *E. coli* or *S. aureus* being shed in milk. Treatments were controls, which received intramammary infusions of either sterile saline, antibiotic treatment with 10 g of gentamycin[9] or a commercial preparation of oregano oil[10] given at single or double dose; all treatments were administered twice a day for 3 days. Treatment with saline and oregano oil resulted in swelling of some quarters. Milk SCC were decreased in the quarters treated with gentamycin and oregano oil. In the gentamycin and oil of oregano groups, *E. coli* or *S. aureus* were not detected in milk at cessation of treatment (study day 4). Treatment with gentamycin and oil of oregano also decreased the white blood cell numbers in blood. Other essential oils have been evaluated in vitro for their antibacterial activities against mastitic pathogens (Ananda Baskaran et al. 2009). The compounds[11] studied were carvacrol and thymol from oregano oil, eugenol from oil of clove, and *trans*-cinnamaldehyde from cinnamon bark extract. Test organisms were *Streptococcus agalactiae*, *Streptococcus dysgalactiae*, *Streptococcus uberis*, *S. aureus*, and *E. coli*. Of these, *trans*-cinnamaldehyde was the most effective in killing bacteria, and its killing activity persisted for 14 days. Carvacrol, thymol, and eugenol also were effective in killing the five pathogens but less effective than *trans*-cinnamaldehyde. A proprietary herbal gel[12] was studied as a treatment for subclinical mastitis (Bhatt et al. 2014). Ten grams of the herbal gel contained 200 mg *Eucalyptus globulus*, 200 mg *Glycyrrhiza glabra*, 40 mg *Curcuma longa*, 1.0 g *Cedrus deodara*, 40 mg *Paederia foetida*, and 1 g sulfur. The herbal gel was applied to the udder after the morning and evening milking for 5 days. Parameters studied were cytokine and milk SCC and bacteria colony-forming units in milk. The transcriptional activity of cytokines in milk (IL-6, IL-8, IL-12, GM-CSF, IFN-γ, and TNF-α) was significantly increased on study day 5 and significantly reduced on study day 21. The shedding of bacteria in milk significantly decreased on study days 5 and 21, but there were no reductions in milk SCC.

2.4 Honey

Honey, a plant source food-store made by honey bees, is one of the oldest reported traditional medicines (Mandal and Mandal 2011). The antibacterial activity of honey is variable and is linked to its floral species makeup (Stagos et al. 2018). Honey is a nutraceutical prepared by bees from selected sugar-containing plant material. The honey bee (*Apis mellifera*) is the predominate producer of honey used in traditional and allopathic medicine. However, honey made

[8] Control of Pharmaceutical and Biological Product (Beijing, China)

[9] Gentamicin Cream, Daesung Microbiological Labs (Korea)

[10] Eco-Mast 10 mL/tube containing 0.9 mL oregano oil, Daehan New Pharm (Korea)

[11] Sigma-Aldrich Chemical Co. (St Louis, MO)

[12] Mastilep, Dabur Ayurvet Ltd. (Ghaziabad, India)

by other bees is also used and is credited for substances not found in honey made by *Apis mellifera* (Cortopassi-Laurino et al. 2006). A branch of alternative medicine known as apitherapy is focused on the use of honey as a remedy. The chemistry of honey is in part determined by the plant source of the nectar. For example, honey from *Leptospermum scoparium* (manuka honey) has demonstrated antibacterial activity against Gram-positive, Gram-negative, aerobic, and anaerobic bacteria. Unresponsive wounds and infections have rekindled an interest in the use of honey as a therapeutic. The antibacterial activity of Egyptian honey and essential oils from black cumin and onion was studied in mastitic pathogens isolated from sheep and goats (Abdalhamed et al. 2018). The authors concluded that Egyptian honey and essential oils from onion and black cumin have a strong antibacterial activity against mastitic bacteria isolated from sheep and goats. The essential oils had more antibacterial activity against Gram-positive and Gram-negative bacteria when compared to honey. The honey from non-stinging bees in Australia has antibacterial activity against Gram-positive and Gram-negative bacteria and is used in traditional medicine (Boorn et al. 2010). The pH is 3.85, 25% water, and reducing sugar activity of 54.2/100 g. The plant source of honey precursors is important in its antibacterial activity (Almasaudi et al. 2017). The antibacterial activities of honey from manuka, *Nigella sativa* L., and Sidr (*Ziziphus spina-christi* L.) flowers against *S. aureus* were studied in vitro. Honey from the manuka flower showed a bactericidal effect on both methicillin-resistant and methicillin-sensitive *S. aureus*. Honey from the Sidr and *N. sativa* flowers had a bacteriostatic effect. The antibacterial activity of honey is credited to the hydrogen peroxide, polyphenolic content, and undefined proteinaceous compounds.

2.5 Ginseng Root

Ginseng root from *Panax ginseng* Meyer. has been used as a phytopharmaceutical in traditional Oriental traditional medicine for over 2000 years. It is considered one of the most valuable herbs in Korea, Japan, and China. Ginseng is known to contain polyacetylene, ginsenoside, acid polysaccharide, insulin-like acid peptide, and anti-oxidative aromatic compound (Choi 2008). The biological response modification of ginseng Rg_1 extract was studied in a mouse model of induced *S. aureus* mastitis (Silvestrini et al. 2017). The extract was 27% Rg_1 ginsenoside[13] and did not contain endotoxins (<0.05 ng/mL limit of detection). Irritation tests were done to determine the tolerance of intramammary Rg_1 by infusing 100 μL of 3 mg/mL, 10 mg/mL, or 50 mg/mL of Rg_1 into the teat ducts of lactating mice. There was no observable macroscopic pathology, but there was histopathology. For the mammary glands receiving infusions of 3 and 10 mg Rg_1/mL, some neutrophilic and mononuclear exudates were observed in the interlobular stroma and in the lumen of the alveoli at 6, 24, 48, and 72 h post-infusion. The infusion of the 50 mg RG1/mL solution caused, at 24 h, a moderate exudate of neutrophils, macrophages, and lymphocytes. At 48 h, there were neutrophils, eosinophils, and mast cells surrounding the alveoli and milk ducts. Seventy-two hours later there were massive numbers of neutrophils, eosinophils, and mast cells in the interstitium and in the lumen of milk ducts and alveoli. Mice in Group 1 receive milk duct infusions of 100 μL sterile saline followed 72 h later with 100 μL intraductal inoculation of 10^6 colony-forming units of *S. aureus* solution. Mice in Group 2 received 100 μL of 50 mg GR1/mL followed 72 h later with an intraductal infusion of 100 μL *S. aureus* solution. Mice in Group 3 received an intraductal infusion of 100 μL *S. aureus* solution. The pretreatment with Rg_1 significantly increased clearance of *S. aureus*. Rg_1 infusion significantly increased mammary gland expression of mRNA expression for IL-1α, TNF-α, TLR2, and TLR4 and the secretion of NF-κB-p65. This study demonstrated the potential for ginseng extract to act as a biological response modifier by increasing the immune response to *S. aureus* infection in the mammary gland. A efficacy study was done on Rg_1 in dairy cows for eliminating subclinical *S. aureus* mastitis (Hu et al. 2001). Cows with clinical mastitis were injected subcutaneously with 8 mg of Rg_1 for 6 days. Control cows received injections of sterile saline. Treatment with Rg_1 tended to decrease SCC/mL of milk and the numbers of *S. aureus*-infected quarters. The in vitro phagocytosis and oxidative burst were significantly increased 1 week after RG1 treatment regimen. Another study on ginseng extract in dairy cattle in late lactation showed that the ginseng (Indena SPA[14])-induced inflammatory reactions would likely favor removal of organisms causing chronic mastitis (Baravalle et al. 2011). The 23.9% ginseng extract consisted of protopanaxatriol ginsenosides Rg_1, R_f, and R_e, calculated as Rg_1, and protopanaxadiol ginsenosides R_c, R_d, Rb_2, and Rb_1 calculated as Rb_1. The final solution contained 3 mg ginseng extract/mL in 0.89% saline. The experimental unit was a mammary quarter, which was grouped as control (no injections), saline injection, and 10 mL of the 3 mg ginseng extract/mL. There was cessation of milking following the intramammary infusions. Milk was collected at 24, 48, and 72 h following treatment, and the cows were slaughtered 7 days after treatment and mammary tissues examined by histopathology, histochemistry, and Western blot procedure. At 24 h, moderate swelling was observed in the ginseng extract-treated quarters. The

[13] Indena®, SpA (Milan, Italy)

[14] Indena SpA (Milan, Italy)

mammary gland pro-inflammatory TNF-α was significantly increased in quarters receiving the ginseng extract treatment as was the number of monocytes/macrophages (determined with CD14 antibody). A follow-up study on ginseng extract (Indena SPA®) provided insights into the mechanisms of actions (Baravalle et al. 2015). Cows with laboratory and clinical signs of mastitis at the termination of milking were used on the study. The experimental unit was a mammary gland quarter. Quarters were infused with 10 mL of a 3 mg ginseng extract/mL extract, saline, or no treatment. Cows were not milked after infusion. This study showed that ginseng extract stimulates the expression of TRL2 and TRL4 in mammary tissue.

2.6 Candidate and Proprietary Herbal Remedies

Tinospora cordifolia has traditional history as a phytoremedy in human and veterinary medicine and has antibacterial, immunomodulatory, and antioxidant effects. Hydromethanolic (50:50) extract of *Tinospora cordifolia* Wild. was studied as a remedy in mastitic cows (Mukherjee et al. 2010). Cows in groups 1 and 2 did not have laboratory or clinical evidence of mastitis, and cows in groups 3 and 4 had had evidence of clinical mastitis and were shedding pathogenic bacteria in their milk. For groups 2 and 3, a solution of 100 mg of powdered *Tinospora cordifolia* extract diluted to 7.5 mL in sterile phosphate buffer was administered intramammary for 5 days, and group 4 was administered phosphate buffer without *T. cordifolia* extract. Infusion of the extract initially increased milk SCC, and then milk SCC were significantly reduced on study day 15. The total bacterial counts in milk were reduced on study day 3. Infusion of *T. cordifolia* extract increased the phagocytic activity of polymorphonuclear cells and increased their lysosomal enzymes in milk. In cows treated with *T. cordifolia* extract, the level of interleukin-8 increased. This study shows that the hydromethanolic extract of *T. cordifolia* is a candidate phytopharmaceutical for the treatment of bovine mastitis.

A proprietary product (Phyto-Mast[15]) containing extracts of *Angelica sinensis*, *Gaultheria procumbens*, *Thymus vulgaris*, *Glycyrrhiza uralensis*, vitamin E, extracted thymol, methyl salicylate, glycyrrhizin, and α-pinene was evaluated as a treatment for bovine mastitis (Pinedo et al. 2013). In Colorado (USA), from February to September, dairy cows on one organic farm with clinical mastitis were enrolled in the study, and cows with systemic signs (hyperpyrexia) were excluded. Mastitis was scored on a 1–3 scale with number 3 being the most severe. Treatment cows, after milking and striping, received 15 mL intramammary infusion of the proprietary product every 12 h for 3 days. Control cows were milked and stripped and did not receive any infusion. Milk samples for microbiology were taken before treatment and on study days 14 and 48. There were no significant differences between groups for parity, days in milk, and mastitis severity scores occurring before treatment. Pathogens identified in milk were 10.3% *S. aureus*, 20.1% *E. coli*, and 40.3% coagulase (-) streptococci, and there were no differences between groups for pretreatment occurrences of *S. aureus* and *E. coli*. Two cows in each group were removed from the study because of systemic signs of mastitis. There were no differences between groups for bacteriological cures and clinical cures. There was a significant effect in the treated group for reduced milk SCC after clinical mastitis. A pharmacokinetic study on this phytoceutical (Phyto-Mast) in lactating goats (alpine genetics) showed thymol was absorbed systemically following one 5 mL intramammary treatment (McPhee et al. 2011). After intramammary treatment, thymol was detected in plasma at 15 min and in milk 12 h later and was not detected in plasma 4 h later. Methyl salicylate was not detected in milk. Phytopharmaceutical mastic products were tested for their antibacterial actions in milk (Mullen et al. 2017). These products were intramammary Phyto-Mast, topical Uddersol,[16] and intravulvar Dr. Paul's CEG tincture.[17] Methods used were the Delvotest®-P[18] and Charm® SL Beta-lactam.[19] None of these phytopharmaceuticals gave a positive test in milk at concentrations expected to occur in raw milk.

3 Dry Cow Treatments

Dry cow therapy is treating dairy animals with medications and teat sealers at the end of lactation (drying off). Mullen et al. (2014) reported a multi-year study, comparing proprietary antibiotic dry cow treatment to dry cow treatments with proprietary herbal products. The first treatment group was intramammary antibiotic (penicillin–dihydrostreptomycin[20]) and a bismuth subnitrate[21]-based teat canal sealant. A second group was treated with a proprietary phytopharmaceutical (Phyto-Mast), the third group received Phyto-Mast and a phyto-based teat canal sealant (Cinnatube),[22] and the fourth group did not receive any treatment. Antibiotic treatment was limited to the experimental farm because of the inclusion of organic dairy farms. The production data was standardized to 305-day mature equivalent lactation. Indicators of effect were change in milk somatic cell score from dry-off sample to freshening for cow and quarter data, changes in milk microbiology from dry-off to subsequent

[15] Phyto-Mast®, Penn Dutch Cow Care (Narvon, PA)

[16] Ralco Animal Health (Marshall, MN, USA)

[17] Cayenne, echinacea, and garlic (Arcadia, WI, USA)

[18] https://www.dsm.com

[19] https://www.charm.com

[20] Quartermaster, Zoetis (Florham Park, NJ)

[21] Orbeseal, Zoetis (Florham Park, NJ)

[22] New AgriTech Enterprises (Locke, NY)

lactation and cull rate. Comparing the pre- and posttreatment lactations, no significant differences were observed in milk production, milk SCC, and cure rates (19% infected quarters at dry off). The rate of new infections was not significantly different except the Cinnatube significantly decrease reinfections (15 ± 7%) compared to controls (35 ± 11%). Overall, the herbal dry cow treatments were similar to antibiotic therapy, and no side effects were observed with the herbal products. Active ingredients in Cinnatube are olive oil, tea tree oil, beeswax, calendula oil, and eucalyptus oil.[23] A proprietary teat sealer (intra-teat canal) was compared to homeopathic drying off treatments (per oral) for preventing a recurrence of mastitis (Klocke et al. 2010). Eight homeopathic remedies were selected as a best fit from the Veterinary Materia Medica.[24] The homeopathic remedies represented were mercurius solubilis, lachesis mutus, sulfur, calcium carbonicum, calcium phosphoricum, *Pulsatilla pratensis*, sepia, and silica. The homeopathic remedies were given orally over the last 5 days of lactation. Treatment groups were untreated cows, teat sealer, and homeopathic remedies. No clinical mastitis was observed during the dry period. During the 100 days following calving, eight clinical cases of mastitis were observed, and the occurrences were not significantly different between treatment groups. The occurrences were 3% for the untreated control group, 9% for the homeopathic group, and 11% for the teat sealer group. Eighty percent of the quarters were culture negative, and there were not differences between groups.

4 Homeopathy

Homeopathy, a medical system based on like cures, is encouraged by the European Union Regulations for organic farming and is commonly used on organic dairy farms. It is estimated that in parts of Europe, 34–51% of occurrences of clinical mastitis are treated by homeopathic remedies (Keller and Sundrum 2018). Organic agriculture in Europe encourages the use of homeopathic products before the use of conventional medications. For mastitis, dairy farmers intermingle conventional veterinary therapies with homeopathy. Their choice of therapies is linked to experience, clinical efficacies, severity of the disease, costs, and a desire to use fewer synthetic chemicals in agricultural production. Studies on the efficacy of homeopathy treatments are difficult because practitioners of homeopathy vary in their choices of remedies. There are differences in research findings on the efficacy of homeopathy in the treatment of ruminant mastitis (Mathie and Clausen 2014). A randomized double-blind study in Germany found no difference between homeopathy treatments and placebo (Ebert et al. 2017). In a Norwegian study, herds using homeopathy therapies were not significantly different in milk yield, culling rates, mastitic pathogens, bulk milk SCC, and dry mammary quarters (agalactia due to disease) (Hektoen 2004). A study in Norwegian dairy cows, using a stratified semi-crossover design, found that there were no differences between homeopathic and antibiotic treatment on study days 7 and 28 (Hektoen et al. 2004). Treatment groups were placebo, homeopathic remedy, and a standardized antibiotic regimen. Comparison was done on Score 1 and Score 2 with Score 1 being tallies of acute signs of mastitis and Score 2 being tallies of chronic signs of mastitis. Observable changes in milk without other signs of intramammary inflammation were defined as mild mastitis. The presence of acute inflammation of the mammary gland without systemic clinical signs was defined as moderate mastitis, whereas severe mastitis was defined as moderate mastitis plus systemic signs of disease, e.g., elevated body temperature, etc. For all groups, Score 1 was significantly reduced by study day 7. For percent reductions in Score 1 for days 0–7, there was no significant differences between homeopathic and placebo. There was a significantly larger reduction in percentage Score 1 for cows on the antibiotic regimen. For Score 1, comparisons of the area under the curve (AUC) for the interval of days 0–28 showed the homeopathy was not significantly different from the antibiotic group and the placebo group. The AUC for the antibiotic group was significantly different from the placebo group. For all groups, Score 2 percentage was significantly reduced from day 0 to day 28. From day 0 to 28, the homeopathic group showed the largest percentage reductions in Score 2 followed by the antibiotic group and placebo group, but the groups were not significantly different from each other. The AUCs for the groups were not significantly different from each other. The homeopathy treatment had more microbiological negative outcomes. The resistance of Gram-positive organisms to antibiotics was not considered a factor in this study. A study using 180 lactating dairy cows (milk yield of 6500–10,000 kg of milk/cow/year) with clinical mastitis[25] found that antimicrobial or alternative remedies should be based on milk pathogens identified (Keller and Sundrum 2018). Cows meeting the criteria of mastitis for homeopathic treatment were repertorized individually according to Hahnemann's theory and standardized using a software repertory of mastitic remedies and choices made by a veterinary homeopath. Antibiotics were commercial mastitic products used according to label and the German guidelines for prudent veterinary use of antimicrobial pharmaceutics. Milk samples were collected before treatment and on study days 7, 14, and 28 and tested for the pathogens and the milk SCC determined. Isolated pathogens were *S. aureus*, *Streptococcus uberis*, *Streptococcus dysgalactiae*, *E. coli*, *Klebsiella* spp., *Enterococcus* species, *Serratia* spp., other coliform bacteria, *Streptococcus*

[23] http://newagritech.com/

[24] Steingassner HM (1998) Homoopathische Materia Medica fur Veterinarmediziner (Wien: Wilhelm Maudrich, Vienna)

[25] Criteria of International Dairy Federation (Brussels, Belgium)

dysgalactiae, other aesculin-positive streptococci, coagulase (-) staphylococci, *Corynebacterium bovis*, and yeasts. On day 7, cows treated with antibiotics had 81%, homeopathic remedies 43%, and controls 45%, respectively, elimination of mastitic pathogens. Similar results were observed on study day 14. On day 28, the cows treated with antibiotics had the higher bacteriological cures and the lowest nonresponsive rate. In cows with clinical mastitis and negative pretreatment milk cultures, there were no differences between treatment and control cows. Decrease in milk SCC was 39%, 34%, and 27%, respectively, for antibiotic, homeopathic, and control groups. Total cure rate did not differ between treatment methods. Cows that achieved a somatic cell count of 100,000 cells after treatment were independent of treatment strategy. There was an increase in the number of cows in the antibiotic treatment group that became nonresponsive to treatment. There was evidence that antibiotic treatment of infections caused by *E. coli* is contraindicated. For non-*E. coli* infections, antibiotics were the best treatment for udder recovery.

5 Probiotics, Bacteriocins, and Bacteriophages

5.1 Background

There is evidence that the lactating mammary gland can have its own unique microbiome. The emerging concept is that milk as it leaves the nipple is not sterile and is an important source of bacteria for "building" the gut microbiome of the neonate (Fernandez et al. 2013; Gomez-Gallego et al. 2016). Some authors view breast milk as a source of probiotics for the infant. The emerging concept for infectious mastitis in humans is that a cause can be an upset in the microbiome of the mammary gland. Factors influencing the mammary gland microbiome of lactating women include mode of delivery (vaginal vs. cesarean section), previous antimicrobial chemotherapy, stage of lactation, gestational age, body mass index, concurrent pathophysiology, the time of day the milk sample is collected, and geographic location (Gomez-Gallego et al. 2016; Kumar et al. 2016). There is evidence that the secretory mammary gland of ruminants does not have a unique microbiome (Rainard 2017). The mammary-associated microbiome of ruminants may exist at the teat orifice and in the teat canal. For example, suckling of ewes by lambs dynamically changes the microbiota of the teat duct and can transfer infectious organisms (Gougoulis et al. 2008). The multiple small milk ducts exiting at the nipple could be reservoirs for the human mammary microbiome. Studies of the microbiome associated with each species of animal are influencing medical recommendations and altering the criteria for prescribing antibiotics in the management of microbial infections.

The intramammary formation of biofilms by coagulase (-) staphylococci, *S. aureus*, *E. coli*, and *Enterococcus faecalis* is likely important in chronic and subclinical mastitis (Olson et al. 2002; Gomes and Henriques 2016; Gomes et al. 2016). There is evidence that when a biofilm is formed, there is less expression of virulence factors that excite clinically evident intramammary immune responses (Le and Otto 2015). Sessile bacteria in biofilms can be 10–1000 times less sensitivity to antibiotics that the planktonic bacteria living outside the biofilm. Bacterial gene expression of virulence can be down regulated in the biofilm versus the virulence gene expression in non-biofilm planktonic forms. This is evidence that strains of *S. aureus* isolated from animals in the same herd can produce biofilms with uniquely differing compositions (Ceotto-Vigoder et al. 2016). *Lactobacillus* spp. can inhibit the formation of biofilms by species of *Streptococcus*, and epigallocatechin gallate in green tea is effective against formation of biofilms by some organisms (Yang et al. 2012). The polysaccharide intercellular adhesin/poly-N-acetylglucosamine is important in the formation of biofilms along with proteins. Biofilms provide pathogens with a mechanism that disrupts immune defenses and decrease the susceptibility of pathogens to antibiotics. Some bacteriocins are effective against biofilms. Eighteen strains of *S. aureus* isolated from cattle were found to produce biofilms (Ceotto-Vigoder et al. 2016). Eight of these strains produced distinctive biofilms and were selected for determining their sensitivity to nisin and lysostaphin. For planktonic *S. aureus*, the minimum inhibitor concentration of nisin ranged from 15.6 to 500 μg/mL and 3.9 to 50 μg/mL for lysostaphin. Detachment of the biofilm and death of sessile cells were observed at 0.4 μg lysostaphin/mL, and 100 μg nisin/mL caused notable reduction in *S. aureus* viability. This study suggests that lysostaphin alone or in combination with nisin is candidate for control of *S. aureus* bovine mastitis. The use of plant extracts in inhibiting biofilm formation is a promising area for combination therapeutics. *S. aureus* "hides" intracellularly from the immune system, and this is considered to be one of the mechanisms for the existence of chronic infectious mastitis.

5.2 *Lactococcus lactis* and Bacteriocins

Antibiotic resistance has increased the interest in bacteriocins and bacteria producing bacteriocins. Bacteriocins are natural peptides that are ribosomally synthesized and posttranslationally modified to augment functionality (Allen et al. 2014). This group of peptides are produced by Gram-positive lactic acid bacteria (Bedard and Biron 2018). Some bacteriocins, e.g., nisin, have governmental approval for use by the food industry. Non-aureus staphylococci are heterogeneous group of approximately 50 species that are commonly isolated from aseptically collected milk samples. This is evidence that the bacteriocins produced by the resident non-aureus staphylococci protect the mammary gland from mastitic pathogens by the production of bacteriocins. The antibiotic resistance of *S. auras* isolated from dairy cows with subclinical mastitis was studied in eastern

Poland (Szweda et al. 2014). The sensitivity of *S. auras* was tested against 20 antibiotics and the bacteriocins lysostaphin and nisin. The decreasing order of antibiotic resistance was streptomycin > β-lactams > amoxicillin > ampicillin. All of the isolates resistant to amoxicillin and ampicillin were also resistant to penicillin. The minimum inhibitory concentration for lysostaphin was 0.008–0.5 μg/mL, and all the *S. auras* isolates were considered to be sensitive. For nisin, 18/39 *S. auras* isolates were considered to be above 32 μg/mL and considered to be resistant. A study in Flanders region of Belgium found that herds participating in a veterinary herd health management program had decreased overall use of antibiotics for mastitis control (Stevens et al. 2016). Selectively drying off cows and treating cows with homeopathic substances also decreased antibiotic usage. The use of critically important antibiotics (fluoroquinolones and third- and fourth-generation cephalosporins) was associated with the way subclinical mastitis was managed. Studies provide evidence that mammary gland-sourced non-aureus staphylococci genomes provide evidence that >21% of these isolates are possible producers of bacteriocins (Carson et al. 2017). *Lactococcus lactis* strain V7, a lactic acid bacterium isolated from healthy cows in Brazil, was studied in bovine mammary epithelial cell culture as pathogenicity modifiers of *S. aureus* and *E. coli* (Assis et al. 2015). V7 *L. lactis* inhibited the internalization of *S. aureus* and *E. coli* into the epithelial cells but had limited activity against the adhesion of *S. aureus* to cell surfaces. Also, it did not inhibit the adhesion of *E. coli* to epithelial cells. The V7 *L. lactis* itself induced production of CXCL8 cytokine which increased the CXCL8 response to *E. coli*. *Lactobacillus* spp. have been isolated from dairy cattle and studied as candidate probiotics for prevention and treatment of bovine mastitis (Espeche et al. 2012; Frola et al. 2012). A study on the antagonistic activity of *Lactobacillus perolens* CRL 1724 and *Lactobacillus plantarum* CRL 1716 showed that *L. perolens* CRL 1724 was superior in growth antagonizing co-aggregation of mastitic pathogens (Frola et al. 2012). CRL 1716 also had greater adhesion index to mammary epithelial cells. The teat canals of two lactating cows were infused with 10^3 and 10^6 colony-forming units of *L. perolens*. At 24 h after inoculation, no clinical signs of mastitis were observed and a twofold increase in milk SCC was observed. Since the 10^6 colony-forming units were well tolerated, nine quarters in three cows were inoculated. There was a significant increase in milk SCC at 24 h and 48 h and then returned to pre-inoculation values. At study day 15, 22% of the quarters continued to shed *L. perolens* CRL 1724. A study in a mouse mastitis model showed that *Lactobacillus lactis lactis* LMG[26] 7930 infusion into the mammary gland was unpredictable in the severity of the resulting mastitis (Camperio et al. 2017). The histopathology of intramammary inoculation of *Lactobacillus perolens* CRL 1724 was studied in dairy cattle (Frola et al. 2013). Dairy cows in late lactation (average of 14 kg of milk/day), with no clinical and laboratory evidence of mastitis, were inoculated intramammary after the evening milking with one million colony-forming units of *L. perolens* CRL 1724 in 1 mL. The unit of study was a mammary quarter with three quarters being infused and one quarter used as control. Following infusion of 1 mL of 10^6 colony-forming units of *L. perolens* CRL 1724 per quarter, there were no physical signs of mastitis or changes in the appearances of milk stripped from the udder. The milk SCC increased to 4.5×10^6 cells/mL and returned to 10^6 cells/mL in 7 days. Histopathology was increased as was neutrophilic exudate in the epithelium of the teat cistern and congestion was observed. There were colonies of Gram-positive bacteria adhered to the cistern epithelium. The immune response following the intramammary infusion of *Lactococcus lactis* DPC 3147, a food-grade organism, was studied in dairy cattle (Beecher et al. 2009). This strain of *L. lactis* also produces the lacticin 3147 bacteriocin. Following intramammary infusion, *L. lactis* was recovered in milk up to 72 h, and no *L. lactis* was recovered from the mammary quarters that were not infused with live bacteria. At 7 h post-infusion with *L. lactis*, signs of inflammation were observed including swollen mammary gland, elevated body temperature, and elevated milk SCC, and clots were observed in the milk. All infused cows continued to have signs of udder inflammation at 7 days post-infusion. A notable increase was observed in immune gene expression, and these were for interleukin (IL)-1b and IL-8, with highest expression corresponding to peaks in milk SCC. A study in dairy cattle with chronic or acute mastitis compared mammary infusions of *L. lactis* DPC3147 (food grade) to infusions of antibiotic approved for intramammary use (Klostermann et al. 2008). The antibiotic infusion[27] contained amoxicillin (200 mg), clavulanic acid (50 mg), and prednisolone (10 mg). In treatment of chronic mastitis, live cultures of *L. lactis* DPC3147 were efficacious in treating infectious mastitis (*Staphylococcus aureus*) with 7 infected quarters becoming pathogen-free compared to 5 of the 11 quarters treated with antibiotic. In the treatment of acute mastitis, 15/25 cases *L. lactis* DPC3147 and 18 of 25 cases treated with antibiotic did not show clinical signs following treatment. A concurrent study investigated the mammary gland immune response to *L. lactis* DPC3147 (Crispie et al. 2008). Compared to control mammary quarters, the quarters infused with *L. lactis* DPC3147 had a large increase in milk leukocytes. The recruitment of leukocytes in the quarters treated with antibiotic treatment (formulation contained prednisolone) was significantly less. Infusion of quarters with heat-killed *L. lactis* DPC3147 produced an immunocyte response similar to the live culture. Milk amyloid A and haptoglobin were increased by infusion of

[26] BCCM/LMG Bacteria Collection (Belgium)

[27] Synulox, Pfizer Animal Health (New York, USA)

both the live bacteria and freeze-dried bacteria with the highest response occurring with infusion of the freeze-dried cells.

Lactococcus lactis LMG 7930 was studied in vitro as a candidate probiotic for mastitis control (Armas et al. 2017). The *L. lactis* LMG 7930 strain is a known nisin producer and is used in the production of Swiss cheese. This strain was shown to be sensitive to antibiotics, have medium surface hydrophobicity, not accept electrons, have low auto-aggregation, and have no co-aggregation ability with pathogens. The strain has antagonism against many microbial pathogens isolated from the mammary glands of sheep and cattle. In the bovine mammary epithelial cell line BME-UV1d, *L. lactis* LMG 7930 was adhesive with epithelial cells and had low internalization rate. In a study on the efficacy of live cultures of *Lactococcus lactis* in Italian ewes, mammary infusions of *L. lactis* caused transient clearance of mastitic pathogens and *L. lactis* itself caused mild to moderate mastitis (Mignacca et al. 2017). Live culture was chosen because it has increased immunomodulatory activities compared to heat-killed cells and cell-free extracts. In mammary glands infected with coagulase-negative staphylococci and treated with *L. lactis*, 92% were negative for coagulase (-) staphylococci 3 days after treatment and coagulase (-) staphylococci reappeared in milk cultures after treatment was stopped (day 7). This is evidence that *L. lactis* were unable to colonize the mammary gland. The milk SCC increased with infusion of *L. lactis*. The *L. lactis* infusions were ineffective against *Staphylococcus aureus*. However, IL-1β, IL-8, and chemokine receptor *CXCR1* expression was increased on the average of 7000-fold, 4400-fold, and 2700-fold, respectively, and the increases corresponded to peaks in milk SCC.

5.2.1 Nisin

The bacteriocin nisin, produced by *L. lactis*, has been studied as a treatment for clinically evident mastitis in dairy cows (Cao et al. 2007). Milk samples were collected immediately before treatment and 1 and 2 weeks after treatment for bacteriology and milk SCC. Intramammary infusions were 2.5 million international units (IU) of nisin (nisin Z[28]) or 800 mg gentamycin.[29] The clinical cure rate was 90.2% and 91.1% for nisin and gentamycin, respectively. The *S. aureus* bacteriology cure rate at 2 weeks was 6/11 for nisin and 2/6 for gentamycin, and for *Streptococcus agalactiae* the bacteriology cure rate was (15/18) for nisin and 11/22 for gentamycin. Milk SCC at 2 weeks were not statically different between treatment groups but had an apparent trend to be higher for the nisin-treated quarters. For *S. aureus*, the resistance rate to gentamycin was 35.3% and penicillin 82.5%, and there was no observed resistance to nisin. Inhibition of milk fermentation by lactic streptococci was 36 h for nisin and 72 h for gentamycin. The irritation of nisin Z was studied in a cow that did not have clinical and laboratory evidence of mastitis (Wu et al. 2007). Three quarters were injected with 1.25 million, 2.5 million, or 5.0 million IU of nisin in 20 mL volume, and one quarter was injected with 20 mL of sterile saline. The milk SCC and N-acetyl-beta-D-glucosaminidase (NAGase, an indirect measure of milk SCC and a possible measure of epithelial damage) tended to increase with the IUs of nisin infused into the mammary gland. Five million units of nisin Z caused clinical signs of mammary inflammation. The efficacy of nisin Z (2.5 million IU) as a treatment for subclinical infectious mastitis was also studied in 90 cows. Bacteria isolated from the mammary glands were *S. aureus*, coagulase (-) staphylococci, and *Streptococcus agalactiae* at 27.8%, 31.1%, and 33.0% of the cows, respectively. Unspecified bacteria were isolated from 7.8% of the cows. The treated cows received intramammary infusions of 2.5 million IU of nisin Z/day (morning) for 3 days, and control cows were not treated. Milk SCC and NAGase were reduced with treatment. Nisin Z treatment reduced the intramammary infections by 43.5% and 65.2% at week 1 and 2, respectively. Treatment with nisin Z resulted in bacteriologically cures for *S. aureus* and *Streptococcus agalactiae* at 50% and 90.9%, respectively. Fifteen percent of the control cows had bacteriological recoveries, and no decrease was observed in milk SCC. There were 20 isolates of *S. aureus*, and the resistance to penicillin, gentamicin, cefamezin, norfloxacin, and sulfamethoxazole–trimethoprim combination was 80%, 45%, 5%, 75%, and 90%, respectively. Nisin Z was detected in milk at 75.8 ± 36.5 IU/mL and 5.7 ± 7.3 IU/ml at 24 and 48 h, respectively, after termination of therapy. The minimum inhibitory concentration of nisin Z for *S. aureus* was 75.5 ± 70.8 IU/mL. The efficacy of nisin Z[30] was studied in combination with a bacterin made from *S. aureus* strain CQ399RP (Guan et al. 2017). The criteria for chronic mastitis were $\geq 5 \times 10^5$ white blood cells/mL of milk and one quarter infected with *S. aureus*. Cows (75) meeting study criteria were randomized into treatment groups. Group 1 received an injection of bacterin into the supramammary lymph node 1 week before the start of nisin Z infusions and a booster 1 week after nisin infusions. Nisin Z (2.5 million IU) was infused into the mammary gland once a day for three consecutive days. Group 2 received the three nisin Z infusions, and Group 3 consisted of untreated control cows. For Group 1, bacteriological cures were 68.0%, 72.0%, and 72.0%, respectively, at 2, 4, and 6 weeks after treatment and 44.0%, 40.0%, and 40.0%, respectively, for Group 2. Nisin A[31] and cefazolin[32]

[28] Zhejiang Silver-Elephant Bioengineering Co., Ltd. (Tiantai, Zhejiang, China)

[29] Jilin Animal Health Products Co., Ltd. (Jilin, China)

[30] Zhejiang Silver-Elephant Bio-Engineering Co., Ltd. (Tiantai, China)

[31] Omu Milk Products Co., Ltd. (Fukuoka, Japan)

[32] Meiji Seika Pharma Co., Ltd. (Tokyo, Japan)

combination was studied in vitro as a possible treatment for mastitis (Kitazaki et al. 2017). Nisin A in combination with cefazolin showed synergistic interactions against *S. aureus* and *Enterococcus faecalis* and additive interactions against *Staphylococcus intermedius*, *Streptococcus agalactiae*, *Streptococcus dysgalactiae*, and *E. coli*. The organisms were isolates from cases of clinical and subclinical mastitis in the Fukuoka Prefecture of Japan, and the antimicrobial interactions were determined in a checkerboard assay.

5.3 Experience in Human Medicine

Women that have previously had mastitis are at increased risk for developing mastitis in subsequent lactations. *Lactobacillus salivarius* PS2 was studied as an orally administered probiotic in women that experienced mastitis in a previous lactation (Fernandez et al. 2016). Women in the treatment group received 9 $\log_{10}$ colony-forming units of *L. salivarius*/day from week 30 of pregnancy until delivery. The number of women that developed mastitis was significantly lower in the treatment group. Women volunteers with non-abscessed staphylococcal mastitis refractory to cloxacillin, clindamycin, clindamycin, amoxicillin/clavulanic acid, and erythromycin were selected for a double-blind study using nisin isolated from *Lactococcus lactis* ESI 515 (Fernandez et al. 2008). Criteria were pain during breastfeeding, fissures in the nipple and surrounding area, staphylococcal milk counts $>4\log_{10}$/mL, milk leukocyte counts >6 $\log_{10}$/mL, and no antibiotic treatment for the 2 previous weeks. Treatment was 2 weeks of applying ~0.1 nisin solution (6 μg/mL) to the nipple and surrounding area after each nursing episode. Treatment with nisin significantly lowered milk bacterial colony-forming units. A study was done in women using *Lactobacillus fermentum* CECT5716 and *L. salivarius* CECT5713 isolated from non-mastitic breast milk as an oral treatment for mastitis (Arroyo et al. 2010). The orally administered probiotic treatments were compared to physician prescribed antibiotic regimens. Antibiotic treatments were amoxicillin–clavulanic acid, amoxicillin, cotrimoxazole, cloxacillin, and erythromycin. Before treatment the dominant bacterial species isolated from breast milk were *S. epidermidis* (73% of the women), *S. aureus* (43%), and *S. mitis* (30%), other bacterial species (<5%), and *Lactobacilli* spp. (0%). Pain in the breasts was scored from 0 (very painful) to 10 (no pain). On day 21 of treatment, the bacterial counts in the women receiving oral *L. fermentum* CECT5716 and *L. salivarius* CECT5713 treatments were significantly lower than the women receiving antibiotic treatments. Breast pain was correlated with bacterial plate counts, and the probiotic groups were not significantly different but had significantly lower pain scores compared to antibiotic groups. *L. fermentum* CECT5716 and *L. salivarius* CECT5713 were isolated from the milk of women receiving these probiotics. Women in the probiotic groups had lower recurrences of mastitis.

6 Bacteriophages and Their Products

Bacteriophages are viruses that attack and kill bacteria (Gomes and Henriques 2016). A problem with using bacteriophages is they may not infect bacteria inside the mammary gland because of the interfering–inactivating actions of milk and other mammary secretions. Bacteriophages can be specific in attacking one species of mastitis causing *Staphylococcus*, or the bacteriophage can be polyvalent including activities against biofilms. Specific proteins isolated from bacteriophages are being tested in vivo for their antibacterial activity. Two bacteriophage proteins are expressed inside the bacteria; the holing protein creates a pore in the cell membrane allowing endolysin to pass through the pore and hydrolyze the peptidoglycan in the cell wall liberating the newly formed bacteriophages. Endolysin produced by recombinant procedures in *E. coli* was tested as a therapeutic for *S. aureus* mastitis in dairy cattle with clinical signs of mild mastitis (Fan et al. 2016). The endolysin gene sequence (trx-SA1) was isolated from a bacteriophage identified as IME-SAG1 in the Myoviridae family. Five udder quarters with *S. aureus* mastitis were treated with the Trx-SA1 recombinant endolysin by intramammary infusion. Twenty milligrams of endolysin was dissolved in physiological saline and infused once a day for 3 days. Milk SCC and shedding of pathogens decreased with remission of clinical signs. Treatment with endolysin was not effective in mammary quarters infected with *E. coli* and *Streptococcus agalactiae*.

7 Vitamin and Mineral Supplementation

Vitamin E has been studied as a nutraceutical to prevent mastitis in dairy cattle. Dairy cattle in the immediate peripartum and postpartum period is at risk for oxidative stress, ketosis, insulin resistance, and hepatic steatosis. Decreased immune functions can be associated with oxidative stress. The effects of vitamin E supplementation during the dry period (interval between lactations) were investigated in a double-blind study for its effects on reducing occurrences of clinical and subclinical mastitis (Bouwstra et al. 2010a, b). This study was in Holstein heifers and in cows producing 8000–10,000 kg of milk/year and having a mean dry period of 6 weeks. The study was controlled for housing, method of milking, season of the year, and diet. Animals in the low supplemented group received 100 g of a mineral mix/day/cow providing 135 IU of DL-α-tocopheryl-acetate/day/cow

and cows and heifers in the high group received 3000 IU of DL-α-tocopheryl-acetate/day/cow. Supplementation was started at dry off and continued until parturition (mean of 8 weeks). Blood was collected at dry off, 4 and 2 weeks before calculated parturition, and 24 h after calving. Subclinical mastitis was defined as SCC >100,000 cells/mL of milk. Cows in the group receiving 3000 IU vitamin E/day had higher occurrences of clinical and subclinical mastitis than cows receiving 135 IU vitamin E/day. Blood levels of vitamin E were increased by vitamin E supplementation as was the vitamin E/cholesterol ratio. Additionally, this study showed that blood level of vitamin E above 14.5 μmol/L is a risk factor for increased clinical mastitis. This risk was 3 times higher in the unsupplemented group and 1.5 times higher in the supplemented groups. The blood levels of heifers at the start of the study were 9.10 μmol/L. The mechanism of action can be excessive vitamin E interfering with oxidative reactions normally occurring in immune system responses. A parenterally administered proprietary product[33] was studied for its effect on reducing subclinical mastitis (Ganda et al. 2016). The study was conducted from January to April in upstate New York, USA, and enrolled 620 cows. The cows were on a total mixed ration that met or exceeded their nutrient requirements as recommended by the US National Research Council. Enrollment requirements were SCC >200,000 cells/mL milk, <150 days pregnant, and no treatment with antibiotics in the current lactation. Treated cows received 5 mL of the proprietary product administered once parenterally, and control cows received 5 mL of saline. Each mL of the test product contained 60 mg zinc, 10 mg manganese, 5 mg selenium, and 15 mg copper. A random selection of cows had blood drawn on study day 0 and again on study day 30 and the serum analyzed for calcium, magnesium, phosphorus, copper, iron, potassium, manganese molybdenum, selenium, and zinc. Body condition scores, lactation number, milk yield, days in milk, and linear scores of somatic cell numbers at enrollment were also parameters in the study, and these parameters were not significantly different. There was a 10-month follow-up period. Serum calcium and magnesium were significantly higher in the control cows, and also there was a tendency for molybdenum to also be higher in the control cows. There was no difference between groups for the other elements measured in serum. There were no significant differences between groups for cures from subclinical mastitis and milk SCC and no significant interactions between treatment and lactation on milk SCC. There was a tendency within lactation for the treatment to cure subclinical mastitis in cows with three or more lactations. There was an overall tendency for treatment to reduce chronic clinical mastitis especially in primiparous cows. Independent of treatment, cows with lower serum zinc levels at mastitis diagnosis and higher serum levels of selenium and phosphorus 30 days later had more cures. A Swedish study on vitamin E (RRR-α-tocopheryl-acetate[34]) supplementation of dairy cattle did not reduce the occurrences of mastitis (Persson Waller et al. 2007). The cows were in herds with high occurrences of mastitis and were supplemented with 1.61 g of RRR-α-tocopheryl-acetate as a top dressing for 4 weeks before calculated calving and 2 weeks after actual calving.

8 Teat Dips

The teat duct is a portal of entry for pathogens, and the first responder is the innate immune system (Mavrogianni et al. 2007). Maintaining teat health is important in preventing mammary infections. Teat dips are used to decrease bacterial numbers on the teat especially at the teat orifice. The IMAU 80065 and IMAU 10155 strains of *Lactobacillus plantarum* were studied as a teat dip for cattle (Yu et al. 2017). Cows with milk SCC >200,000 cells/mL were selected for the study. Teats were dipped after milking in a preparation of *L. plantarum* (5×10^{10} colony-forming units/mL) or a commercial[35] teat dip. Teat dipping with the probiotic lactic acid bacteria was considered efficacious in reducing mastitis-associated bacteria. The milk SCC of the cows with teats dipped in probiotic lactic acid bacteria decreased and were significantly lower than cows that had teats dipped in the commercial teat dip. A teat dip made from a recipe using 2–3 drops of liquid soap, tea tree oil, peppermint oil, lavender oil, and grape seed extract at one drop each added to 350 mL of potable water was found to be the most likely source of *Pseudomonas aeruginosa* causing mastitis in dairy goats (Kelly and Wilson 2016). The teat dip itself also supported the growth of *P. aeruginosa*. *Prototheca* microalgae can be a cause of mastitis and is difficult to treat because of its resistance to antimicrobial chemotherapeutics. A proprietary[36] vegetable rinse formulation was studied for its antiseptic activity against *Prototheca zopfi* (Grzesiak et al. 2018b). The *P. zopfi* strains tested were isolated from cows with subclinical mastitis. The minimal microbicidal concentrations of were 0.002–0.019%. The ingredients in this product are decyl polyglucose, cornstarch, and coconut oil. The active ingredient is decyl polyglucose, a compound widely used in cosmetics. The finding suggests that this product has potential as a teat wash and teat dip.

[33] Multimin 90, Multimin North America Inc. (Fort Collins, CO)

[34] Natur-E granulat 40%, Pharmalett A/S (Kolding, Denmark)

[35] Dipal Concentrate 1C4 (Delaval, Tianjin, China)

[36] SunSmile® Fruit & Vegetable Rinse (Sunrider International, http://www.sunrider.com/eng/worldwide/offices#)

9 Immunotherapy

Egg yolk immunoglobulin IgY from hens vaccinated against mastitic strains of *S. aureus* were evaluated in cows with experimentally induced and naturally infected *S. aureus* mastitis (Zhen et al. 2009). Criteria for cows with natural infections were clinical signs of mastitis, milk SCC >500,000 cells/mL and bacterial plate counts of 100–200 *S. aureus* colony-forming units/mL of milk, and no antibiotic treatment in the past 90 days. For *S. aureus*-induced mastitis, the cows before inoculation were clinically normal, milk SCC <100,000 cells/mL of milk, <10 colony-forming units of *S. aureus*/mL of milk, and no antibiotic treatment in the past 90 days. The *S. aureus* inoculation to induce mastitis was 10^7 colony-forming units/mL. Treatments were intramammary infusions of 10 mL of 20 mg of IgY/mL or 10 mL of 100 mg of penicillin/mL immediately after morning and evening milkings or no infusions. The cure rates for IgY were 83.3% for experimentally induced mastitis and 50% for clinical mastitis. Cure rate for penicillin treatment was 66.7% and 33.3%, respectively, for experimental and clinical mastitis. The treated cows were significantly different from control untreated cows for milk SCC and bacterial counts. Bovine platelet concentrate has been investigated as a therapeutic for mastitis (Lange-Consiglio et al. 2014). A healthy cow on each of three farms was used to donate platelets. The quarters of cows with mastitis were divided into three treatment groups: antibiotics, antibiotics plus platelets, and platelets without antibiotics. Cows with acute and chronic mastitis were enrolled in the study. The platelet concentrate was standardized at 10^9 platelets/mL. Cows in the groups receiving platelets received 5 mL of the platelet concentrate for 3 days. For acute mastitis, platelet concentrate group was not statistically different from the antibiotic alone group, and for chronic mastitis, platelet concentrate alone was significantly better than antibiotic alone. For both acute and chronic mastitis, antibiotic plus platelet concentrate was significantly better than antibiotic alone, and this combination also significantly reduces the rate of relapses.

10 Concluding Remarks and Future Directions

Nutraceuticals have increased use for prevention and treatment of mastitis. Medical reasons include increased antimicrobial resistance to approved antimicrobial chemotherapeutics, public health concerns regarding human exposure to antibiotic-resistant microorganisms in animal-source foods, restrictions on antibiotic use in organic farming, and increase interest in the use of alternative medicine. The interactions between the immune system and nutrition and body antioxidant status as it pertains to health of the mammary gland are important in prevention of mastitis. Advances in metabolomics will likely provide strategic profiles for mammary gland health. Increased understanding of the microbiome and the interactions of microbial populations in maintaining mammary health and immune modulation will increase.

References

Abdalhamed AM, Zeedan GSG, Zeina H (2018) Isolation and identification of bacteria causing mastitis in small ruminants and their susceptibility to antibiotics, honey, essential oils, and plant extracts. Vet World 11(3):355–362

Abuelo A, Hernandez J, Benedito JL et al (2015) The importance of the oxidative status of dairy cattle in the periparturient period: revisiting antioxidant supplementation. J Anim Physiol Anim Nutr (Berl) 99 (6):1003–1016

Aghamohammadi M, Haine D, Kelton DF et al (2018) Herd-level mastitis-associated costs on Canadian dairy farms. Front Vet Sci 5:100

Akers RM, Nickerson SC (2011) Mastitis and its impact on structure and function in the ruminant mammary gland. J Mammary Gland Biol Neoplasia 16(4):275–289

Alekish MO, Ismail ZB, Awawdeh MS et al (2017) Effects of intramammary infusion of sage (*Salvia officinalis*) essential oil on milk somatic cell count, milk composition parameters and selected hematology and serum biochemical parameters in Awassi sheep with subclinical mastitis. Vet World 10(8):895–900

Allen HK, Trachsel J, Looft T et al (2014) Finding alternatives to antibiotics. Ann N Y Acad Sci 1323:91–100

Almasaudi SB, Al-Nahari AAM, Abd El-Ghany ESM et al (2017) Antimicrobial effect of different types of honey on *Staphylococcus aureus*. Saudi J Biol Sci 24(6):1255–1261

Al-Qumber M, Tagg JR (2006) Commensal bacilli inhibitory to mastitis pathogens isolated from the udder microbiota of healthy cows. J Appl Microbiol 101(5):1152–1160

Amber R, Adnan M, Tariq A et al (2018) Antibacterial activity of selected medicinal plants of northwest Pakistan traditionally used against mastitis in livestock. Saudi J Biol Sci 25(1):154–161

Ananda Baskaran S, Kazmer GW, Hinckley L et al (2009) Antibacterial effect of plant-derived antimicrobials on major bacterial mastitis pathogens *in vitro*. J Dairy Sci 92(4):1423–1429

Armas F, Camperio C, Marianelli C (2017) In vitro assessment of the probiotic potential of *Lactococcus lactis* LMG 7930 against ruminant mastitis-causing pathogens. PLoS One 12(1):e0169543

Arroyo R, Martin V, Maldonado A et al (2010) Treatment of infectious mastitis during lactation: antibiotics versus oral administration of *Lactobacilli* isolated from breast milk. Clin Infect Dis 50 (12):1551–1558

Arsenault J, Dubreuil P, Higgins R et al (2008) Risk factors and impacts of clinical and subclinical mastitis in commercial meat-producing sheep flocks in Quebec, Canada. Prev Vet Med 87(3–4):373–393

Assis BS, Germon P, Silva AM et al (2015) *Lactococcus lactis* V7 inhibits the cell invasion of bovine mammary epithelial cells by *Escherichia coli* and *Staphylococcus aureus*. Benef Microbes 6 (6):879–886

Baravalle C, Dallard BE, Cadoche MC et al (2011) Proinflammatory cytokines and CD14 expression in mammary tissue of cows following intramammary inoculation of *Panax ginseng* at drying off. Vet Immunol Immunopathol 144(1–2):52–60

Baravalle C, Silvestrini P, Cadoche MC et al (2015) Intramammary infusion of *Panax ginseng* extract in bovine mammary gland at cessation of milking induces changes in the expression of toll-like

receptors, MyD88 and NF-kB during early involution. Res Vet Sci 100:52–60
Bedard F, Biron E (2018) Recent progress in the chemical synthesis of class ii and s-glycosylated bacteriocins. Front Microbiol 9:1048
Beecher C, Daly M, Berry DP et al (2009) Administration of a live culture of *Lactococcus lactis* DPC 3147 into the bovine mammary gland stimulates the local host immune response, particularly IL-1beta and IL-8 gene expression. J Dairy Res 76(3):340–348
Bhatt VD, Shah TM, Nauriyal DS et al (2014) Evaluation of a topical herbal drug for its in-vivo immunomodulatory effect on cytokines production and antibacterial activity in bovine subclinical mastitis. Ayu 35(2):198–205
Bhattarai D, Worku T, Dad R et al (2018) Mechanism of pattern recognition receptors (PRRs) and host pathogen interplay in bovine mastitis. Microb Pathog 120:64–70
Boorn KL, Khor YY, Sweetman E et al (2010) Antimicrobial activity of honey from the stingless bee *Trigona carbonaria* determined by agar diffusion, agar dilution, broth microdilution and time-kill methodology. J Appl Microbiol 108(5):1534–1543
Boothe DM (2004) Balancing fact and fiction of novel ingredients: definitions, regulations and evaluation. Vet Clin North Am Small Anim Pract 34(1):7–38
Bouwstra RJ, Nielen M, Stegeman JA et al (2010a) Vitamin E supplementation during the dry period in dairy cattle. Part I: Adverse effect on incidence of mastitis postpartum in a double-blind randomized field trial. J Dairy Sci 93(12):5684–5695
Bouwstra RJ, Nielen M, Newbold JR et al (2010b) Vitamin E supplementation during the dry period in dairy cattle. Part II: Oxidative stress following vitamin E supplementation may increase clinical mastitis incidence postpartum. J Dairy Sci 93(12):5696–5706
Byung-Wook C, Chun-Nam C, Soo-Mi L et al (2015) Therapeutic effect of oregano essential oil on subclinical bovine mastitis caused by *Staphylococcus aureus* and *Escherichia coli*. Korean J Vet Res 55 (4):253–257
Camperio C, Armas F, Biasibetti E et al (2017) A mouse mastitis model to study the effects of the intramammary infusion of a food-grade *Lactococcus lactis* strain. PLoS One 12(9):e0184218
Cao LT, Wu JQ, Xie F et al (2007) Efficacy of nisin in treatment of clinical mastitis in lactating dairy cows. J Dairy Sci 90 (8):3980–3985
Carson DA, Barkema HW, Naushad S et al (2017) Bacteriocins of non-aureus *Staphylococci* isolated from bovine milk. Appl Environ Microbiol 83(17):e01015-17
Ceotto-Vigoder H, Marques SL, Santos IN et al (2016) Nisin and lysostaphin activity against preformed biofilm of *Staphylococcus aureus* involved in bovine mastitis. J Appl Microbiol 121 (1):101–114
Cho B-W, Cha C-N, Lee S-M et al (2015) Therapeutic effect of oregano essential oil on subclinical bovine mastitis caused by *Staphylococcus aureus* and *Escherichia coli*. Korean J Vet Res 55 (4):253–257
Choi KT (2008) Botanical characteristics, pharmacological effects and medicinal components of Korean *Panax ginseng* CA Meyer. Acta Pharmacol Sin 29(9):1109–1118
Cooper S, Huntley SJ, Crump R et al (2016) A cross-sectional study of 329 farms in England to identify risk factors for ovine clinical mastitis. Prev Vet Med 125:89–98
Cortopassi-Laurino M, Imperatriz-Fonseca VL, Roubik DW et al (2006) Global meliponiculture: challenges and opportunities. Apidologie 37:275–292
Crispie F, Alonso-Gomez M, O'Loughlin C et al (2008) Intramammary infusion of a live culture for treatment of bovine mastitis: effect of live lactococci on the mammary immune response. J Dairy Res 75 (3):374–384
Ebert F, Staufenbiel R, Simons J et al (2017) Randomized, blinded, controlled clinical trial shows no benefit of homeopathic mastitis treatment in dairy cows. J Dairy Sci 100(6):4857–4867
Espeche MC, Pellegrino M, Frola I et al (2012) Lactic acid bacteria from raw milk as potentially beneficial strains to prevent bovine mastitis. Anaerobe 18(1):103–109
Fan J, Zeng Z, Mai K et al (2016) Preliminary treatment of bovine mastitis caused by *Staphylococcus aureus*, with Trx-SA1, recombinant endolysin of *S. aureus* bacteriophage IME-SA1. Vet Microbiol 191:65–71
Fernandez L, Delgado S, Herrero H et al (2008) The bacteriocin nisin, an effective agent for the treatment of staphylococcal mastitis during lactation. J Hum Lact 24(3):311–316
Fernandez L, Langa S, Martin V et al (2013) The human milk microbiota: origin and potential roles in health and disease. Pharmacol Res 69(1):1–10
Fernandez L, Cardenas N, Arroyo R et al (2016) Prevention of infectious mastitis by oral administration of *Lactobacillus salivarius* PS2 during late pregnancy. Clin Infect Dis 62(5):568–573
Francoz D, Wellemans V, Dupre JP et al (2017) Invited review: a systematic review and qualitative analysis of treatments other than conventional antimicrobials for clinical mastitis in dairy cows. J Dairy Sci 100(10):7751–7770
Frola ID, Pellegrino MS, Espeche MC et al (2012) Effects of intramammary inoculation of *Lactobacillus perolens* CRL1724 in lactating cows' udders. J Dairy Res 79(1):84–92
Frola ID, Pellegrino MS, Magnano G et al (2013) Histological examination of non-lactating bovine udders inoculated with *Lactobacillus perolens* CRL 1724. J Dairy Res 80(1):28–35
Ganda EK, Bisinotto RS, Vasquez AK et al (2016) Effects of injectable trace mineral supplementation in lactating dairy cows with elevated somatic cell counts. J Dairy Sci 99(9):7319–7329
Gomes F, Henriques M (2016) Control of bovine mastitis: old and recent therapeutic approaches. Curr Microbiol 72(4):377–382
Gomes F, Saavedra MJ, Henriques M (2016) Bovine mastitis disease/pathogenicity: evidence of the potential role of microbial biofilms. Pathog Dis 74(3):ftw006
Gomez-Gallego C, Garcia-Mantrana I, Salminen S et al (2016) The human milk microbiome and factors influencing its composition and activity. Semin Fetal Neonatal Med 21(6):400–405
Gougoulis DA, Kyriazakis I, Tzora A et al (2008) Effects of lamb sucking on the bacterial flora of teat duct and mammary gland of ewes. Reprod Domest Anim 43(1):22–26
Grzesiak B, Kolodziej B, Glowacka A et al (2018a) The effect of some natural essential oils against bovine mastitis caused by *Prototheca zopfii* isolates in vitro. Mycopathologia 183(3):541–550
Grzesiak B, Krukowski H, Glowacka A (2018b) The in vitro efficacy of SunSmile® Fruit & Vegetable Rinse against pathogenic strains of *Prototheca* algae that cause mastitis in cows. J Mycol Med 28 (2):300–304
Guan R, Wu JQ, Xu W et al (2017) Efficacy of vaccination and nisin Z treatments to eliminate intramammary *Staphylococcus aureus* infection in lactating cows. J Zhejiang Univ Sci B 18(4):360–364
Hamilton C, Emanuelson U, Forslund K et al (2006) Mastitis and related management factors in certified organic dairy herds in Sweden. Acta Vet Scand 48:11
Hektoen L (2004) Investigations of the motivation underlying Norwegian dairy farmers' use of homoeopathy. Vet Rec 155(22):701–707
Hektoen L, Larsen S, Odegaard SA et al (2004) Comparison of homeopathy, placebo and antibiotic treatment of clinical mastitis in dairy cows – methodological issues and results from a randomized-clinical trial. J Vet Med A Physiol Pathol Clin Med 51(9–10):439–446
Holmes MA, Zadoks RN (2011) Methicillin resistant *S. aureus* in human and bovine mastitis. J Mammary Gland Biol Neoplasia 16 (4):373–382

Honorato LA, Machado Filho LC, Barbosa Silveira ID et al (2014) Strategies used by dairy family farmers in the south of Brazil to comply with organic regulations. J Dairy Sci 97(3):1319–1327

Hu S, Concha C, Johannisson A et al (2001) Effect of subcutaneous injection of ginseng on cows with subclinical *Staphylococcus aureus* mastitis. J Vet Med B Infect Dis Vet Public Health 48(7):519–528

Jamali H, Barkema HW, Jacques M et al (2018) Invited review: incidence, risk factors, and effects of clinical mastitis recurrence in dairy cows. J Dairy Sci 101(6):4729–4746

Kaithwas G, Mukerjee A, Kumar P et al (2011) *Linum usitatissimum* (linseed/flaxseed) fixed oil: antimicrobial activity and efficacy in bovine mastitis. Inflammopharmacology 19(1):45–52

Keller D, Sundrum A (2018) Comparative effectiveness of individualised homeopathy and antibiotics in the treatment of bovine clinical mastitis: randomised controlled trial. Vet Rec 182(14):407

Kelly EJ, Wilson DJ (2016) *Pseudomonas aeruginosa* mastitis in two goats associated with an essential oil-based teat dip. J Vet Diagn Investig 28(6):760–762

Kher MN, Sheth NR, Bhatt VD (2018) In vitro antibacterial evaluation of *Terminalia chebula* as an alternative of antibiotics against bovine subclinical mastitis. Anim Biotechnol:1–8. https://doi.org/10.1080/10495398.2018.1451752

Kitazaki K, Koga S, Nagatoshi K et al (2017) *In vitro* synergistic activities of cefazolin and nisin A against mastitis pathogens. J Vet Med Sci 79(9):1472–1479

Klocke P, Ivemeyer S, Butler G et al (2010) A randomized controlled trial to compare the use of homeopathy and internal teat sealers for the prevention of mastitis in organically farmed dairy cows during the dry period and 100 days post-calving. Homeopathy 99(2):90–98

Klostermann K, Crispie F, Flynn J et al (2008) Intramammary infusion of a live culture of *Lactococcus lactis* for treatment of bovine mastitis: comparison with antibiotic treatment in field trials. J Dairy Res 75(3):365–373

Kromker V, Leimbach S (2017) Mastitis treatment-reduction in antibiotic usage in dairy cows. Reprod Domest Anim 52(Suppl 3):21–29

Kumar R, Bharati KA (2013) New claims in folk veterinary medicines from Uttar Pradesh, India. J Ethnopharmacol 146(2):581–593

Kumar H, du Toit E, Kulkarni A et al (2016) Distinct patterns in human milk microbiota and fatty acid profiles across specific geographic locations. Front Microbiol 7:1619

Lainesse C, Gehring R, Pasloske K et al (2012) Challenges associated with the demonstration of bioequivalence of intramammary products in ruminants. J Vet Pharmacol Ther 35(Suppl 1):65–79

Lange-Consiglio A, Spelta C, Garlappi R et al (2014) Intramammary administration of platelet concentrate as an unconventional therapy in bovine mastitis: first clinical application. J Dairy Sci 97(10):6223–6230

Lans C, Turner N, Khan T et al (2007) Ethnoveterinary medicines used for ruminants in British Columbia, Canada. J Ethnobiol Ethnomed 3:11

Le KY, Otto M (2015) Quorum-sensing regulation in staphylococci – an overview. Front Microbiol 6:1174

Li X, Lin J, Han W et al (2012) Antioxidant ability and mechanism of rhizoma *Atractylodes macrocephala*. Molecules 17(11):13457–13472

Loken T (2001) Alternative therapy of animals-homeopathy and other alternative methods of therapy. Acta Vet Scand Suppl 95:47–50

Lu Y, Hu YL, Kong XF et al (2008) Selection of component drug in activating blood flow and removing blood stasis of Chinese herbal medicinal formula for dairy cow mastitis by hemorheological method. J Ethnopharmacol 116(2):313–317

Mandal MD, Mandal S (2011) Honey: its medicinal property and antibacterial activity. Asian Pac J Trop Biomed 1(2):154–160

Mason SE, Mullen KAE, Anderson KL et al (2017) Pharmacokinetic analysis of thymol, carvacrol and diallyl disulfide after intramammary and topical applications in healthy organic dairy cattle. Food Addit Contam Part A 34(5):740–749

Mathie RT, Clausen J (2014) Veterinary homeopathy: systematic review of medical conditions studied by randomised placebo-controlled trials. Vet Rec 175(15):373–381

Mavrogianni VS, Cripps PJ, Fthenakis GC (2007) Bacterial flora and risk of infection of the ovine teat duct and mammary gland throughout lactation. Prev Vet Med 79(2–4):163–173

McPhee CS, Anderson KL, Yeatts JL et al (2011) Milk and plasma disposition of thymol following intramammary administration of a phytoceutical mastitis treatment. J Dairy Sci 94(4):1738–1743

Mignacca SA, Dore S, Spuria L et al (2017) Intramammary infusion of a live culture of *Lactococcus lactis* in ewes to treat staphylococcal mastitis. J Med Microbiol 66(12):1798–1810

Mukherjee R, De UK, Ram GC (2010) Evaluation of mammary gland immunity and therapeutic potential of *Tinospora cordifolia* against bovine subclinical mastitis. Trop Anim Health Prod 42(4):645–651

Mullen KA, Anderson KL, Washburn SP (2014) Effect of 2 herbal intramammary products on milk quantity and quality compared with conventional and no dry cow therapy. J Dairy Sci 97(6):3509–3522

Mullen KA, Beasley E, Rizzo JQ et al (2017) Potential of phytoceuticals to affect antibiotic residue detection tests in cow milk in a randomised trial. Vet Rec Open 4(1):e000214

Mushtaq S, Shah AM, Shah A et al (2018) Bovine mastitis: an appraisal of its alternative herbal cure. Microb Pathog 114:357–361

Nobrega DB, De Buck J, Barkema HW (2018a) Antimicrobial resistance in non-aureus staphylococci isolated from milk is associated with systemic but not intramammary administration of antimicrobials in dairy cattle. J Dairy Sci 101:7425–7436

Nobrega DB, Naushad S, Naqvi SA et al (2018b) Prevalence and genetic basis of antimicrobial resistance in non-aureus staphylococci isolated from Canadian dairy herds. Front Microbiol 9:256

Olson ME, Ceri H, Morck DW et al (2002) Biofilm bacteria: formation and comparative susceptibility to antibiotics. Can J Vet Res 66(2):86–92

Persson Waller K, Hallen Sandgren C, Emanuelson U et al (2007) Supplementation of RRR-alpha-tocopheryl acetate to periparturient dairy cows in commercial herds with high mastitis incidence. J Dairy Sci 90(8):3640–3646

Pinedo P, Karreman H, Bothe H et al (2013) Efficacy of a botanical preparation for the intramammary treatment of clinical mastitis on an organic dairy farm. Can Vet J 54(5):479–484

Rainard P (2017) Mammary microbiota of dairy ruminants: fact or fiction? Vet Res 48(1):25

Ribeiro ES, Gomes G, Greco LF et al (2016) Carryover effect of postpartum inflammatory diseases on developmental biology and fertility in lactating dairy cows. J Dairy Sci 99(3):2201–2220

Ruegg PL (2009) Management of mastitis on organic and conventional dairy farms. J Anim Sci 87(13 Suppl):43–55

Saini V, McClure JT, Scholl DT et al (2013) Herd-level relationship between antimicrobial use and presence or absence of antimicrobial resistance in gram-negative bovine mastitis pathogens on Canadian dairy farms. J Dairy Sci 96(8):4965–4976

Silvestrini P, Beccaria C, Pereyra EAL et al (2017) Intramammary inoculation of *Panax ginseng* plays an immunoprotective role in *Staphylococcus aureus* infection in a murine model. Res Vet Sci 115:211–220

Spelman K, Duke JA, Bogenschutz-Godwin MJ (2006) The synergy principle at work with plants, pathogens, insects, herbivores, and humans. In: Cseke LJ, Kirakosyan A, Kaufman PB et al (eds) Natural products from plants. CRC, Boca Raton, FL, pp 475–501

Spinella M (2002) The importance of pharmacological synergy in psychoactive herbal medicines. Altern Med Rev 7(2):130–137

Stagos D, Soulitsiotis N, Tsadila C et al (2018) Antibacterial and antioxidant activity of different types of honey derived from Mount Olympus in Greece. Int J Mol Med 42(2):726–734

Stevens M, Piepers S, De Vliegher S (2016) Mastitis prevention and control practices and mastitis treatment strategies associated with the consumption of (critically important) antimicrobials on dairy herds in Flanders, Belgium. J Dairy Sci 99(4):2896–2903

Szweda P, Schielmann M, Frankowska A et al (2014) Antibiotic resistance in *Staphylococcus aureus* strains isolated from cows with mastitis in eastern Poland and analysis of susceptibility of resistant strains to alternative nonantibiotic agents: lysostaphin, nisin and polymyxin B. J Vet Med Sci 76(3):355–362

Tavakol M, Riekerink RG, Sampimon OC et al (2012) Bovine-associated MRSA ST398 in the Netherlands. Acta Vet Scand 54:28

Uncini Manganelli RE, Camangi F, Tomei PE (2001) Curing animals with plants: traditional usage in Tuscany (Italy). J Ethnopharmacol 78(2–3):171–191

Upadhyay B, Singh KP, Kumar A (2011) Ethno-veterinary uses and informants consensus factor of medicinal plants of Sariska region, Rajasthan, India. J Ethnopharmacol 133(1):14–25

Wang X, Feng S, Ding N et al (2018) Anti-inflammatory effects of berberine hydrochloride in an LPS-induced murine model of mastitis. Evid Based Complement Alternat Med 2018:5164314

Wu J, Hu S, Cao L (2007) Therapeutic effect of nisin Z on subclinical mastitis in lactating cows. Antimicrob Agents Chemother 51 (9):3131–3135

Xu W, Guan R, Lu Y et al (2015) Therapeutic effect of polysaccharide fraction of *Atractylodis macrocephalae* Koidz in bovine subclinical mastitis. BMC Vet Res 11:165

Yang L, Liu Y, Wu H et al (2012) Combating biofilms. FEMS Immunol Med Microbiol 65(2):146–157

Yu J, Ren Y, Xi X et al (2017) A novel lactobacilli-based teat disinfectant for improving bacterial communities in the milks of cow teats with subclinical mastitis. Front Microbiol 8:1782

Zhen YH, Jin LJ, Li XY et al (2009) Efficacy of specific egg yolk immunoglobulin (IgY) to bovine mastitis caused by *Staphylococcus aureus*. Vet Microbiol 133(4):317–322

Nutraceuticals in Immune Disorders

Moges Woldemeskel

Abstract

Immune disorders in animals and humans may occur due to derangements in the body immune system in responses to various substances such as allergens, infections, chronic immune deficiency diseases, autoimmune diseases, and disruption in the immune system due to various other conditions. Modem medicine has been in use for long to treat different types of immune disorders. Many of these in fact have unintended side effects, although may be effective in treating diseases or alleviating the associated symptoms. Nutraceuticals are in use in traditional medicine throughout the world for centuries despite emergence of modem medicine. Nowadays, there is an increased tendency to look into nutraceuticals for the treatment of various diseases and conditions in humans and animals due to their efficacy in treating diseases and the fact that they cause no unintended side effects. This chapter briefly highlights on the use of nutraceuticals in immune disorders.

Keywords

Nutraceuticals · Immune disorders · Veterinary medicine

1 Introduction

The term "nutraceutical" was coined in 1989 by Stephen DeFelice and combines two words: "nutrient" and "pharmaceutical" (Kalra 2003). Nutraceutical is a broad term used to describe any product derived from food sources that provide health benefits including prevention and treatment of disease beyond its intrinsic nutritional value (Kalra 2003; Larussa et al. 2017). It was proposed that nutraceuticals can be grouped into three broad categories: (1) substances with established nutritional functions, such as vitamins, minerals, amino acids, and fatty acids, also defined as nutrients; (2) herbs or botanical products as concentrates and extracts, often called herbals; and (3) reagents such as pyruvate, chondroitin sulfate, and steroid hormone precursors that are derived from other sources and serve specific functions, such as sports nutrition, weight-loss supplements, and meal replacements, also indicated as dietary supplements (Chauhan et al. 2013). Medicinal plants constitute a natural reservoir for medicines worldwide and are part and parcel of nutraceuticals. They serve mainstream therapeutics and are central in folklore medicine (Akhtar et al. 2017).

Natural plant products have been used as the foundation of several medical treatments. Although modern western medicine has become the forefront of clinical practice today, natural plant products continue to be used as remedies in alternative medicine throughout the world. It is estimated that 80% of individuals in developing countries depend primarily on natural products to meet their healthcare needs. In the United States, it has been found that around one in three Americans uses natural medicinal products daily. Natural products are not only effective but are relatively nontoxic and have therapeutic doses well below their toxic levels (Fadus et al. 2017). In general, nutraceuticals are natural bioactive compounds or foodstuff that provide extra health benefits in addition to basic nutritional values. Nutraceuticals often refer to active ingredients found in functional foods and involve extracting, purifying, concentrating, and assaying such ingredients (Alkhatib et al. 2017). Various nutraceuticals have been in use for centuries to heal ailments in humans and animals as part of traditional medicine. This chapter gives a brief account of the use of nutraceuticals in diseases and condition with an underlying immune disorders or dysfunction, including degenerative and metabolic diseases, and diseases associated with hypersensitive or overactive immune system.

M. Woldemeskel (✉)
Tifton Veterinary Diagnostic and Investigational Laboratory, Department of Pathology, College of Veterinary Medicine, University of Georgia, Tifton, GA, USA
e-mail: mwoldem@uga.edu

R. C. Gupta et al. (eds.), *Nutraceuticals in Veterinary Medicine*, https://doi.org/10.1007/978-3-030-04624-8_39

2 Nutraceuticals in Regulation of Immune System

Immune system comprises of various cells, proteins, molecules, tissues, and organs that protect the body from harmful agents. Appropriate nutrients are necessary for the varied cells of the immune system to function optimally and respond to injury and invading viruses and bacteria. Dietary extremes, especially protein-energy malnutrition, have profound effects in decreasing immune function and increasing risk of opportunistic infections (Nieman and Mitmesser 2017). It was indicated in the 2016 Global Nutrition Report that one in three people worldwide is malnourished in one form or another (Global Nutritional Report 2016, 3).

Nutritional deficiencies have a negative effect in body defense against various infections by lowering the immune system. For example, Tsuji et al. (2015) citing various reports on animal models indicated that selenium (Se) deficiency impairs immune response to infection, cancer, and other stimuli, indicating the importance of the role of selenium in immune response. Examples include a reduction in CD4+ T-cell response in selenium-deficient mice challenged with a peptide/adjuvant, increased tumor growth and spread in a mouse model of breast cancer, enhanced type I allergic response in a mouse model of active cutaneous anaphylaxis, and increased immunotoxicity resulting from arsenic exposure (Tsuji et al. 2015).

More recently, natural substances mainly food supplements derived from plants that have been fed to farm animals evoked attention as a substitute to antibiotic growth promoters (Zhang et al. 2017). They are found to be beneficial in improving growth performance, digestive function, and the absorption of nutrients. They are also helpful in improving the ability to fight against infection and reducing the incidence of diarrhea. Recent studies have demonstrated that isoquinoline alkaloids can regulate metabolic processes, innate immune system**,** and digestive functioning in animals. Supplementation with isoquinoline alkaloids from *Macleaya cordata* extract increases feed intake and weight gain. It was found that ingestion of extract of *Macleaya cordata* increases the expression of ZO-1 and claudin-1 and hence helps in preventing substances with allergenic properties and toxigenic substances entering the intestines (Montagne et al. 2007). This indicates and supports the understanding that the use of extract of *Macleaya cordata* as a feed additive can promote intestinal mucosal growth and improve defense systems (Liu et al. 2016). It was also demonstrated that dietary supplementation with alkaloids reduced the level of serum amyloid A (SAA), a group of apolipoproteins produced in reaction to cytokines stimulated by monocytes or macrophages. Serum amyloid A apolipoproteins are closely associated with inherent immunity and are involved in the pathogenesis of chronic inflammatory diseases (Eklund et al. 2012). Hence, dietary supplementation with isoquinoline alkaloids extracted from *Macleaya cordata* boosts the immune system and regulates metabolic process and finally promotes growth and development. It is reported to be beneficial to swine and poultry through enhancing increase in feed consumption, body mass, and weight gain, as well as the concentration of serum amino acids. It boosts the innate immune system by regulating phagocytes, haptoglobin, and amyloid A (Ni et al. 2016).

Furthermore, in vitro studies and studies on inflammatory bowel disease-animal models have shown that nutraceuticals, such as herbal products or vitamins, are generally accepted as safer alternative or supplementation to conventional therapy. They are involved in several biological processes, including antioxidant defenses, cell proliferation, and gene expression, which could account for their role in the maintenance of the mucosal barrier integrity, the control of the inflammatory pathways, and the modulation of the immune response (Larussa et al. 2017). For example, it was demonstrated in experimental animal model that levels of interferon-γ and other cytokines were reduced in selenium-deficient mice. Additional studies in mouse knockouts have demonstrated that the selenoproteome, as well as individual selenoproteins such as Selk and Sep15, appears to play direct roles in supporting immune function. The important roles that selenoproteins play in regulating cellular oxidation states, catalyzing redox reactions with protein and chemical substrates, Ca^{2+} signaling, protein folding, and the downstream effects of these functions will likely be identified as the molecular mechanisms to explain the importance of dietary selenium in supporting the immune systems (Tsuji et al. 2015). Mushroom polysaccharides have also been demonstrated to have positive effects in the function of the immune system. Alpha (α)- and β-glucans derived from fungi and other food sources have been shown to possess effective immunostimulating activity (Zhang et al. 2017).

3 Anti-inflammatory Effects of Nutraceutical

Inflammation and inflammatory reactions underlie many human and animal diseases. Several anti-inflammatory drugs are prescribed to manage inflammatory reactions and inflammation-associated pain and diseases. However, the side effects of drugs used to treat inflammatory conditions particularly during chronic inflammatory diseases such as rheumatoid arthritis with various medications such as steroidal, nonsteroidal, and immunosuppressive drugs may be more difficult to manage than the disease itself. Safe anti-inflammatory nutraceuticals that can be used as

complementary to anti-inflammatory drugs may lead to the reduction in dose level of such drugs, thereby reducing their side effects (Al-Okbi 2014).

Various nutraceuticals are known to exert anti-inflammatory effect and help in treating and managing many diseases and conditions in humans and animals. Foods rich in antioxidants and anti-inflammatory bioactive constituents, for example, phenolic compounds, polyunsaturated fatty acids, phytosterols, tocopherols, and carotenoids, may be a good source of nutraceuticals of beneficial effect toward chronic inflammatory disease. Among many others, fish oil, primrose oil, black cumin, fenugreek, liquorice, coriander, tomato, carrot, sweet potato, broccoli, green tea, rosemary, hazelnut, walnut, wheat germ, and date extracts are examples of such sources (Al-Okbi 2014). For example, oral administration of methanol and aqueous extracts of edible portion of date fruits is reported to suppress inflammation significantly by 67.8% and 61.3%, respectively (Al-Okbi 2014). Ayurvedic herb which belongs to the family Berberidaceae is also used since ancient times as an anti-inflammatory agent for osteoporosis, joint pain, fever, eyes, and skin infections. Furthermore, topical instillation of aqueous extract of *Berberis aristata* showed potent anti-inflammatory activity against endotoxin-induced uveitis as well as against turpentine liniment-induced ocular inflammation in rabbits (Nimisha et al. 2017).

Among many other natural products, curcumin is an anti-inflammatory agent that has been used for treating medical conditions for many years (Fadus et al. 2017). Curcumin belongs to the family of natural compounds collectively called curcuminoids. It is the principal natural curcuminoid, which is a class of phenols found in the plant *Curcuma longa*, a rhizomatous herbaceous perennial flowering plant commonly used as a spice, food preservative, and a coloring agent in foods (Vecchi Brumatti et al. 2014; Larussa et al. 2017). Curcumin possesses remarkable beneficial properties as an antioxidant, anti-inflammatory, anticancer, and neuroprotective agent (Vecchi Brumatti et al. 2014). Several experimental and pharmacologic trials have demonstrated efficacy of curcumin as an anti-inflammatory agent. It has been shown to be effective in treating and managing chronic conditions such as rheumatoid arthritis, inflammatory bowel disease, Alzheimer's disease, and other common malignancies (Fadus et al. 2017).

The anti-inflammatory mechanism of action exerted by various nutraceuticals is different and depends on their nature and structure whether steroidal or nonsteroidal (Al-Okbi 2014). For example, the anti-inflammatory mechanism of curcumin works mainly through the suppression of the nuclear factor kappa-light-chain-enhancer of activated B-cell (NF-κB)-related inflammatory pathway, with subsequent inhibition of tumor necrosis factor (TNF)-α, interleukin (IL)-12 and IL-2, thus affecting the immune response modulation and representing a safe and promising agent (Vecchi Brumatti et al. 2014; Larussa et al. 2017) as is reported in the suppression of inflammation in inflammatory bowel disease (Vecchi Brumatti et al. 2014).

Polyphenols from various plants also have potent anti-inflammatory effects. They are naturally occurring compounds found largely in fruits such as grapes, apple, pear, cherries, and berries, in vegetables, cereals, and beverages (Pandey and Rizvi 2009). They are secondary plant metabolites which also have been shown to exert anti-oxidative and anti-inflammatory effects in cell culture, rodent, and human studies (Gessner et al. 2016).

Due to the fact that oxidative stress and inflammation are highly prevalent in farm animals, polyphenols are considered as promising feed additives in the nutrition of farm animals. A large number of studies with either cell cultures, such as intestinal cells, immune cells, endothelial and smooth muscle cells, adipocytes, or experimental animal models of inflammation including intestinal inflammation, systemic inflammation associated with obesity, metabolic syndrome, and atherosclerosis, demonstrated that isolated polyphenolic compounds or extracts, from polyphenol-rich plants such as green tea, hop, cocoa, and grape, suppress experimentally induced inflammatory processes (Gessner et al. 2016). Effects of polyphenols against inflammation are mediated by complex cellular mechanisms, most of which are linked with an inhibition of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), the major regulator of inflammation. Polyphenols are able to block the activation of NF-κB by inhibiting phosphorylation and proteasomal degradation of inhibitory kappa B (IκB), an effect which is in part due to the antioxidant properties of polyphenols (Vendrame and Klimis-Zacas 2015). In general, increasing evidence from studies with pigs, poultry, and cattle indicates that plant polyphenols are helpful to alleviate both local and systemic inflammatory conditions, which are of particular relevance during the weaning phase in monogastric species and during the periparturient period in dairy cattle, and thereby improve animal's performance. The main reason for improvements of animal's performance, such as increases in gain-to-feed ratio and milk yield, in response to plant polyphenols is that phenols reduce the effect of inflammatory condition that decreases feed intake, increases energy requirement for the production of fever, and induces several hormonal changes shifting the metabolism into a more catabolic state in farm animals (Gessner et al. 2016).

4 Nutraceuticals in Degenerative and Metabolic Diseases

Degenerative and metabolic diseases develop due to chronic or continuous insults to cells, tissues, and organs in humans and animals resulting in increased disruption of morphology and functions (metabolism) of tissues and organs over time.

Normal age-associated bodily wear or lifestyle choices such as exercise or nutritional habits may underlie development of degenerative and metabolic diseases. Natural feed supplements are known to prevent or slow the development of degenerative diseases and alleviate the disease signs, symptoms, and pain associated with clinical diseases. For example, natural dietary supplement, consisting of extract from five plant sources that contained *Cynara scolymus*, *Silybum marianum*, *Taraxacum officinale*, *Curcuma longa* (curcumin), and *Commiphora mukul*, is reported to prevent nonalcoholic fatty liver disease and atherogenesis by modulating the expression of different genes involved and avoiding renin-angiotensin system imbalance. The natural dietary supplement prevents liver fat accumulation and development of atherosclerotic lesions and improves hyperlipidemia (Amato et al. 2017).

A randomized controlled trial (Williams et al. 2017) in obese, older individuals also showed that fruit and vegetable concentrate supplementation has the potential to improve the metabolic profile of overweight and obese individuals by reducing blood lipid levels and systemic inflammation, as well as improving body composition. The degree of the improvements is found to be clinically significant, as the reduction in total cholesterol observed in the full cohort is estimated to be equivalent to a weight loss of 4 kg and an 8–9% reduction in cardiovascular disease risk. All obese individuals are likely to gain some benefit from supplementation with fruit and vegetable concentrate; however, it appears likely that the greatest improvements are in those with high baseline systemic inflammation or blood lipids. All in all, it was indicated that in obese individuals, who typically have a low fruit and vegetable intake, supplementation with fruit and vegetable concentrate may be beneficial to improve the metabolic profile and consequently reduce the risk of developing chronic inflammatory disease (Williams et al. 2017) and thereby decrease the development of various metabolic diseases.

Chronic inflammation is associated with a broad spectrum of neurodegenerative diseases of aging in humans including Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, Parkinson-dementia complex of Guam, all of the tauopathies, and age-related macular degeneration, as well as various degenerative diseases such as osteoarthritis, rheumatoid arthritis, atherosclerosis, and myocardial infarction (McGeer and McGeer 2004). Therefore, nutraceuticals such as fruit and vegetable concentrates that reduce chronic inflammation would help to alleviate the clinical disease or development of such degenerative diseases. For example, polysaccharides and other constituents of mushrooms have been demonstrated to repair oxidative damage and suppress neuroinflammation displaying a great potential to develop therapies for neurodegenerative conditions (Zhang et al. 2017).

A systematic review (Zhai et al. 2017) provided some evidence that coenzyme Q10 (CoQ10) supplementation may partly improve the process of inflammatory state in patients with metabolic diseases, although further well-designed studies, with larger sample size, are needed to confirm its efficacy in metabolic disease-associated inflammation.

5 Nutraceuticals in Hypersensitivity (Allergic) Disorders and Other Inflammatory Diseases

Hypersensitivity or allergic reactions and diseases occur in humans and animals when the body immune system overreacts after exposure to various foreign agents often known as allergens that illicit such reaction. This will be manifested in various forms depending upon the affected tissues, organs, or systems in the body. It could be seen as allergic dermatitis in skin or inflammatory bowel disease in the gastrointestinal tract. Various nutraceuticals including those from plant origin are used in treating and/or reducing the clinical effects of allergic reaction-associated diseases. For example, aloe vera gel, a plant extract known for medicinal purposes in several cultures for centuries, is one of the common herbal therapies used for inflammatory bowel disease, despite a lack of large trials confirming its efficacy (Hilsden et al. 2003). Langmead et al. (2004) demonstrated the induction of clinical response in patients with ulcerative colitis after a 4-week treatment with oral administration of aloe vera gel, although no significant effects were observed on endoscopic and histological outcomes.

In another study (Del Pinto et al. 2015), inflammatory bowel disease was found to be significantly associated with deficiency of vitamin D. Evidence supports an immunological role of vitamin D in inflammatory bowel disease, both promoting tissue barrier formation through the expression of cell adhesion proteins and stabilization of tight junctions between epithelial cells and inhibiting the production of pro-inflammatory cytokines through the activation of vitamin D receptor (Mouli and Ananthakrishnan 2014). Furthermore, Larussa et al. (2017) citing several authors indicated that supplementation with vitamin D is reported to result in lower relapse rates and improvement in Crohn's disease activity index and is demonstrated to have a beneficial effect in patients with ulcerative colitis. Dietary peptides and amino acids (AAs) have also been shown to modulate intestinal immune functions and influence inflammatory responses, being involved in reducing inflammation, oxidative stress, and apoptosis in the gut and may be useful as alternative or ancillary treatments in Inflammatory Bowel Disease (Zhang et al. 2015).

6 Concluding Remarks and Future Directions

Nutraceuticals in different forms including herbs and other plant products have been in use for centuries. Despite development of modern medicine, traditional medicine that uses herbs and nutraceuticals is still in use with an increasing tendency throughout the world. With the exposure of humans and animals to polluted environment, synthetic products for day-to-day uses, and food contaminated with chemicals and pollutants, various diseases including allergic reactions and immune mediated diseases are befalling humans and animals in increased rates. Due to unintended side effects modern drugs used to treat various diseases, and the efficacy of natural products in the treatment and management of various diseases in animals and humans without out much side effects, nutraceuticals have a lot in store in the future as part of alternative medicine.

References

Akhtar MS, Hossain MA, Said SA (2017) Isolation and characterization of antimicrobial compound from the stem-bark of the traditionally used medicinal plant, Adenium obesum. J Tradit Complement Med 7 (2017):296–300

Alkhatib A, Tsang C, Tiss A et al (2017) Functional foods and lifestyle approaches for diabetes prevention and management. Nutrients 2017 (9):1310. https://doi.org/10.3390/nu9121310

Al-Okbi SY (2014) Nutraceuticals of anti-inflammatory activity as complementary therapy for rheumatoid arthritis. Toxicol Ind Health 30(8):738–749

Amato A, Caldara G-F, Nuzzo D et al (2017) NAFLD (Non-alcoholic fatty liver disease) and atherosclerosis are prevented by a natural dietary supplement containing curcumin, silymarin, guggul, chlorogenic acid and inulin in mice fed a high-fat diet. Nutrients 9:492. https://doi.org/10.3390/nu9050492

Chauhan B, Kumar G, Kalam N et al (2013) Current concepts and prospects of herbal nutraceutical: a review. J Adv Pharm Technol Res 4:4–8

Del Pinto R, Pietropaoli D, Chandar AK et al (2015) Association between inflammatory bowel disease and vitamin D deficiency: a systematic review and meta-analysis. Inflamm Bowel Dis 21:2708–2717

Eklund KK, Niemi K, Kovanen PT (2012) Immune functions of serum amyloid A. Crit Rev Immunol 32(4):335–348

Fadus MC, Lau C, Bikhchandani J, Lynch HT (2017) Curcumin: an age-old anti-inflammatory and anti-neoplastic agent. J Tradit Complement Med 7:339–346

Gessner DK, Ringseis R, Eder K (2016) Potential of plant polyphenols to combat oxidative stress and inflammatory processes in farm animals. J Anim Physiol Anim Nutri 101:605–628

Global Nutrition Report (2016) From promise to impact: ending malnutrition by 2030. International Food Policy Research Institute (IFPRI), Washington, DC, USA. http://www.ifpri.org/publication/global-nutrition-report-2016-promise-impact-ending-malnutrition-2030

Hilsden RJ, Verhoef MJ, Best A et al (2003) Complementary and alternative medicine use by Canadian patients with inflammatory bowel disease: results from a national survey. Am J Gastroenterol 98:1563–1568

Kalra EK (2003) Nutraceutical-definition and introduction. AAPS PharmSci 5:E25

Langmead L, Feakins RM, Goldthorpe S et al (2004) Randomized, double-blind, placebo-controlled trial of oral aloe vera gel for active ulcerative colitis. Aliment Pharmacol Ther 19:739–747

Larussa T, Imeneo M, Luzza F (2017) Potential role of nutraceutical compounds in inflammatory bowel disease. World J Gastroenterol 23(14):2483–2492

Liu G, Guan G, Fang J, et al (2016) *Macleaya cordata* extract decreased diarrhea score and enhanced intestinal barrier function in growing piglets. BioMed Res Int, 2016, 1069585, 7 pages

McGeer PL, McGeer EG (2004) Inflammation and the degenerative diseases of aging. Ann N Y Acad Sci 1035:104–116

Montagne L, Boundry G, Favier C et al (2007) Main intestinal markers associated with the changes in gut architecture and function in piglets after weaning. Br J Nutr 97:45–57

Mouli VP, Ananthakrishnan AN (2014) Review article: vitamin D and inflammatory bowel diseases. Aliment Pharmacol Ther 39: 125–136

Ni H, Martínez Y, Guan G et al (2016) Analysis of the impact of isoquinoline alkaloids, derived from *Macleaya cordata* extract, on the development and innate immune response in swine and poultry. BioMed Res Int 2016, 1352146, 7 pages https://doi.org/10.1155/2016/1352146

Nieman DC, Mitmesser SH (2017) Potential impact of nutrition on immune system recovery from heavy exertion: a metabolomics perspective. Nutrients 2017(9):513. https://doi.org/10.3390/nu9050513

Nimisha RDA, Fatima Z, Neema KCD (2017) Antipsoriatic and anti-inflammatory studies of *Berberis aristata* extract loaded nanovesicular gels. Pharmacogn Mag 13(Suppl 3):S587–S594

Pandey KB, Rizvi SI (2009) Plant polyphenols as dietary antioxidants in human health and disease. Oxid Med Cell Longev 2(5):270–278

Tsuji PA, Carlson BA, Anderson CB et al (2015) Dietary selenium levels affect selenoprotein expression and support the interferon- and IL-6 immune response pathways in mice. Nutrients 7:6529–6549

Vecchi Brumatti L, Marcuzzi A, Tricarico PM et al (2014) Curcumin and inflammatory bowel disease: potential and limits of innovative treatments. Molecules 19:21127–21153

Vendrame S, Klimis-Zacas D (2015) Anti-inflammatory effect of anthocyanins via modulation of nuclear factor- jB and mitogen-activated protein kinase signaling cascades. Nutr Rev 73:348–358

Williams EJ, Baines KJ, Berthon BS et al (2017) Effects of an encapsulated fruit and vegetable juice concentrate on obesity-induced systemic inflammation: a randomised controlled trial. Nutrients 2017(9):116. https://doi.org/10.3390/nu9020116

Zhai J, Bo Y, Lu Y et al (2017) Effects of coenzyme Q10 on markers of inflammation: a systematic review and meta-analysis. PLoS One 12 (1):e0170172. https://doi.org/10.1371/journal.pone.0170172

Zhang H, Hu CA, Kovacs-Nolan J et al (2015) Bioactive dietary peptides and amino acids in inflammatory bowel disease. Amino Acids 47:2127–2141

Zhang L, Li CG, Liang H et al (2017) Bioactive mushroom polysaccharides: immunoceuticals to anticancer agents. J Nutr Food Sci 2(2):6

Plant and Food Derived Immunomodulators as Nutraceuticals for Performance Enhancing Activities

Bhanushree Gupta, Vidya Rani Singh, Surabhi Verma, Neha Meshram, Leena Dhruw, Rahul Sharma, Kallol K. Ghosh, and Ramesh C. Gupta

Abstract

The progress in research has prompted a new age for food- and plant-derived products that are of incredible benefit and are widely utilized as immunomodulators and vitality supporters in the form of nutraceuticals. They are even being regarded as agents with the potential to cure numerous diseases. These functional products are processed from plants in the form of phytochemicals, as well as from food sources such as soy products, mushrooms, and milk. A considerable number of these nutraceuticals have relevant physiological functions and important biological activities. The present aggregated information about nutraceuticals undoubtedly provides extraordinary opportunities for use by nutritionists, doctors, food technologists, and chemists.

Keywords

Immunomodulators · Bioactive peptides · Nutraceuticals · Nutritional food · Performance-enhancing nutraceuticals

1 Introduction

In the rapidly growing field of biomedical research, the demand for functional foods (i.e., nutraceuticals) has been highly commercialized in response to increasing awareness of the effects of diet on health (Orlando 2018). As these foods provide additional nutrients to the body, they are broadly utilized as an additional source of energy for the body. Nutraceutical are substances present in food or isolated from food that provide medical or health benefits, including benefits in the treatment and prevention of diseases (DeFelice 1992). They are also known as designer foods and include isolated nutrients, dietary supplements, and herbal products from nature, as well as processed food products such as cereals, soups, and beverages. This chapter focuses on plant-derived and animal-derived nutraceuticals.

From a vast amount of research over the years, plant-derived phytochemicals such as flavonoids and polysaccharides have been distinguished for their immunomodulatory attributes (Jantan et al. 2015). Immunodrugs incorporate natural and synthetic organic operators; for example, cytokines and antibodies for single targets or pathways have been utilized to treat immune-related illnesses, but with only limited success. Various illnesses can be treated with immunomodulation utilizing plants, rather than by other means. The discovery and isolation of particular immunomodulatory operators and power boosters of plant origin have the potential to eliminate side effects and reduce the high manufacturing costs of synthetic drugs (Baxter 2007). This chapter features the importance of plants as creators of active functional molecules of varied types with conceivable uses in animals and their well-being.

The chapter also focuses on the food sector, which has shown uninterrupted growth in food proteins, offering health benefits beyond serving the nutritional needs of the body. Such nutritional interventions can be of immense benefit in enhancing the health of immune-compromised and healthy individuals. Numerous ongoing research studies describe the

Authors Vidya Rani Singh, Surabhi Verma, Neha Meshram, and Leena Dhruw have contributed equally.

B. Gupta (✉) · V. R. Singh · S. Verma · N. Meshram · L. Dhruw
Department of Chemistry, Center for Basic Sciences, Pandit Ravishankar Shukla University, Raipur (C.G.), India

R. Sharma
Department of Plant Physiology, Agricultural Biochemistry, Medicinal and Aromatic Plants, Indira Gandhi Agricultural University, Raipur (C.G.), India

K. K. Ghosh
School of Studies in Chemistry, Pandit Ravishankar Shukla University, Raipur (C.G.), India

R. C. Gupta
Toxicology Department, Breathitt Veterinary Center, Murray State University, Hopkinsville, KY, USA

R. C. Gupta et al. (eds.), *Nutraceuticals in Veterinary Medicine*, https://doi.org/10.1007/978-3-030-04624-8_40

potential for use of fish oils in the treatment of atopic dermatitis and psoriasis. Treatment with dietary n-3 fatty acid supplements offers exciting novel possibilities in malignant diseases (Fürst and Kuhn 2000). The roles of traditional soy foods in disease prevention and treatment have gained worldwide recognition because of their antidiarrheal, hypolipidemic, anticarcinogenic, and antiosteoporotic effects. Bioactive peptides have also been established as being protective against harmful pathogens present in the environment, as well as in humoral and cell-mediated immune functions. Thus, they have prospects of being incorporated as ingredients in functional foods, nutraceuticals, and pharmaceuticals, where their biological activities may assist in the control and prevention of diseases. They can be produced from a wide range of food materials, including those from animal sources (e.g., milk), as they provide immense power-boosting capability and have wide immunoregulatory features (Gill et al. 2000).

Hence, we can conclude that nutraceuticals have an advantage over medicines because of their immense capability as immunomodulators and power boosters. They are also of great advantage as they lack side effects and naturally supplement the diet (Chauhan et al. 2013). A great advantage of nutraceuticals is their safety, while their great disadvantage is their relatively poor effectiveness. However, this apparent disadvantage may sometimes be deceptive.

2 Plant-Derived Immunomodulators

The immune system is responsible for the mechanisms of host defense. Assistance of the body to defend against foreign substances, pathogens, toxins, and noncompatible living cells is provided by the immune system (Licciardi and Underwood 2011). The immune system has been categorized into two broad categories: the adaptive immune system (which provides specific or acquired immunity) and the innate immune system (which provides nonspecific immunity).

Plant-derived compounds have appreciable immunomodulatory characteristics. Alkaloids, flavonoids, diterpenoids, and glycosides are the major types of plant-derived immunomodulatory agents. In addition, curcumin, resveratrol, quercetin, capsaicin, etc., are of therapeutic importance (Jantan et al. 2015).

Use of medicinal plants for immunomodulation is an alternative to ordinary chemotherapy for the treatment of various diseases—for example, immunosuppression. Nutraceuticals may be useful in circumstances such as improper functioning of the immune system. Products of *Emblica officinalis* (from the Euphorbiaceae family)—also known as *amla* or the Indian gooseberry—have significant antifungal, antibacterial, and antidiabetic properties (Dhir et al. 1991). Apart from these properties, this fruit has also demonstrated cytoprotective properties in acute cadmium toxicity (Khandelwal et al. 2002). The juice of *shankhpushpi* (*Evolvulus alsinoides*, from the Convolvulaceae family) has been found to improve the healing of ulcers, and the leaf extract has been used as a remedy for whitlows on fingers and toes (Sethiya et al. 2011).

2.1 Alkaloids

Alkaloids are usually used in the form of salts. Two examples are caffeine and codeine, which have stimulant and analgesic activities, respectively. Some alkaloids, such as salts of nicotine and anabasine, are used as insecticides. Modified alkaloids are the main sources of many synthetic and semisynthetic drugs, which are designed in such a way as to enhance or change the primary effect of the drug and reduce unwanted side effects. Piperine, an immunomodulatory agent, is a type of alkaloid, which has the following activities: it reduces the levels of the proinflammatory cytokines interleukin (IL)-1β, IL-6, and tumor necrosis factor (TNF) α; it downregulates expression of cyclooxygenase (COX)-2, nitric oxide synthase (NOS) 2, and nuclear factor (NF) κB; and it inhibits eicosanoid generation by inhibiting phospholipase A2 (PLA2) and thromboxane A2 (TXA2) synthase activity (Vaibhav et al. 2012).

2.2 Flavonoids

Flavonoids are a large class of plant pigments, which have a structure based on, or similar to, that of flavones. Various forms of flavonoids are flavanols, phloroglucinols, isoflavones, quinones, etc.

As an example, butein is a chalcone that suppresses nitric oxide (NO) production by attenuating inducible nitric oxide synthase (iNOS) expression, and it inhibits translocation of NF-κB (Wang et al. 2014). Quercetin—a flavanol involved in decreased expression of proinflammatory cytokines, NF-κB, and iNOS—also reduces expression of vascular cell adhesion molecule (VCAM) 1 and E-selectin. Epigallocatechin-3-gallate, a member of the flavanol class, inhibits reactive oxygen species (ROS) generation, mitogen-activated protein kinase (MAPK) phosphorylation, adhesion molecule expression, and signal transducer and activator of transcription (STAT) 3 and activating transcription factor (ATF) 2 translocation through induction of heme oxygenase (HO) 1 and suppressor of cytokine signaling (SOCS) 3 expression. The isoflavone daidzein decreases TNF-α, IL-1β, monocyte chemoattractant

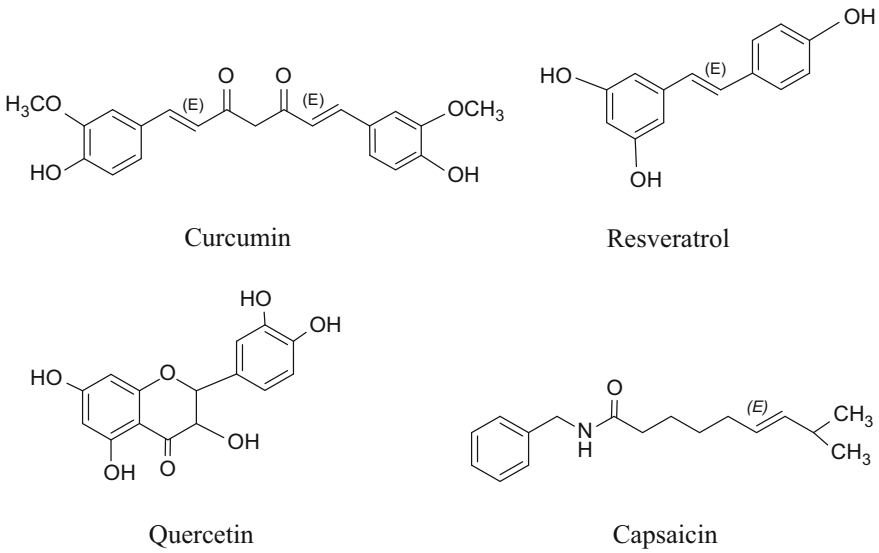


Fig. 1 Chemical structures of selected plant-derived immunomodulators

protein (MCP) 1, NO, and iNOS expression at the messenger RNA (mRNA) level (Rabinovitch et al. 1996). The phloroglucinol myrtucommulone inhibits prostaglandin E_2 (PGE_2) production by inhibiting microsomal prostaglandin E synthase (mPGES) 1 activity without significantly inhibiting COX enzyme activity. Thymoquinone, an active principle in *Nigella sativa* L., belongs to the quinines and performs the mechanisms of lipopolysaccharide (LPS)-induced fibroblast proliferation and H_2O_2-induced 4-hydroxynonenal generation (Wang et al. 2014).

2.3 Terpenoids

Terpenoids are the substances that are responsible for the odor of plants and flowers. They are found broadly in the leaves and products of higher plants such as conifers, citrus, and eucalyptus. 1,4-Deoxyandrographolide (from *Andrographis paniculata*) is a terpenoid that has an immunomodulatory action and enhances multiplication of lymphocytes (Kumar et al. 2004) (Fig. 1).

2.4 Curcumin

Curcumin is a characteristic diarylheptanoid compound found in the rhizome of *Curcuma longa* and related species. The therapeutic advantages of curcumin have been known for hundreds of years. An assortment of natural and pharmacological properties of curcumin have been described, including inhibition of cancer cell growth, angiogenesis, and cell proliferation; and induction of apoptosis (Bhaumik et al. 2000; Surh et al. 2001).

2.5 Resveratrol

Resveratrol (which synthetically is (5-[(E)-2-(4-hydroxyphenyl) ethenyl benzene-1,3-diol) is a subsidiary of stilbene and phytoalexin. It is found in different dietary items and plants, including grapevines, red wine, and peanuts. Like curcumin, resveratrol has been found to exert a number of pharmacological effects, such as antimicrobial, chemopreventive, anticancer/proapoptotic, mitigating, and cell reinforcement properties. Provocative atoms are emphatically hindered by resveratrol.

Resveratrols are chiefly found in grapes, nuts that abates myeloperoxidase (MPO) movement and mPGES-1 to basal levels; inhibits iNOS and COX-2 articulation (Youn et al. 2009); reduces the expert incendiary cytokines IL-8, TNF-α, IFN-γ, and IL-1α; and increases levels of the mitigating cytokine IL-10 (Vang et al. 2011).

2.6 Quercetin

The flavanol quercetin (which chemically is 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy chromen-4-one) belongs to the family of polyphenols, representing very extensively spread secondary plant metabolites. Quercetin is found in a variety of food such as tea, capers, red onions, broccoli, berries, grapes, and apples. Quercetin has been found to exert antimutagenic, antioxidative, anti-inflammatory, anticancer/chemopreventive, neuroprotective, antihypertensive, and blood glucose–lowering effects (Middleton et al. 2000). Quercetin activates various kinases, which phosphorylate eukaryotic initiation factor (eIF) 2, thus inhibiting cell translation (Ito et al. 1999). The mechanism

behind these actions is decreased expression of proinflammatory cytokines, NF-κB, iNOS, VCAM-1, and E-selection.

2.7 Capsaicin

Capsaicin (which synthetically is (E)-N-[(4-hydroxy-3-methoxyphenyl) methyl]-8-methylnon-6-enamide) is a hydrophobic alkaloid found in stew peppers (*Capsicum* species from the Solanaceae family) and is responsible for the trademark zestiness/sharpness of the natural products of this sort. It has been utilized in customary drugs for counteraggravation and as a topical rubefacient to calm pain in joints and muscles. An 8% capsaicin cutaneous patch has recently been approved to treat neuropathic pain in nondiabetic adults in the European Union (EU) and to treat neuropathic pain associated with postherpetic neuralgia in the USA (Caterina et al. 1997). Capsaicin has been found to inhibit the NF-κB pathway, iNOS expression, and COX-2 activity in macrophages in a transient receptor potential vanilloid (TRPV) 1–independent manner (Kim et al. 2004).

3 Food-Derived Immunomodulators

Immunomodulatory agents derived in the form of food from animals and plants have several regulatory functions in the immune system (Hartmann et al. 2000). Through use of these immunomodulatory agents, long-lasting immunity and other beneficial effects can be achieved in animals. Food production efficiency can be enhanced with the aid of these agents, as they boost the immune system and its functioning in animals, regulating certain immune factors (through suppression or stimulation). Bioactive peptides are widely known in the field of science because of their beneficial biological functionality, induced by food protein hydrolysates. They can be produced from the by-products of food protein bioprocessing and also from a wide range of food materials from both animals (egg, cheese, milk, beef, and chicken and fish skin) and plants (soy, soy products, wheat, and biofortified crops).

3.1 Bioprocessing of Bioactive Peptides from Proteins

Bioactive peptides serve as immunomodulatory agents because of the presence of different functional groups that have various structure-dependent activities. These functional properties of bioactive compounds can be determined on the basis of their unique three-dimensional structures. This determination can be done according to the type and nature of the amino acids present in the primary sequences of the food protein hydrolysates. Proteins can be produced from bioactive peptides by various reported methods, such as:

1. Enzymatic hydrolysis with digestive enzymes
2. Fermentation of food proteins with proteolysis starter cultures
3. Proteolysis by enzymes derived from microorganisms or plants

3.1.1 Enzymatic Hydrolysis with Digestive Enzymes

The most commonly used method for obtaining bioactive peptides is enzymatic hydrolysis of entire molecules. The enzymes utilized are from plants, bacteria, or fungi, or can be of gastrointestinal origin. Protein source production by proteolytic enzymes and their precursors, by use of recombinant DNA technology, has been reported (Korhonen and Pihlanto 2006). Ordinarily used enzymes are chymotrypsin, pepsin, alkalase, trypsin, papain, and pancreatin, which are either utilized alone or mixed with other enzymes. By enzymatic hydrolysis, production of bioactive peptides is performed using the optimized condition of the enzyme(s), and the type of peptides generated is subjected to the hydrolytic specificity of the enzyme(s). Additionally, with this method, specific enzyme combinations can be used to determine the fate of a protein molecule when it goes through the digestive system. For example, sequentially digested *Brassica carinata* protein—isolated in vitro with trypsin, chymotrypsin, and carboxypeptidase A—has been used to produce peptides with cancer prevention properties and angiotensin-converting enzyme inhibitor activities (Pedroche et al. 2007). Bioactive peptides generated with this type of enzyme combination are believed to stimulate those produced by the digestive system (Pedroche et al. 2007). In addition, the production of numerous novel peptides is led by combination of various enzyme treatments. The use of immobilized catalysts over the conventional soluble enzymes for production of bioactive peptides is gaining momentum. In a study by Pedroche et al. (2007), bioactive peptides were produced from *B. carinata* hydrolyzed by immobilized enzymes on a glyoxal agarose support.

3.1.2 Fermentation of Food Proteins with Proteolysis Starter Cultures

Another widely known technique for bioactive peptide production is fermentation of food proteins with proteolytic starter cultures. The proteolytic system of lactic acid bacteria (LAB) offers shifted enzymatic specificities. In the production of bioactive peptides, LAB offer cell envelope–associated proteinases (CEPs) and also intracellular peptidases—for example, endopeptidases, aminopeptidases, tripeptidases, and dipeptidases—for possible application. For

the production of probiotics and bioactive peptides, the probiotic microbes of the genera *Lactobacillus* and *Bifidobacterium* have been broadly studied (Alhaj et al. 2010). The activities of peptidases are influenced by the development states of the production microorganisms; thus, the development of peptides by this method can be controlled to some extent (Korhonen and Pihlanto 2006).

3.1.3 Proteolysis by Enzymes Derived from Microorganisms or Plants

Research on commercial production of bioactive peptides is progressing and is made possible by the development of optimized production techniques. Advances in bioseparation technologies (utilized in sanitizing and isolating biomolecules) combined with chemical engineering convective mass exchange principles could offer a practical and versatile production stage for immunomodulatory peptides. To date, the strategies that have been utilized for peptide fractionation and advancement are those that enable to regain bioactive peptides with minimal destruction. Briefly, they include ion exchange, ultrafiltration, and gel filtration technologies. Coupled techniques—for example, ultrafiltration in a membrane reactor followed by enzymatic hydrolysis and, likewise, ion exchange membrane chromatography—have been utilized (Chabeaud et al. 2009; Pedroche et al. 2007). Utilization of a combination of techniques is also becoming popular for filtration, production, separation, and identification of bioactive peptides. Such methods lead to products of high yield, as well as high purity. For isolation and enrichment of bioactive peptides, the electromembrane filtration technique is utilized (Bargeman et al. 2002). This technique combines membrane filtration and electrophoresis and thus is a very effective one for use on strongly charged biomolecules. Moreover, key operating parameters—for example, the electrical field strength, the kind of membrane, the salination of the hydrolysates, and the hydrolysate concentration—can be controlled to enhance the product transfer and separation rates (Bargeman et al. 2002).

3.2 Biological Activities of Bioactive Peptides

Various biological activities such as antihypertensive, antioxidant, immunomodulatory, anticancer, antimicrobial, and lipid-lowering activities have been exhibited by food protein hydrolysates.

3.2.1 Antihypertensive Activity

Among the vast number of bioactive peptides, most peptides from exogenous sources are antihypertensive peptides—for example, ovalbumin, which is present in egg white protein. These food-derived antihypertensive peptides not only have been well researched but also have wide applications, such as in functional and designer foods. Nowadays, hypertension has become a major and genuine medical issue in animals, particularly in developed nations, and is considered a risk factor for developing cardiovascular disease. Antihypertensive peptides have been attracting interest because of their wide effectiveness in lowering blood pressure. They have proved to be effective in preventing or treating hypertension, mainly by inhibiting angiotensin-converting enzymes, which play a key role in regulation of blood pressure and electrolyte homeostasis. Ile-Pro-Pro (IPP) and Val-Pro-Pro (VPP) peptides have been, for the most part, described and analyzed as inhibitors of angiotensin I–converting enzymes. Angiotensin I–converting enzyme (ACE; peptidyl dipeptide hydrolase; EC 3.4.15.1) is a key enzyme in the renin–angiotensin system. This enzyme controls extracellular fluid volume and arterial vasoconstriction either by converting angiotensin I to the vasoconstrictor angiotensin II or by inactivating bradykinin (a vasodilatory peptide) and enkephalin. Inhibition of ACE in this manner results in an decrease in blood pressure, helping to control hypertension (Petrillo and Ondetti 1982).

3.2.2 Antioxidant Activity

Oxidation plays a critical role in the body for the survival of cells and also in nourishment. A side effect of oxidative digestion, being fundamental for the survival of cells, is generation of free radicals and other ROS, resulting in oxidative changes. At the point when an excess of free radicals is formed, they can overpower protective enzymes such as superoxide dismutase, catalase and peroxidase, causing dangerous and deadly impacts on cells, similar to apoptosis, by oxidizing cell proteins, membrane lipids, DNA, and enzymes, and thereby disrupting cell processes (Sharma et al. 2011). Proteins, protein hydrolysates, singular peptides, and amino acids appear to exhibit significant antioxidant activity. Antioxidative bioactive peptides have been derived from many hydrolyzed food proteins such as caseins, whey proteins, egg yolk protein, porcine myofibrillar proteins, and aquatic by-product proteins (Pihlanto 2006). They are effective against enzymatic and nonenzymatic peroxidation of lipids and fundamental fatty acids such as free radical scavengers and metal particle chelators. During hydrolysis by proteolytic enzymes, caseins can release antioxidative peptides (Korhonen and Pihlanto 2006). Peptides derived from α-s-casein have demonstrated free radical–scavenging activity and inhibited enzymatic and nonenzymatic lipid peroxidation (Sharma et al. 2011). Other than being critical for survival of cells in an organism, inhibition of oxidative forms is of great importance for food quality. Development of free radicals results in detectable effects on food quality such as

rancid flavor, unacceptable taste, and reduction of shelf life, while intake of food containing lipid oxidation products has been linked to different diseases, including cancer, diabetes, and cardiovascular disease (Ryan et al. 2011).

3.2.3 Cytomodulatory and Anticancer Activities

Proteins, peptides, and amino acids have been implicated in preventing the development of different types of cancer. Milk proteins and their peptide derivatives play roles in cancer prevention. Casein phosphopeptides (CPPs) have demonstrated anticarcinogenic activity (Bouhallab and Bouglé 2011). The anticancer activities of these proteins may be at least partially attributable to encrypted bioactive peptides. Numerous peptides in different sizes from various sources have demonstrated anticancer effects in in vivo studies (Yu et al. 2014). There is increased evidence that by acting as specific signals that may affect the viability of cancer cells, milk-derived peptides may have cytomodulatory activities (Gobbetti et al. 2007). Bioactive peptides with cytomodulatory activities have been found during bacterial hydrolysis of casein in commercial yogurt starter cultures, which affected colon cell Caco-2 kinetics in vitro (Macdonald et al. 1994). Bioactive peptides with antiproliferative activity toward leukemia cells have been found during digestion of bovine skimmed milk with a cell-free extract of the yeast *Saccharomyces cerevisiae* (Roy et al. 1999). Modulation of cell viability, such as through proliferation and apoptosis, in different human cell culture models has been shown by many purified peptides equivalent to sequences of casein. Hartmann et al. (2000) reported that cytomodulatory peptides derived from casein fractions inhibit cancer cell growth or stimulate the activity of immune-competent cells and neonatal intestinal cells. The fragments 1–18 and 105–117 from β-casein have been shown to influence the viability—as well as the proliferation, differentiation, and apoptosis—of different cell types (Phelan et al. 2009).

3.3 Immunomodulatory Effects

The association between nutrition and immunity has long been recognized. Bioactive peptides derived from different protein sources have been shown to exert immunomodulatory effects in in vitro and in vivo studies. However, such studies have generally focused on evaluation of the impacts of peptides and protein hydrolysates on particular immune systems, and only a limited number of investigations have analyzed their effects on nonspecific (intrinsic) immune systems (Shahidi and Zhong 2008). Bioactive peptides from caseins and whey proteins are known to have immunomodulatory impacts.

3.4 Functional Ingredients of Bioactive Peptides as Immunomodulators

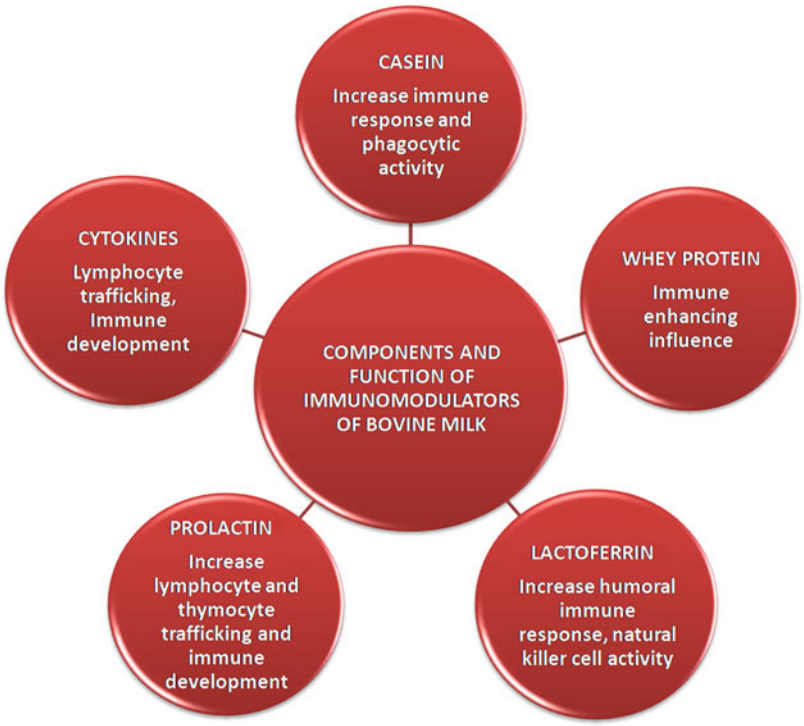
The functional groups present in various biologically active peptides serve as an immense source of energy and also act as immunomodulators when taken in the form of nutraceuticals. The main sources of bioactive peptides that act as immunomodulators are milk, mushrooms, eggs, cheese, beef, seafood, rice, corn, soy, and wheat (Dominic and Danquah 2012).

3.4.1 Milk Components as Immunomodulators

Milk is possibly the best-known source of nutrients. Aside from this, some milk components such as casein, lactoferrin, prolactin, and cytokines are milk growth factors. Immunoglobulin from milk facilitates passive immunity and contributes to protection against harmful environmental pathogens. Milk-derived proteins and peptides have wide immunoregulatory features, making milk the most important food consumed worldwide. Table 1 lists the components of milk that are responsible for immunomodulatory properties.

1. Casein: This is the major protein in milk and is a source of amino acids for young animals. The action of mammalian intestinal proteinases results in production of some peptides, derived from caseins, which have been found to retain immunoenhancing properties in vitro. The phosphopeptide residues 59–75 from bovine casein (generated by bovine trypsin digestion) and residues 1–25 from b-casein demonstrate mitogenic activity in mouse spleen cells, mouse thymocytes, or rabbit Peyer's patch cells; and a stimulatory effect on immunoglobulin production in mouse spleen cells (Otani et al. 2010).
2. Whey proteins: These are important immunoregulators for animals. Bovine whey proteins have immunoenhancing effects in mice. They include various protein such as γ-globulin, β-lactoglobulin, α-lactalbumin (αLA), bovine serum albumin (BSA), lactoferrin (LF), lactoperoxidase (LP), and some growth factors such as interleukins, epidermal growth factor, insulin-like growth factor, and transforming growth factor. These proteins have important biological activities. Some major and minor whey proteins are now commercially available as a result of advances in milk protein fractional technology (Wong et al. 1997).
3. Lactoferrin: This is an 80-KDa glycoprotein, which is involved in binding of iron and occurs in the milk of all mammals. It shows diverse effects on the host defense system. Apart from its function as a growth factor and its antimicrobial action, lactoferrin has been found to exhibit various immunomodulatory effects. In cell culture experiments it has been shown that lactoferrin and peptides derived from it influence the production of

Table 1 Bovine milk and its Immunomodulatory components with their bioactivities (Park 2009)



cytokines involved in immune and inflammatory actions in the body (Yada et al. 2002).

4. Prolactin: This is a 23-KDa single-chain polypeptide secreted from lactotroph cells of the anterior pituitary gland in vertebrates. It enhances immune function in fish. Prolactin stimulate the phagocytic activity of fish leukocytes. Prolactin is necessary to maintain circulating levels of immunoglobin in rainbow trout (Yada et al. 2002).
5. Cytokines: These are present in mammary glands and play important roles in development of the immune system in neonates. Immune factors are transferred from mothers to neonates through the placenta or via breast feeding and protect offspring from pathogens until maturation of the immune system (Nguyen et al. 2007).

3.4.2 Mushrooms

Mushrooms have great importance in medicines and nutraceuticals used to improve health. Some species of mushroom—such as *Lentinula edodes*, *Schizophyllum commune*, *Ganoderma lucidum*, and *Coriolus versicolor*—have been used as immunomodulators and anticancer agents.

Polysaccharide K (PSK; Krestin) and polysaccharopeptide (PSP) are both isolated from *C. versicolor* and are used as immune and chemotherapeutic agents for the treatment of cancer. PSP inhibits tumor cell proliferation through immunomodulatory activity. Schizophyllan (SPG) is a mushroom-derived polysaccharide, isolated from *S. commune*, which stimulates the immune system and activates NK cells, spleen cells, lymphoid cells, and bone marrow cells; and enhances the production of immunomodulatory cytokines. Two immunomodulatory β-glucans derived from *G. lucidum* are polysaccharides with a β-D-(1–3)-glucan backbone and branching characterized by β-D-(1–6) linkage, and have immunomodulatory and antioxidant properties.

Factors Influencing Bioactivity of Mushroom-Derived Immunomodulators

The major factors influencing the activity and confirmation of polysaccharides (glycosidic linkage and helical structure) are as follows:

1. The presence of (1–3)-β linkage in the main chain of glucans is an important structural feature for immunostimulatory and anticancer activity.
2. The triple helical structure of (1–3)-β-glucan can be recognized by glucan receptors, correlating with immunomodulatory activity.

Another factor that influences the immunomodulatory activities of polysaccharides is the degree of branching. Polysaccharides with a greater degree of branching demonstrate higher immunomodulatory and anticancer activities.

Immunoceutical Potential of Mushroom Polysaccharides

Glucans, mannans, galactans, and fructans are the most important immunostimulatory and anticancer polysaccharides. Among all of them, glucans are the most auspicious immunostimulatory and anticancer polysaccharides. Glucans are D-glucose-based polymers, mostly joined by β-glycosidic linkages and, in some cases, by α linkages. While the most active immunostimulators are β-glucans, α-glucans are nonactive, but α-glucans with (1–4) and (1–6) glycosidic linkages shows immunostimulatory activity (Zhang et al. 2017).

3.4.3 Eggs

Chicken egg white derivative (CWD), active egg white product (AEWP), and chicken egg white–derived immunoactive peptide (EF 203) have been shown to enhance nonspecific immunity in mice, piglets, cattle, and rainbow trout (Hirota and Yang 1995).

4 Clinical Applications of Food-Derived Nutraceuticals

Nutraceuticals have been used in the prevention and treatment of various common diseases in animals such as cardiovascular disease, cognitive dysfunction, cancer, osteoarthritis, and periodontal disease, providing evidence for their pharmacological potential in a wide range of animal diseases in numerous clinical trials. Nutraceuticals have been administered in animal supplements in many forms such as capsules, tablets, and powders, with or without prescription by veterinarians (Lerman and Lockwood 2007).

5 Concluding Remarks and Future Directions

Various diseases can be treated by immunomodulation through utilization of therapeutic plants rather than pharmacotherapy. Immunomodulatory peptides may likewise be included in the categories of nourishment and medication, and their use may be expanded in the form of supplements. Food proteins have been shown to offer health benefits beyond the need for bodily nourishment. There has been critical advancement in our understanding of the structures and bioactivity of polysaccharides, obtained from organisms and other nourishment sources, which appear to have viable immune-empowering actions. Studies have established the effects of certain lifestyle-related practices on a few parameters of the immune system. By implication, peptides with antihypertensive, antimicrobial, and immunomodulatory properties might be utilized to help prevent or control lifestyle-related diseases such as hypertension, proliferation, and cardiovascular disease. Further research on the bioactivity of polysaccharides is expected to lead to development of novel therapeutic and nutraceutical agents for curative and preventive applications against diseases, including cancer.

References

Alhaj OA, Kanekanian AD, Peters AC et al (2010) Hypocholesterolaemic effect of *Bifidobacterium animalis* subsp. lactis (Bb12) and trypsin casein hydrolysate. Food Chem 123(2):430–435

Bargeman G, Koops GK, Houwing J et al (2002) The development of electromembrane filtration for the isolation of bioactive peptides: the effect of membrane selection and operating parameters on the transport rate. Desalination 149(1e3):369–374

Baxter D (2007) Active and passive immunity, vaccine types, excipients and licensing. Occup Med 57:552–556

Bhaumik S, Jyothi MD, Khar A (2000) Differential modulation of nitric oxide production by curcumin in host macrophages and NK cells. FEBS Lett 483:78–82

Bouhallab S, Bouglé D (2011) Mineral-binding peptides from food. In: Hettiarachchy NS, Sato K, Marshall MR, Kannan A (eds) Bioactive food proteins and peptides: applications in human health. CRC Press, Boca Raton, FL, pp 117–130

Caterina MJ, Schumacher MA, Tominaga M et al (1997) The capsaicin receptor: a heat-activated ion channel in the pain pathway. Nature 389:816–824

Chabeaud A, Vandanjon L, Bourseau P et al (2009) Performances of ultrafiltration membranes for fractionating a fish protein hydrolysate: application to the refining of bioactive peptide fractions. Sep Purif Technol 66(3):463–471

Chauhan B, Kumar G, Kalam N et al (2013) Current concepts and prospects of herbal nutraceutical: a review. J Adv Pharm Technol Res 4(1):4–8

DeFelice SL (1992) The nutraceutical initiative: a recommendation for US economic and regulatory reforms. Genet Eng News 12:13–15

Dhir H, Roy AK, Sharma A et al (1991) Modification of clastogenicity of lead and aluminium in mouse bone marrow cells by *Phyllanthus emblica* fruit extract. Mutation Res 24:305–312

Dominic A, Danquah MK (2012) Rethinking food-derived bioactive peptides for antimicrobial and immunomodulatory activities. Trends Food Sci Technol 23(2):62–69

Fürst P, Kuhn KS (2000) Fish oil emulsions: what benefits can they bring? Clin Nutr 19:7–14

Gill HS, Doull F, Rutherfurd KJ et al (2000) Immunoregulatory peptides in bovine milk. Br J Nutr 84(Suppl. 1):S111–S117

Gobbetti M, Minervini F, Rizzello CG (2007) Bioactive peptides in dairy products. In: Hui YH (ed) Handbook of food products manufacturing. Wiley, Hoboken

Hartmann R, Gunther S, Martin D et al (2000) Cytochemical model systems for the detection and characterization of potentially bioactive milk components. Kiel Milchwirtschaftliche Forschungsberichte 52:61–85

Hirota Y, Yang MP (1995) Enhancing effect of chicken egg white derivatives on the phagocytic response in the dog. J Vet Med Sci 57(5):825–829

Ito T, Warnken SP, May SW (1999) Protein synthesis inhibition by flavonoids: roles of eukaryotic initiation factor 2 alpha kinases. Biochem Biophys Res Commun 265:589–594

Jantan I, Ahmad W, Nasir S, Bukhari NS (2015) Plant-derived immunomodulators: an insight on their preclinical evaluation and clinical trials. Front Plant Sci 6:655. https://doi.org/10.3389/fpls.2015.00655

Khandelwal S, Shukla LJ, Shanker R (2002) Modulation of acute cadmium toxicity by *Emblica officinalis* fruit in rat. Ind J Exp Biol 40:564–570

Kim S, Shin HJ, Kim SY et al (2004) Genistein enhances expression of genes involved in fatty acid catabolism through activation of PPAR alpha. Mol Cell Endocrinol 220:51–58

Korhonen H, Pihlanto A (2006) Bioactive peptides: production and functionality. Int Dairy J 16(9):945–960

Kumar RA, Sridevi K, Kumar NV et al (2004) Anticancer and immunostimulatory compounds from *Andrographis paniculata*. J Ethnopharmacol 92:291–295

Lerman A, Lockwood B (2007) Nutraceuticals in veterinary medicine. Pharm J 278:51–55

Licciardi PV, Underwood JR (2011) Plant-derived medicines: a novel class of immunological adjuvants. Int Immunopharmacol 11 (3):391–398

Macdonald RS, Thornton WH, Marshall RT (1994) A cell culture model to identify biologically active peptides generated by bacterial hydrolysis of casein. J Dairy Sci 77:1167–1175

Middleton E, Kandaswami C, Theoharides TC (2000) The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer. Pharmacol Rev 52:673–751

Nguyen TV, Yuvan L, Saif LJ et al (2007) Transfer of maternal cytokines to suckling piglets: in vivo and in vitro models with implications for immunomodulation of neonatal immunity. Vet Immunol Immunopathol 117:236–248

Orlando JM (2018) Behavioral nutraceuticals and diets. Vet Clin N Am Small Anim Pract 48:473–495

Otani H, Kihara Y, Park M (2010) The immunoenhancing property of dietary casein phosphopeptide preparation in mice. Food Agr Immunol 12:165–173

Park YW (ed) (2009) Bioactive components in milk and dairy products. Wiley, Hoboken

Pedroche J, Yust MM, Lqari H et al (2007) Obtaining of *Brassica carinata* protein hydrolysates enriched in bioactive peptides using immobilized digestive proteases. Food Res Int 40(7):931–938

Petrillo EW Jr, Ondetti MA (1982) Angiotensin converting enzyme inhibitors: medicinal chemistry and biological actions. Med Res Rev 2:1–41

Phelan M, Aherene A, Fitz Gerald RJ et al (2009) Casein-derived bioactive peptides: biological effects, industrial uses, safety aspects and regulatory status. Int Dairy J 19:643–654

Pihlanto A (2006) Antioxidative peptides derived from milk proteins. Int Dairy J 16:1306–1314

Rabinovitch A, Suarez-Pinzon WL, Sorensen O et al (1996) Inducible nitric oxide synthase (iNOS) in pancreatic islets of nonobese diabetic mice: identification of iNOS expressing cells and relationships to cytokines expressed in the islets. Endocrinology 137(5):2093–2099

Roy MK, Watanabe Y, Tami Y (1999) Induction of apoptosis in HL-60 cells by skimmed milk digested with a proteolytic enzyme from the yeast *Saccharomyces cerevisiae*. J Biosci Bioeng 88:426–432

Ryan JT, Ross RP, Bolton D et al (2011) Bioactive peptides from muscle sources: meat and fish. Nutrients 3:765–791

Sethiya NK, Alok N, Dixit VK et al (2011) Cognition boosting effect of Canscoradecussata (a South Indian Shankhpushpi). Eur J Integr Med 4:113–121. ELSEVIER

Shahidi F, Zhong Y (2008) Bioactive peptides. J AOAC Int 91 (4):914–931

Sharma S, Singh R, Rana S (2011) Bioactive peptides: a review. Int J Bioautomation 15(4):223–250

Surh YJ, Chun KS, Cha HH et al (2001) Molecular mechanisms underlying chemopreventive activities of antiinflammatory phytochemicals: down-regulation of COX-2 and iNOS through suppression of NF-activation. Mutat Res 481:243–268

Vaibhav K, Shrivastava P, Javed H et al (2012) Piperine suppresses cerebral ischemia-reperfusion-induced inflammation through the repression of COX-2, NOS-2, and NF-κB in middle cerebral artery occlusion rat model. Mol Cell Biochem 367:73–84

Vang O, Ahmad N, Baile CA et al (2011) What is new for an old molecule? Systematic review and recommendations on the use of resveratrol. PLos One 6(6):e19981

Wang Z, Lee Y, Eun JS et al (2014) Inhibition of adipocyte inflammation and macrophage chemotaxis by butein. Eur J Pharmacol 738:40–48

Wong CW, Seow HF, Husband AJ et al (1997) Effects of purified bovine whey factors on cellular immune functions in ruminants. Vet Immunol Immunopathol 56:85–96

Yada T, Uchida K, Kajimura S et al (2002) Immunomodulatory effects of prolactin and growth hormone in the tilapia, *Oreochromis mossambicus*. J Endocrinol 173:483–492

Youn J, Lee JS, Na HK et al (2009) Resveratrol and piceatannol inhibit iNOS expression and NF-κB activation in dextran sulfate sodium--induced mouse colitis. Nutr Cancer 61:847–854

Yu L, Yang L, An W et al (2014) Anticancer bioactive peptide-3 inhibits human gastric cancer growth by suppressing gastric cancer stem cells. J Cell Biochem 115(4):697–711

Zhang L, Guang Li C, Liang H et al (2017) Bioactive mushroom polysaccharides: immunoceuticals to anticancer agents. J Nutr Food Sci 2:1–5

Nutraceuticals for the Prevention and Cure of Cancer

Subash Chandra Gupta, Anurag Sharma, Shruti Mishra, and Nikee Awasthee

Abstract

Despite better healthcare system and advanced technology, the morbidity and mortality due to cancer are high in the western countries as compared to that in eastern world. Conversely, an increase in cancer incidence has been reported in the eastern world during recent years most likely due to the changes in lifestyle and food habits. The available cancer drugs provide only little benefits to the patients. Accumulating evidence suggests that Mother Nature has been a great source of medicine in as much as almost 70% of the current drugs have originated from Nature. Nutraceutical that refers to the food or food products with nutritional values has emerged as novel agents for cancer prevention and cure. Contrary to rationally designed drugs, nutraceuticals are multi-targeted and inexpensive and are reportedly safe. Chemically, nutraceuticals are diverse and can target multi-steps of tumor development. The anticancer potential of nutraceuticals has been demonstrated at the molecular, cellular, animal, and human levels. The cancer-related molecular targets of nutraceuticals include pro-inflammatory molecules, protein kinases, receptors, transcription factors, etc. Some of the common nutraceuticals with anticancer activities include caffeic acid, capsaicin, curcumin, epigallocatechin gallate, flavopiridol, genistein, resveratrol, sanguinarine, and tocotrienol. Nutraceuticals have also been structurally modified for better bioavailability and efficacy. These agents are also used in combination to enhance the efficacy of existing drugs. In this chapter, we discuss the therapeutic potential of nutraceuticals against cancer. The advantages and disadvantages associated with the use of nutraceuticals are discussed.

Keywords

Cancer · Efficacy · Nutraceutical · Prevention · Safety · Treatment

Abbreviation

5-LOX	5-lipoxygenase
8-OHdG	8-hydroxy-2′ -deoxyguanosine
AKBA	acetyl-11-keto-beta-boswellic acid
AMPK	5′ adenosine monophosphate-activated protein kinase
AP-1	activator protein-1
APC	adenomatous polyposis coli
Bax	bcl-2-associated X protein
Bcl-2	B-cell lymphoma-2
Bcr-abl	breakpoint cluster region-Abelson murine leukemia viral oncogene
CAPE	caffeic acid phenethyl ester
COX-2	cyclooxygenase-2
CRC	colorectal cancer
DNA	deoxyribonucleic acid
EGCG	epigallocatechin gallate
EGFR	epidermal growth factor receptor
ERK1/2	extracellular signal-regulated kinase 1/2
GAS-5	growth arrest specific-5
GSH	glutathione
GST	glutathione S-transferase
HIF-1	hypoxia-inducible factor-1
ICAM	intercellular adhesion molecule
IGF-1	insulin-like growth factor-1
IGF1-R	insulin-like growth factor 1 receptor
IGFBP-3	insulin-like growth factor binding protein-3
IKK	IκB kinase
IL	interleukin

S. C. Gupta (✉) · S. Mishra · N. Awasthee
Laboratory for Translational Cancer Research, Department of Biochemistry, Institute of Science, Banaras Hindu University, Varanasi, India
e-mail: sgupta@bhu.ac.in

A. Sharma
Division of Environmental Health and Toxicology, Nitte University Centre for Science Education and Research (NUCSER), Mangaluru, India

R. C. Gupta et al. (eds.), *Nutraceuticals in Veterinary Medicine*, https://doi.org/10.1007/978-3-030-04624-8_41

Keap-1	Kelch-like ECH-associated protein-1
lncRNA	long noncoding ribonucleic acid
M1G	pyrimido[1,2-a]-purin-10(3H)-one
MDA	malondialdehyde
MEG-3	maternally expressed-3
MMP	matrix metalloproteinase
mTOR	mammalian target of rapamycin
NF-κB	nuclear factor kappa-light-chain-enhancer of activated B cells
NIK	NF-κB-inducing kinase
NO	nitric oxide
NOS	nitric oxide synthase
Nrf2	nuclear factor erythroid 2 (NFE2)-related factor 2
PAI-1	plasminogen activator inhibitor-1
PAK	p21-activated kinase-1
PGC-1α	peroxisome proliferator-activated receptor gamma coactivator 1-alpha
PGE_2	prostaglandin E_2
PI3K	phosphoinositide 3-kinase
PMA	phorbol myristate acetate
PPAR-γ	peroxisome proliferator-activated receptor gamma
PSA	prostate-specific antigen
RASSF-1α	Ras association domain-containing family 1 alpha
SIRT-1	sirtuin 1
STAT3	signal transducer and activator of transcription 3
TIMP-2	tissue inhibitor of metalloproteinase-2
TNF-α	tumor necrosis factor alpha
VCAM	vascular cell adhesion molecule
VEGFR	vascular endothelial growth factor receptor
Wnt	wingless

1 Introduction

In 1971, an extensive campaign was launched by Richard Nixon, the then President of the USA for the cure of cancer. Since this campaign, the pathogenesis of the disease is relatively well understood. However, the disease continues to be major causes of death worldwide. The incidence and death because of cancer are likely to double by 2030 as compared to that in 2002. Unless we rethink the approaches for the cure, the mortality and morbidity due to cancer are unlikely to be significantly reduced.

Carcinogenesis is a multi-step process during which a series of events determine the fate of a cell from normal to a malignant state. Majority of the cancer-related deaths are due to the metastasis of the tumor. However, most drugs are designed to kill the cancer cells and not to prevent the spread. It is estimated that more than 500 genes are dysregulated in any given cancer. Yet, most current cancer therapeutics are based on the modulation of only a few targets, which is unlikely to be effective. The current focus for cancer therapy is either to design a drug that can hit several targets or to combine multiple mono-targeted agents. Accumulating evidence suggests that a multi-targeted approach has a higher possibility of success as compared to mono-targeted therapy. Some of the rationally designed multi-targeted drugs made by pharmaceutical companies are lapatinib, sunitinib and sorafenib. These drugs have added a little to the overall survival of the patients. However, they produce numerous side effects when taken for a long period of time. Additionally, these drugs are highly expensive and cannot be afforded by more than 80% of the world population. For example, a combination of Erbitux and Avastin for colon cancer treatment is known to make the therapy worst rather than better (Mayer 2009).

It is estimated that as much as 90% of cancer-related deaths are linked with inappropriate lifestyle and diet. For example, tobacco use and food habits significantly contribute to the development of gastric cancer (Pavithra et al. 2018). The association of lifestyle factors with cancer incidence is reported for the cancer of the bladder (Fankhauser and Mostafid 2018), breast (Bougnoux et al. 2010), gastrointestinal tract (Bjelakovic et al. 2008), kidney (Tahbaz et al. 2018), lung (Cranganu and Camporeale 2009; Goralczyk 2009), and prostate (Er et al. 2017). Implication of these statements suggests that much of cancer can be prevented by changing lifestyle and food habits. For example, the consumption of foods rich in vegetables and fruits is negatively related with the cancer risk (Block et al. 1992; Steinmetz and Potter 1996; Reddy et al. 2003; Benetou et al. 2008; Freedman et al. 2008). Approximately 35% of cancer deaths can be avoided by the use of the appropriate nutrition (Doll and Peto 1981; Hardy et al. 2003). Further, appropriate diet can help to reduce as much as 90% of certain cancers (Doll and Peto 1981; Hardy et al. 2003). The dietary intake of vegetables, fruits, and grains reduces the incidence of ovarian cancer in women (Plagens-Rotman et al. 2018).

During recent years, an emphasis has been given to the use of nutritional agents called nutraceuticals for cancer prevention and treatment. To our knowledge, the word nutraceutical (nutrition + pharmaceutical) was first coined in 1989 by Stephen L. DeFelice (Brower 1998; Kalra 2003). The food or food component that can provide health benefits is referred to as nutraceuticals (Brower 1998; Zeisel 1999). The Mother Nature is a great source of nutraceuticals. Nutraceuticals have demonstrated potential against breast cancer, colorectal cancer, pancreatic cancer, prostate cancer, squamous cell carcinoma, and many others (Gupta et al. 2010). Nutraceuticals can target multi-steps of tumor development. The nutraceuticals are diverse regarding their chemical

structures. A number of epidemiological studies support the benefits of nutraceuticals in the cancer prevention and cure.

2 Anticancer Activities of Nutraceuticals

2.1 Molecular and Cellular Level

The molecular basis for the anticancer activities of nutraceuticals is relatively well explored. We now know that nutraceuticals can hit the cancer cells at the molecular, cellular, animal, and human levels. The common cancer-related molecular targets modulated by nutraceuticals include growth factor receptors [epidermal growth factor receptor (EGFR), insulin-like growth factor 1 receptor (IGF1-R), vascular endothelial growth factor receptor (VEGFR)], pro-inflammatory mediators [5-lipoxygenase (5-LOX), cyclooxygenase-2 (COX-2), interleukins, tumor necrosis factor alpha (TNF-α)], protein kinases [5′ adenosine monophosphate-activated protein kinase (AMPK), breakpoint cluster region-Abelson murine leukemia viral oncogene (Bcr-abl), mammalian target of rapamycin (mTOR), phosphoinositide 3-kinase (PI3K), Ras/Raf], and transcription factors [activator protein 1 (AP-1), β-catenin/Wnt, nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), nuclear factor erythroid 2 (NFE2)-related factor 2 (Nrf2), peroxisome proliferator-activated receptor gamma (PPAR-γ), sonic hedgehog, and signal transducer and activator of transcription 3 (STAT3)]. Additionally, nutraceuticals are known to trigger proteasome and epigenetic changes leading to the cancer prevention and treatment (Pandey et al. 2017). Being a crucial regulator of tumorigenic proteins, transcription factors represent an important target of nutraceuticals (Shishodia et al. 2007b). Among all nutraceuticals, much is known about curcumin. Chemically, curcumin is a polyphenol derived from the Indian spice turmeric (Gupta et al. 2012). This polyphenol can suppress NF-κB activation induced by TNF-α in leukemia cells by inhibiting IκBα phosphorylation and degradation (Singh and Aggarwal 1995). Curcumin also suppresses TNF-α-induced COX-2 expression and NF-κB activation in human colonic epithelial cells (Plummer et al. 1999). The polyphenol also suppressed IκB degradation and the activities of NF-κB-inducing kinase (NIK) and IκB kinase (IKK) that may contribute to its inhibitory effects on NF-κB activation. The potential of curcumin in suppressing proliferation and metastasis and inducing apoptosis is mediated through its negative effects on NF-κB activation (Bharti et al. 2003, Philip and Kundu 2003). Curcumin can also sensitize cancer cells to chemotherapeutic agents through its inhibitory effects on NF-κB activation (Kunnumakkara et al. 2009). Apart from transcription factors, curcumin can also modulate the activities of protein kinases. For example, curcumin downregulated EGFR and the activity of extracellular signal-regulated kinase 1/2 (ERK1/2) in pancreatic and lung cancer cells (Lev-Ari et al. 2006). In malignant glioma cells, curcumin suppresses PI3K/AKT pathway (Aoki et al. 2007). Other protein kinases negatively regulated by curcumin include the phosphorylase kinase, pp60c-src tyrosine kinase, protein kinase C, and protamine kinase (Shishodia et al. 2007b).

Bharangin is a poorly studied diterpenoid quinone methide that is derived from *Pygmacopremna herbacea*. Our group recently demonstrated the anticancer activities of this diterpenoid against breast cancer (Awasthee et al. 2018). Moreover, okadaic acid-induced NF-κB activation was also suppressed by this diterpenoid. For the first time, we also demonstrated that the diterpenoid modulates the expression of long noncoding ribonucleic acids (lncRNAs). While the expression of tumor suppressor lncRNAs such as growth arrest specific-5 (GAS-5) and maternally expressed-3 (MEG-3) was induced, the expression of H19 (oncogenic lncRNA) was suppressed by the diterpenoid. It is likely that bharangin exhibits anticancer activities by modulating lncRNA expression and abrogating NF-κB activation. We are further exploring the in-depth mechanism for the activities of bharangin against breast cancer.

Resveratrol is a polyphenol and phytoalexin, the major source of which is grapes (Gupta et al. 2011). This phytoalexin suppresses constitutive NF-κB activation and induces pro-apoptotic activities in breast (Banerjee et al. 2002) and pancreatic cancer cells (Mouria et al. 2002). Sanguinarine is an alkaloid that suppresses NF-κB activation in response to inducers such as interleukin (IL)-1, okadaic acid, phorbol ester, and TNF (Chaturvedi et al. 1997). Caffeic acid phenethyl ester (CAPE) suppresses NF-κB activity in a direct manner. More specifically, this agent inhibits the binding between p65-p50 complex and DNA and leading to the suppression of NF-κB-dependent target gene transcription (Natarajan et al. 1996). Epigallocatechin gallate (EGCG) is an antioxidant chiefly present in green tea. This antioxidant suppresses NF-κB activation and malignant transformation of mouse JB6 cell line (Nomura et al. 2000). EGCG also suppresses p65 acetylation and NF-κB-associated gene expression induced by a range of stimuli (Choi et al. 2009b). Gallic acid obtained from gallnuts, green tea, oak bark, and sumac can also abrogate NF-κB activity (Choi et al. 2009c).

The nutraceuticals can also induce cell cycle arrest in cancer cells. The bark of *Boswellia serrata* is a rich source for acetyl-11-keto-beta-boswellic acid (AKBA) which is a pentacyclic triterpene. This polyphenol exhibits anticancer activities against colon cancer cells by inducing G1 phase cell cycle arrest (Liu et al. 2006). Similarly, a decrease in the activities of cyclin-D1 and p21-activated kinase (PAK)-1 by curcumin was associated with its ability to suppress G1/S

phase transition (Cai et al. 2009). β-Escin also induces G1/S phase cell cycle arrest in HT-29 colon cancer cells (Patlolla et al. 2006). Similarly, thymoquinone induces G1/S phase cell cycle arrest in prostate cancer cells (Kaseb et al. 2007). Concomitant with these observations, an upregulation in the expression of p21 and p27 while downregulation in the expression of androgen receptor and E2F-1 was observed (Kaseb et al. 2007). Other nutraceuticals known to exhibit antiproliferative effects by the induction of cell cycle arrest include berberine (Liu et al. 2009), deguelin (Murillo et al. 2009), emodin (Kuo et al. 2002), fisetin (Khan et al. 2008), guggulsterone (Shishodia et al. 2007a), piceatannol (Lee et al. 2009), silibinin (Mateen et al. 2010), silymarin (Ramakrishnan et al. 2009), sulforaphane (Bryant et al. 2010), and quercetin (Hung 2007). In human colon cancer cells, fisetin downregulated COX-2 expression and inhibited prostaglandin E_2 secretion (Suh et al. 2009). This was accompanied by decreased activities of wint (Wnt) signaling, EGFR, and NF-κB and a concomitant suppression in cyclin-D1 expression (Suh et al. 2009). Some nutraceuticals have also been shown to suppress cancer cell proliferation by suppressing NF-κB activation and the gene products regulated by this transcription factor. Examples of such nutraceuticals are anacardic acid (Sung et al. 2008), diosgenin (Shishodia and Aggarwal 2006), isoeoxyelephantopin (Ichikawa et al. 2006), noscapine (Sung et al. 2010), pinitol (Sethi et al. 2008), and ursolic acid (Shishodia et al. 2003).

The invasion and metastasis which are the major causes of cancer-related deaths can also be suppressed by nutraceuticals. The molecular basis for the negative effects of nutraceuticals on the invasiveness of cancer cells is most likely through modulation of matrix metalloproteinases (MMPs) and inhibition of NF-κB activation. For example, resveratrol can suppress the migration and invasion of lung cancer cells (Liu et al. 2010). The suppression in invasion activity by resveratrol was accompanied by the suppressed activity of NF-κB and reduced expression of MMPs (Liu et al. 2010). Similarly, silibinin inhibited the invasion of SCC-4 tongue cancer and A459 lung cancer cells (Chu et al. 2004, Chen et al. 2006). This was accompanied by suppression in the expression of MMP-2 and u-PA and increased expression of tissue inhibitor of metalloproteinase (TIMP)-2 and plasminogen activator inhibitor-1 (PAI-1) (Chu et al. 2004, Chen et al. 2006). The invasiveness of MCF-7 breast cancer cells induced by phorbol myristate acetate (PMA) was suppressed by silibinin possibly through its inhibitory effects on MMP-9 expression (Lee et al. 2007). Other nutraceuticals known to suppress cancer cell invasion include lycopene (Huang et al. 2005), myricetin (Ko et al. 2005), piperine (Pradeep and Kuttan 2004), quercetin (Vijayababu et al. 2006), and sanguinarine (Choi et al. 2009a).

2.2 Animal Level

The anticancer potential of nutraceuticals is demonstrated in cancer-bearing rodent models. For example, curcumin inhibited the ovarian tumor growth in orthotopic mice model most likely by negatively regulating the activities of NF-κB and STAT3 pathway (Lin et al. 2007). In mice implanted with Caki-I renal carcinoma cells, caffeic acid suppressed hypoxia-inducible factor-1 (HIF-1) and VEGF expression, angiogenesis, and vascularization (Jung et al. 2007). Capsaicin abrogates skin tumor formation and NF-κB activation induced by PMA in mice (Han et al. 2001). Silibinin suppresses tumor growth in mice model implanted with human colorectal carcinoma (Singh et al. 2008). Silibinin also induced antiangiogenic activities and downregulated the expression of COX, HIF-1α, nitric oxide synthase (NOS), and VEGF in nude mice (Singh et al. 2008). Diallyl sulfide can reduce the VEGF level in the serum of mice implanted with B16F-10 melanoma cells (Thejass and Kuttan 2007). Ursolic acid also suppresses capillary formation in C57BL/6 mice implanted with melanoma cells (Kanjoormana and Kuttan 2010). Ursolic acid can also suppress the levels of nitric oxide (NO) pro-inflammatory cytokines and VEGF in the serum of tumor-bearing animals (Kanjoormana and Kuttan 2010). Sulforaphane can inhibit lung metastasis and the activation of MMPs in the mice model of melanoma (Thejass and Kuttan 2006). Vanillin suppresses tumor cell angiogenesis induced by hepatocyte growth factor in a mouse model (Lirdprapamongkol et al. 2009). The suppression in angiogenesis by vanillin was mediated through its inhibitory effects on PI3K/AKT signaling and VEGF expression (Lirdprapamongkol et al. 2009).

2.3 Clinical Potential of Nutraceuticals

The pleiotropic activities of nutraceuticals have provided a basis for examining their clinical potential for cancer prevention and treatment. The pharmacokinetics, safety, and efficacy of nutraceuticals have been demonstrated in cancer patients. Nutraceuticals are known to modulate molecular targets such as 8-hydroxy-2′ -deoxyguanosine (8-OHdG), AMPK, BCL-2-associated X protein (Bax), B-cell lymphoma 2 (Bcl-2), caspases, COX-2, glutathione (GSH), glutathione S-transferase (GST), intercellular adhesion molecule (ICAM), IGF-1, insulin-like growth factor binding protein-3 (IGFBP-3), IKKβ, interleukins, kelch-like ECH-associated protein 1 (Keap-1), pyrimido[1,2-a]-purin-10(3H)-one (M1G), MMPs, malondialdehyde (MDA), NF-κB, Notch, Nrf-2, p53, phosphorylated protein kinase strain Ak thymoma (pAkt), peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α), prostaglandin E_2 (PGE_2), prostate-specific antigen (PSA), STAT3, Ras

association domain-containing family 1 alpha (Rassf-1α), sirtuin 1 (SIRT-1), TNF, vascular cell adhesion molecule 1 (VCAM-1), VEGF, and wingless/integrated (Wnt) in cancer patients (Gupta et al. 2013; Pandey et al. 2017). In the clinical trial, nutraceuticals have been used both individually and in combination. Curcumin is probably one of the extensively studied nutraceuticals in the human subjects. The promising effects of this nutraceutical have been reported in patients with bladder, breast, cervical, colorectal, liver, oral, skin, and pancreatic cancer (Hsu and Cheng 2007, Gupta et al. 2013). Curcumin's anticancer potential in cancer patients was probably first demonstrated in 1987 in a clinical trial comprising of 62 patients (Kuttan et al. 1987). The daily intake of 3.6 g curcumin for 29 days reduces inducible prostaglandin E_2 (PGE_2) production in blood samples of colorectal cancer (CRC) patients (Sharma et al. 2004). In phase II clinical trial, curcumin's potential in 25 advanced pancreatic cancer patients was evaluated. Curcumin was administered orally at 8 g per day (Dhillon et al. 2008). While limited absorption was observed, curcumin was well-tolerated and produced beneficial effects in some patients (Dhillon et al. 2008).

Resveratrol has demonstrated therapeutic potential in patients with breast cancer, multiple myeloma, and colon cancer. In one study, the effects of resveratrol on prostaglandin PGE_2 expression and DNA methylation were evaluated in 39 women with breast cancer (Rasyid and Lelo 1999). Resveratrol was administered to the patients at 5 mg and 50 mg/day for 12 weeks. The DNA methylation of cancer-related genes [adenomatous polyposis coli (APC), CCND-2, p16, RASSF-1α] was evaluated after resveratrol administration. Resveratrol was found to significantly suppress RASSF-1α methylation. Furthermore, the level of PGE_2 was also decreased in the nipple aspirate fluid of patients. These observations provide evidence for the health benefits of resveratrol against breast cancer (Rasyid and Lelo 1999). More studies involving a larger cohort of patients will further confirm the potential of resveratrol against breast cancer (Rasyid and Lelo 1999). When administered in combination with ellagic acid and quercetin, resveratrol was found safe and well tolerated in prostate cancer patients (Zuccotti et al. 2009).

Capsaicin is known to provide temporary relief in cancer patients with oral mucositis pain (Pyun et al. 2008). The topical application of capsaicin also reduces neuropathic pain in cancer patients post-surgery (Ellison et al. 1997). Ginger, the commonly used spice in the Indian kitchen, has been reported to reduce chemotherapy-associated nausea and vomiting in cancer patients (Levine et al. 2008, Pillai et al. 2011). Flavopiridol is an alkaloid obtained from the bark of *Dysoxylum binectariferum*. This alkaloid can enhance the efficacy of imatinib in hematological malignancies (Bose et al. 2012). In combination with bortezomib, flavopiridol is well tolerated and effective in patients with indolent non-Hodgkin lymphoma of relapsed/refractory multiple myeloma (Holkova et al. 2014). Flavopiridol also enhances the potency of doxorubicin in advanced sarcoma patients (Luke et al. 2012).

3 Concluding Remarks and Future Directions

Nutraceuticals have demonstrated the potential for cancer prevention and cure both by preclinical and clinical studies. The therapeutic potential of nutraceuticals against chronic diseases is based on their ability to disrupt several cancer-related cell signaling pathways. Nutraceuticals have been consumed since ancient time and thus the safety is not a concern. However, the total number of clinical studies on nutraceuticals is far less as compared to preclinical studies. Moreover, most studies are focused on killing the cancer cells. Over 90% of cancer-related deaths are because of the spread of the tumor to secondary organs. More studies are required to examine if nutraceuticals can suppress metastasis of tumor cells. How nutraceuticals discriminate between normal and cancer cells should also be thoroughly examined. Strategies are also required to enhance the bioavailability of nutraceuticals. In our opinion, more emphasis on clinical research is required so that nutraceuticals can be indicated for human and animal ailments.

Acknowledgment The support in the laboratories of Gupta (ECR/2016/000034) and Sharma (ECR/2016/001863) comes from Science and Engineering Research Board. Gupta's laboratory is also supported by University Grants Commission [No. F.30-112/2015 (BSR)]. SM is an ICMR-JRF (3/1/3/JRF-2016/LS/HRD-65-80388).

References

Aoki H, Takada Y, Kondo S et al (2007) Evidence that curcumin suppresses the growth of malignant gliomas in vitro and in vivo through induction of autophagy: role of Akt and extracellular signal-regulated kinase signaling pathways. Mol Pharmacol 72 (1):29–39

Awasthee N, Rai V, Verma SS et al (2018) Anti-cancer activities of Bharangin against Breast Cancer: evidence for the role of NF-kB and lncRNAs. Biochim Biophys Acta 1862(12):2738–2749

Banerjee S, Bueso-Ramos C, Aggarwal BB (2002) Suppression of 7,12-dimethylbenz(a)anthracene-induced mammary carcinogenesis in rats by resveratrol: role of nuclear factor-kappaB, cyclooxygenase 2, and matrix metalloprotease 9. Cancer Res 62(17):4945–4954

Benetou V, Orfanos P, Lagiou P et al (2008) Vegetables and fruits in relation to cancer risk: evidence from the Greek EPIC cohort study. Cancer Epidemiol Biomark Prev 17(2):387–392

Bharti AC, Donato N, Singh S et al (2003) Curcumin (diferuloylmethane) down-regulates the constitutive activation of nuclear factor-kappa B and IkappaBalpha kinase in human multiple myeloma cells, leading to suppression of proliferation and induction of apoptosis. Blood 101(3):1053–1062

Bjelakovic G, Nikolova D, Simonetti RG et al (2008) Antioxidant supplements for preventing gastrointestinal cancers. Cochrane Database Syst Rev 3:CD004183

Block G, Patterson B, Subar A (1992) Fruit, vegetables, and cancer prevention: a review of the epidemiological evidence. Nutr Cancer 18(1):1–29

Bose P, Perkins EB, Honeycut C et al (2012) Phase I trial of the combination of flavopiridol and imatinib mesylate in patients with Bcr-Abl+ hematological malignancies. Cancer Chemother Pharmacol 69(6):1657–1667

Bougnoux P, Hajjaji N, Maheo K et al (2010) Fatty acids and breast cancer: sensitization to treatments and prevention of metastatic re-growth. Prog Lipid Res 49(1):76–86

Brower V (1998) Nutraceuticals: poised for a healthy slice of the healthcare market? Nat Biotechnol 16(8):728–731

Bryant CS, Kumar S, Chamala S et al (2010) Sulforaphane induces cell cycle arrest by protecting RB-E2F-1 complex in epithelial ovarian cancer cells. Mol Cancer 9:47

Cai XZ, Wang J, Li XD et al (2009) Curcumin suppresses proliferation and invasion in human gastric cancer cells by downregulation of PAK1 activity and cyclin D1 expression. Cancer Biol Ther 8 (14):1360–1368

Chaturvedi MM, Kumar A, Darnay BG et al (1997) Sanguinarine (pseudochelerythrine) is a potent inhibitor of NF-kappaB activation, IkappaBalpha phosphorylation, and degradation. J Biol Chem 272 (48):30129–30134

Chen PN, Hsieh YS, Chiang CL et al (2006) Silibinin inhibits invasion of oral cancer cells by suppressing the MAPK pathway. J Dent Res 85(3):220–225

Choi YH, Choi WY, Hong SH et al (2009a) Anti-invasive activity of sanguinarine through modulation of tight junctions and matrix metalloproteinase activities in MDA-MB-231 human breast carcinoma cells. Chem Biol Interact 179(2–3):185–191

Choi KC, Jung MG, Lee YH et al (2009b) Epigallocatechin-3-gallate, a histone acetyltransferase inhibitor, inhibits EBV-induced B lymphocyte transformation via suppression of RelA acetylation. Cancer Res 69(2):583–592

Choi KC, Lee YH, Jung MG et al (2009c) Gallic acid suppresses lipopolysaccharide-induced nuclear factor-kappaB signaling by preventing RelA acetylation in A549 lung cancer cells. Mol Cancer Res 7(12):2011–2021

Chu SC, Chiou HL, Chen PN et al (2004) Silibinin inhibits the invasion of human lung cancer cells via decreased productions of urokinase-plasminogen activator and matrix metalloproteinase-2. Mol Carcinog 40(3):143–149

Cranganu A, Camporeale J (2009) Nutrition aspects of lung cancer. Nutr Clin Pract 24(6):688–700

Dhillon N, Aggarwal BB, Newman RA et al (2008) Phase II trial of curcumin in patients with advanced pancreatic cancer. Clin Cancer Res 14(14):4491–4499

Doll R, Peto R (1981) The causes of cancer: quantitative estimates of avoidable risks of cancer in the United States today. J Natl Cancer Inst 66(6):1191–1308

Ellison N, Loprinzi CL, Kugler J et al (1997) Phase III placebo-controlled trial of capsaicin cream in the management of surgical neuropathic pain in cancer patients. J Clin Oncol 15(8):2974–2980

Er V, Lane JA, Martin RM et al (2017) Barriers and facilitators to healthy lifestyle and acceptability of a dietary and physical activity intervention among African Caribbean prostate cancer survivors in the UK: a qualitative study. BMJ Open 7(10):e017217

Fankhauser CD, Mostafid H (2018) Prevention of bladder cancer incidence and recurrence: nutrition and lifestyle. Curr Opin Urol 28 (1):88–92

Freedman ND, Park Y, Subar AF et al (2008) Fruit and vegetable intake and head and neck cancer risk in a large United States prospective cohort study. Int J Cancer 122(10):2330–2336

Goralczyk R (2009) Beta-carotene and lung cancer in smokers: review of hypotheses and status of research. Nutr Cancer 61(6):767–774

Gupta SC, Kim JH, Prasad S et al (2010) Regulation of survival, proliferation, invasion, angiogenesis, and metastasis of tumor cells through modulation of inflammatory pathways by nutraceuticals. Cancer Metastasis Rev 29(3):405–434

Gupta SC, Kannappan R, Reuter S et al (2011) Chemosensitization of tumors by resveratrol. Ann N Y Acad Sci 1215:150–160

Gupta SC, Patchva S, Koh W et al (2012) Discovery of curcumin, a component of golden spice, and its miraculous biological activities. Clin Exp Pharmacol Physiol 39(3):283–299

Gupta SC, Patchva S, Aggarwal BB (2013) Therapeutic roles of curcumin: lessons learned from clinical trials. AAPS J 15(1):195–218

Han SS, Keum YS, Seo HJ et al (2001) Capsaicin suppresses phorbol ester-induced activation of NF-kappaB/Rel and AP-1 transcription factors in mouse epidermis. Cancer Lett 164(2):119–126

Hardy G, Hardy I, Ball PA (2003) Nutraceuticals--a pharmaceutical viewpoint: part II. Curr Opin Clin Nutr Metab Care 6(6):661–671

Holkova B, Kmieciak M, Perkins EB et al (2014) Phase I trial of bortezomib (PS-341; NSC 681239) and "nonhybrid" (bolus) infusion schedule of alvocidib (flavopiridol; NSC 649890) in patients with recurrent or refractory indolent B-cell neoplasms. Clin Cancer Res 20(22):5652–5662

Hsu CH, Cheng AL (2007) Clinical studies with curcumin. Adv Exp Med Biol 595:471–480

Huang CS, Shih MK, Chuang CH et al (2005) Lycopene inhibits cell migration and invasion and upregulates Nm23-H1 in a highly invasive hepatocarcinoma, SK-Hep-1 cells. J Nutr 135(9):2119–2123

Hung H (2007) Dietary quercetin inhibits proliferation of lung carcinoma cells. Forum Nutr 60:146–157

Ichikawa H, Nair MS, Takada Y et al (2006) Isodeoxyelephantopin, a novel sesquiterpene lactone, potentiates apoptosis, inhibits invasion, and abolishes osteoclastogenesis through suppression of nuclear factor-kappaB (nf-kappaB) activation and nf-kappaB-regulated gene expression. Clin Cancer Res 12(19):5910–5918

Jung JE, Kim HS, Lee CS et al (2007) Caffeic acid and its synthetic derivative CADPE suppress tumor angiogenesis by blocking STAT3-mediated VEGF expression in human renal carcinoma cells. Carcinogenesis 28(8):1780–1787

Kalra EK (2003) Nutraceutical-definition and introduction. AAPS PharmSci 5(3):E25

Kanjoormana M, Kuttan G (2010) Antiangiogenic activity of ursolic acid. Integr Cancer Ther 9(2):224–235

Kaseb AO, Chinnakannu K, Chen D et al (2007) Androgen receptor and E2F-1 targeted thymoquinone therapy for hormone-refractory prostate cancer. Cancer Res 67(16):7782–7788

Khan N, Afaq F, Syed DN et al (2008) Fisetin, a novel dietary flavonoid, causes apoptosis and cell cycle arrest in human prostate cancer LNCaP cells. Carcinogenesis 29(5):1049–1056

Ko CH, Shen SC, Lee TJ et al (2005) Myricetin inhibits matrix metalloproteinase 2 protein expression and enzyme activity in colorectal carcinoma cells. Mol Cancer Ther 4(2):281–290

Kunnumakkara AB, Diagaradjane P, Anand P et al (2009) Curcumin sensitizes human colorectal cancer to capecitabine by modulation of cyclin D1, COX-2, MMP-9, VEGF and CXCR4 expression in an orthotopic mouse model. Int J Cancer 125(9):2187–2197

Kuo PL, Lin TC, Lin CC (2002) The antiproliferative activity of aloe-emodin is through p53-dependent and p21-dependent apoptotic pathway in human hepatoma cell lines. Life Sci 71(16):1879–1892

Kuttan R, Sudheeran PC, Josph CD (1987) Turmeric and curcumin as topical agents in cancer therapy. Tumori 73(1):29–31

Lee SO, Jeong YJ, Im HG et al (2007) Silibinin suppresses PMA-induced MMP-9 expression by blocking the AP-1 activation via MAPK signaling pathways in MCF-7 human breast carcinoma cells. Biochem Biophys Res Commun 354(1):165–171

Lee YM, Lim DY, Cho HJ et al (2009) Piceatannol, a natural stilbene from grapes, induces G1 cell cycle arrest in androgen-insensitive DU145 human prostate cancer cells via the inhibition of CDK activity. Cancer Lett 285(2):166–173

Lev-Ari S, Starr A, Vexler A et al (2006) Inhibition of pancreatic and lung adenocarcinoma cell survival by curcumin is associated with increased apoptosis, down-regulation of COX-2 and EGFR and inhibition of Erk1/2 activity. Anticancer Res 26(6B):4423–4430

Levine ME, Gillis MG, Koch SY et al (2008) Protein and ginger for the treatment of chemotherapy-induced delayed nausea. J Altern Complement Med 14(5):545–551

Lin YG, Kunnumakkara AB, Nair A et al (2007) Curcumin inhibits tumor growth and angiogenesis in ovarian carcinoma by targeting the nuclear factor-kappaB pathway. Clin Cancer Res 13 (11):3423–3430

Lirdprapamongkol K, Kramb JP, Suthiphongchai T et al (2009) Vanillin suppresses metastatic potential of human cancer cells through PI3K inhibition and decreases angiogenesis in vivo. J Agric Food Chem 57(8):3055–3063

Liu JJ, Huang B, Hooi SC (2006) Acetyl-keto-beta-boswellic acid inhibits cellular proliferation through a p21-dependent pathway in colon cancer cells. Br J Pharmacol 148(8):1099–1107

Liu Z, Liu Q, Xu B et al (2009) Berberine induces p53-dependent cell cycle arrest and apoptosis of human osteosarcoma cells by inflicting DNA damage. Mutat Res 662(1–2):75–83

Liu PL, Tsai JR, Charles AL et al (2010) Resveratrol inhibits human lung adenocarcinoma cell metastasis by suppressing heme oxygenase 1-mediated nuclear factor-kappaB pathway and subsequently downregulating expression of matrix metalloproteinases. Mol Nutr Food Res 54(2):S196–S204

Luke JJ, D'Adamo DR, Dickson MA et al (2012) The cyclin-dependent kinase inhibitor flavopiridol potentiates doxorubicin efficacy in advanced sarcomas: preclinical investigations and results of a phase I dose-escalation clinical trial. Clin Cancer Res 18(9):2638–2647

Mateen S, Tyagi A, Agarwal C et al (2010) Silibinin inhibits human nonsmall cell lung cancer cell growth through cell-cycle arrest by modulating expression and function of key cell-cycle regulators. Mol Carcinog 49(3):247–258

Mayer RJ (2009) Targeted therapy for advanced colorectal cancer-more is not always better. N Engl J Med 360(6):623–625

Mouria M, Gukovskaya AS, Jung Y et al (2002) Food-derived polyphenols inhibit pancreatic cancer growth through mitochondrial cytochrome C release and apoptosis. Int J Cancer 98(5):761–769

Murillo G, Peng X, Torres KE et al (2009) Deguelin inhibits growth of breast cancer cells by modulating the expression of key members of the Wnt signaling pathway. Cancer Prev Res (Phila Pa) 2 (11):942–950

Natarajan K, Singh S, Burke TR Jr et al (1996) Caffeic acid phenethyl ester is a potent and specific inhibitor of activation of nuclear transcription factor NF-kappa B. Proc Natl Acad Sci USA 93 (17):9090–9095

Nomura M, Ma W, Chen N et al (2000) Inhibition of 12-O-tetradecanoylphorbol-13-acetate-induced NF-kappaB activation by tea polyphenols, (-)-epigallocatechin gallate and theaflavins. Carcinogenesis 21(10):1885–1890

Pandey MK, Gupta SC, Nabavizadeh A et al (2017) Regulation of cell signaling pathways by dietary agents for cancer prevention and treatment. Semin Cancer Biol 46:158–181

Patlolla JM, Raju J, Swamy MV et al (2006) Beta-escin inhibits colonic aberrant crypt foci formation in rats and regulates the cell cycle growth by inducing p21(waf1/cip1) in colon cancer cells. Mol Cancer Ther 5(6):1459–1466

Pavithra D, Gautam M, Rama R et al (2018) TGFbeta C-509T, TGFbeta T869C, XRCC1 Arg194Trp, IKBalpha C642T, IL4 C-590T Genetic polymorphisms combined with socio-economic, lifestyle, diet factors and gastric cancer risk: a case control study in South Indian population. Cancer Epidemiol 53:21–26

Philip S, Kundu GC (2003) Osteopontin induces nuclear factor kappa B-mediated promatrix metalloproteinase-2 activation through I kappa B alpha/IKK signaling pathways, and curcumin (diferulolylmethane) down-regulates these pathways. J Biol Chem 278 (16):14487–14497

Pillai AK, Sharma KK, Gupta YK et al (2011) Anti-emetic effect of ginger powder versus placebo as an add-on therapy in children and young adults receiving high emetogenic chemotherapy. Pediatr Blood Cancer 56(2):234–238

Plagens-Rotman K, Chmaj-Wierzchowska K, Pieta B et al (2018) Modifiable lifestyle factors and ovarian cancer incidence in women. Ann Agric Environ Med 25(1):36–40

Plummer SM, Holloway KA, Manson MM et al (1999) Inhibition of cyclo-oxygenase 2 expression in colon cells by the chemopreventive agent curcumin involves inhibition of NF-kappaB activation via the NIK/IKK signalling complex. Oncogene 18(44):6013–6020

Pradeep CR, Kuttan G (2004) Piperine is a potent inhibitor of nuclear factor-kappaB (NF-kappaB), c-Fos, CREB, ATF-2 and proinflammatory cytokine gene expression in B16F-10 melanoma cells. Int Immunopharmacol 4(14):1795–1803

Pyun BJ, Choi S, Lee Y et al (2008) Capsiate, a nonpungent capsaicin-like compound, inhibits angiogenesis and vascular permeability via a direct inhibition of Src kinase activity. Cancer Res 68(1):227–235

Ramakrishnan G, Lo Muzio L, Elinos-Baez CM et al (2009) Silymarin inhibited proliferation and induced apoptosis in hepatic cancer cells. Cell Prolif 42(2):229–240

Rasyid A, Lelo A (1999) The effect of curcumin and placebo on human gall-bladder function: an ultrasound study. Aliment Pharmacol Ther 13(2):245–249

Reddy L, Odhav B, Bhoola KD (2003) Natural products for cancer prevention: a global perspective. Pharmacol Ther 99(1):1–13

Sethi G, Ahn KS, Sung B et al (2008) Pinitol targets nuclear factor-kappaB activation pathway leading to inhibition of gene products associated with proliferation, apoptosis, invasion, and angiogenesis. Mol Cancer Ther 7(6):1604–1614

Sharma RA, Euden SA, Platton SL et al (2004) Phase I clinical trial of oral curcumin: biomarkers of systemic activity and compliance. Clin Cancer Res 10(20):6847–6854

Shishodia S, Aggarwal BB (2006) Diosgenin inhibits osteoclastogenesis, invasion, and proliferation through the downregulation of Akt, I kappa B kinase activation and NF-kappa B-regulated gene expression. Oncogene 25(10):1463–1473

Shishodia S, Majumdar S, Banerjee S et al (2003) Ursolic acid inhibits nuclear factor-kappaB activation induced by carcinogenic agents through suppression of IkappaBalpha kinase and p65 phosphorylation: correlation with down-regulation of cyclooxygenase 2, matrix metalloproteinase 9, and cyclin D1. Cancer Res 63(15):4375–4383

Shishodia S, Sethi G, Ahn KS et al (2007a) Guggulsterone inhibits tumor cell proliferation, induces S-phase arrest, and promotes apoptosis through activation of c-Jun N-terminal kinase, suppression of Akt pathway, and downregulation of antiapoptotic gene products. Biochem Pharmacol 74(1):118–130

Shishodia S, Singh T, Chaturvedi MM (2007b) Modulation of transcription factors by curcumin. Adv Exp Med Biol 595:127–148

Singh S, Aggarwal BB (1995) Activation of transcription factor NF-kappa B is suppressed by curcumin (diferuloylmethane) [corrected]. J Biol Chem 270(42):24995–25000

Singh RP, Raina K, Sharma G et al (2008) Silibinin inhibits established prostate tumor growth, progression, invasion, and metastasis and

suppresses tumor angiogenesis and epithelial-mesenchymal transition in transgenic adenocarcinoma of the mouse prostate model mice. Clin Cancer Res 14(23):7773–7780

Steinmetz KA, Potter JD (1996) Vegetables, fruit, and cancer prevention: a review. J Am Diet Assoc 96(10):1027–1039

Suh Y, Afaq F, Johnson JJ et al (2009) A plant flavonoid fisetin induces apoptosis in colon cancer cells by inhibition of COX2 and Wnt/EGFR/NF-kappaB-signaling pathways. Carcinogenesis 30 (2):300–307

Sung B, Pandey MK, Ahn KS et al (2008) Anacardic acid (6-nonadecyl salicylic acid), an inhibitor of histone acetyltransferase, suppresses expression of nuclear factor-kappaB-regulated gene products involved in cell survival, proliferation, invasion, and inflammation through inhibition of the inhibitory subunit of nuclear factor-kappaBalpha kinase, leading to potentiation of apoptosis. Blood 111(10):4880–4891

Sung B, Ahn KS, Aggarwal BB (2010) Noscapine, a benzylisoquinoline alkaloid, sensitizes leukemic cells to chemotherapeutic agents and cytokines by modulating the NF-kappaB signaling pathway. Cancer Res 70(8):3259–3268

Tahbaz R, Schmid M, Merseburger AS (2018) Prevention of kidney cancer incidence and recurrence: lifestyle, medication and nutrition. Curr Opin Urol 28(1):62–79

Thejass P, Kuttan G (2006) Antimetastatic activity of Sulforaphane. Life Sci 78(26):3043–3050

Thejass P, Kuttan G (2007) Antiangiogenic activity of Diallyl Sulfide (DAS). Int Immunopharmacol 7(3):295–305

Vijayababu MR, Arunkumar A, Kanagaraj P et al (2006) Quercetin downregulates matrix metalloproteinases 2 and 9 proteins expression in prostate cancer cells (PC-3). Mol Cell Biochem 287(1–2):109–116

Zeisel SH (1999) Regulation of "nutraceuticals". Science 285 (5435):1853–1855

Zuccotti GV, Trabattoni D, Morelli M et al (2009) Immune modulation by lactoferrin and curcumin in children with recurrent respiratory infections. J Biol Regul Homeost Agents 23(2):119–123

Expanding Metabolic Targets in Cancer by Select Combinations of Vitamin C and EGCG with Different Natural Compounds

Aleksandra Niedzwiecki, Bilwa Bhanap, M. Waheed Roomi, and Matthias Rath

Abstract

Worldwide, cancer is the second leading cause of death. Currently about ninety percent of cancer deaths are attributed to metastasis with no effective treatment options to control it. Current standard cancer treatments such as surgery, chemotherapy, and radiation are associated with multiple side effects. Nutraceuticals including vitamins, minerals, amino acids, and several phytochemicals derived from diet are known to have antioxidant and anticancer properties. However, individual compounds have limited anticancer efficacy as compared to their use in combinations. In this chapter, we describe the efficacy of different combinations of vitamin C and green tea polyphenol (-) epigallocatechin-3-gallate (EGCG) with other natural components and their cancer preventive mechanisms. Properly chosen micronutrient combinations can overcome the metabolic limitations of individual components and enhance the scope of their therapeutic efficacy. In this aspect, our in vitro and in vivo studies have shown that synergy-based interaction of selected micronutrients is effective in controlling key mechanisms of cancer, such as inhibition of cancer cell proliferation, invasion, metastasis, triggering apoptosis, and reducing angiogenesis, among other mechanisms. This nutrient synergy combination is safe and effective in targeting multiple mechanisms of cancer, and it should be investigated further in a clinical setting.

Keywords

Nutraceuticals · Metabolic targets in cancer · Vitamin C · EGCG · Cancer treatment

A. Niedzwiecki (✉) · B. Bhanap · M. Waheed Roomi · M. Rath
Dr. Rath Research Institute, Santa Clara, CA, USA
e-mail: author@drrath.com

1 Introduction

Cancer is a generic term describing more than 125 different types of diseases that share certain common characteristics, such as uncontrolled growth of abnormal cells, invasion, and metastasis. It is the second leading cause of death in the world, and it is estimated that by 2030 over 11 million deaths per year will be attributed to cancer alone worldwide (https://www.cancer.gov/about-cancer/understanding/statistics).

Conventional cancer treatments such as surgery, chemotherapy, and radiation have a limited efficacy and are associated with several devastating side effects (Jemal et al. 2010). A comprehensive evaluation of the benefits of chemotherapy in over 22 types of cancer showed that it can increase the chance of 5-year survival by only 2.1% in the USA and 2.6% in Australia (Morgan et al. 2004). Even the newer cancer drug regimens approved by the US Food and Drug Administration between 2002 and 2014 improved overall survival by merely 2.1 months (Fojo et al. 2014; Mayor 2018).

Since many studies show that high consumption of fruits and vegetables has been associated with prevention, inhibition, and even reversal of cancers, there has been a rapidly growing trend in cancer research to investigate natural compounds (Adlercreutz 1990; Miller 1990; Amin et al. 2009).

Since 90% of cancer deaths occur secondary to metastasis, any successful anticancer treatment has to target this stage of cancer development (Mehlen and Puisieux 2006; Taketo 2011). Rath and Pauling proposed a universal approach to controlling cancer by ensuring optimal synthesis and integrity of connective tissue surrounding cancer cells and, as such, reinforcing the natural barrier curbing their spread and invasion. This process is largely dependent upon nutrients not produced in the human body, such as vitamin C and lysine (Rath and Pauling 1990; Rath and Pauling 1992). Studies following this direction have confirmed the efficacy of this approach. Among others, a study with a mouse model

R. C. Gupta et al. (eds.), *Nutraceuticals in Veterinary Medicine*, https://doi.org/10.1007/978-3-030-04624-8_42

mimicking human metabolism in respect to a lack of endogenous ascorbate synthesis (GULO-/-) demonstrated that dietary vitamin C can inhibit tumor growth and metastasis by facilitating the formation of the collagen barrier around tumors (Cha et al. 2011). This direction also led to the development of a new strategy aimed at simultaneous control of key mechanisms of cancer by using specific combinations of natural compounds and enhancing their efficacy through mutual synergy (Niedzwiecki et al. 2010).

Most published scientific research on the anticancer effects of micronutrients has been conducted using single compounds, and vitamin C was quite extensively studied in various aspects of cancer (Yun et al. 2015; Gonzalez and Miranda-Massari 2014; Cha et al. 2013). As such, many clinical studies investigated effective vitamin C dosage ranges, methods of its delivery (IV or oral intake), and other aspects (Gonzalez and Miranda-Massari 2014). However, there are not many studies focusing on enhancing vitamin C's anticancer effects by combining it with other natural compounds. In addition to vitamin C, there has been steadily increasing interest in dietary phytochemicals in the prevention and treatment of cancers (Donaldson 2004). Although some of the phytochemicals such as EGCG, curcumin, resveratrol, and quercetin have shown anticancer properties in vitro, they have not been very potent as individual compounds in in vivo anticancer studies. This might relate to their metabolism and limited bioavailability after consumption.

In our "nutrient synergy" approach to cancer, we studied different combinations of vitamin C and EGCG with several micronutrients. This pioneering research conducted for over a decade has resulted in developing nutrient combinations with pleiotropic mechanistic effects against cancer (i.e., cell growth, invasion, metastasis, angiogenesis, and apoptosis). These could be achieved with lower doses of individual compounds and, as such, maintaining cellular metabolic balance under control.

This chapter evaluates the anticancer efficacy of vitamin C and (-) epigallocatechin-3-gallate (EGCG) from green tea used in combinations with other individual nutrients, nutrient mixtures, and in synergy with select natural compounds. All of these studies provide further supportive evidence that diet-based protection against cancer may partly derive from synergy among dietary phytochemicals directed against specific molecular targets in responsive cancer cells and provide the basis to explore this principle in designing safe and effective nutrient combinations against cancer.

2 Vitamin C in Combination with Individual Natural Compounds

The majority of micronutrients studied in combination with vitamin C for their anticancer effects were antioxidants and, as such, targeted redox-related cellular mechanisms known to affect cancer cells metabolic pathways involved in cellular growth and death cycles. Experimental and human studies with antioxidant combinations in the treatment of cancer reviewed by Prasad (2004) highlighted the need for nutritional protocols, including high doses of multiple dietary antioxidants and their derivatives, in order to improve oncologic outcomes and decrease the toxicity of conventional treatments. Furthermore, clinical studies involving 8521 patients using beta-carotene; vitamins A, B, C, D_3, E, and K3; and selenium, cysteine, and glutathione as single agents or as an adjunct to radiation or chemotherapy demonstrated that these antioxidants and nutrients did not interfere with therapeutic modalities for cancer but rather increased the anticancer activity and decreased their side effects (Simone et al. 2007).

2.1 Vitamin C with Selenium

Zheng et al. (2002) investigated the effects and mechanisms of the combination of ascorbic acid and sodium selenite on growth and redifferentiation of human gastric cancer cells. They found that the combination induced the redifferentiation of human gastric cancer cells and inhibited cell growth by enhancing the antioxidant enzymes, inducing the formation of H_2O_2, and altering the cell redox status.

2.2 Vitamin C with Copper

A mixture of vitamin C and cupric sulfate administered orally significantly inhibited human mammary tumor growth in mice (Chen et al. 2015). Satoh et al. reported that cytotoxicity induced by sodium ascorbate or sodium 5,6-benzylidene-L-ascorbate and inter-nucleosomal DNA cleavage in human promyelocytic leukemic HL-60 cells were significantly enhanced by the addition of either CuCl or $CuCl_2$. In contrast, addition of either $FeCl_2$ or $FeCl_3$ inhibited the cytotoxic activity of ascorbate. Copper also stimulated gallate or caffeate-induced apoptotic cell death, whereas iron was inhibitory (Satoh and Sakagami 1997a). The apoptotic effects of sodium ascorbate in addition to other antioxidants (gallic acid, n-propyl gallate, and caffeic acid) in human promyelocytic leukemia HL-60 cells were enhanced by $CuCl_2$ or deferoxamine mesylate, an iron chelator, but were reduced by $FeCl_3$. ESR spectroscopy showed that both $CuCl_2$ and $FeCl_3$ enhanced the ascorbyl radical intensity but reduced the gallate and caffeate radical intensity. They concluded that copper and iron ions modified the cytotoxic activity of these antioxidants differently, and their radical intensity was not the sole determinant of cytotoxic activity (Satoh et al. 1997).

2.3 Vitamin C with Glutathione

To address the efficacy of high-dose intravenous ascorbic acid (AA) and intravenous glutathione (GSH), which are often co-administered to cancer patients with unclear efficacy and drug-drug interaction, Chen et al. (2011) tested the effects of AA, GSH, and their combination on cytotoxicity of ten cancer cell lines. Although all treatments (AA, GSH, and AA + GSH) improved survival rate, AA + GSH inhibited the cytotoxic effect of AA alone and failed to provide further survival benefit. Thus, the pro-oxidative anticancer mechanism of pharmacologic AA was confirmed, and the administration of GSH with AA was shown to provide no additional benefit compared with AA alone. The antagonism between ascorbate and glutathione suggests that intravenous AA and high doses of GSH should not be co-administered to cancer patients on the same day. However, the study has been rebuked by others as using an inadequate animal model (athymic mice that make their own vitamin C) and using inappropriate levels of glutathione and, therefore, having little relevance to human cancer patients (Dettman et al. 2012).

2.4 Vitamin C with Methylsulfonylmethane (MSM)

In addition to providing pain relief to cancer patients, a sulfur organic compound, methylsulfonylmethane (MSM), is considered to potentiate anticancer effects of most vitamins and other nutrients, such as vitamin C; coenzyme Q10; all B vitamins; vitamins A, D, and E; amino acids; selenium; calcium; magnesium; and many others. It has been shown that MSM improves the cellular uptake of these nutrients and can prolong lives (Mindell 1997; Ley 1998; Jacob 1983).

2.5 Vitamin C with Retinoic Acid

Retinoic acid, ascorbic acid, and vitamins D and E have been implicated in prevention of the development and progression of breast cancer. Ascorbic acid combined with retinoic acid demonstrated significantly higher inhibition of human breast cancer MCF-7 cell proliferation than did vitamin C or retinoic acid independently (75.7% compared to 20.7% and 23.3%, respectively) (Kim et al. 2006). In addition, evaluation of the gene expression profiles of the treated and untreated cells by radioactive cDNA microarray analysis revealed that 29 genes were upregulated and 38 genes were downregulated after the combination treatment (Kim et al. 2006). The nature of these genes suggests that the mechanism by which retinoid acid and ascorbic acid act synergistically in inhibiting human breast cancer cell proliferation may involve the expression of genes that induce differentiation and block cell growth, in addition to the upregulation of antioxidant enzymes and proteins involved in apoptosis, cell cycle regulation, and DNA repair by altering their gene expression.

2.6 Vitamin C with Quercetin

Quercetin as a multiple cell signaling inhibitor and vitamin C as an antioxidant with potent anticancer activity were combined to investigate their effects on oxidative stress levels in breast cancer cells. The study investigated the induction of nuclear factor erythroid 2-related factor 2 (Nrf2) at the gene and protein levels as a mediator of cellular oxidative stress. The results showed that the combination of these two compounds has a significant effect on Nrf2 genes, especially in MDA-MB231 cells which have higher expression of this factor than the MCF7 cell line and can modulate endogenous levels of oxidative stress (ROS). These aspects are important in decreasing side effects of chemotherapy and overcoming chemo drug resistance (Mostafafavi-Pour et al. 2017).

2.7 Vitamin C with Select Amino Acids and Plant Extracts

Satoh and Sakagami (1997b) investigated the effect of 20 amino acids on the radical intensity of four antioxidants, sodium L-ascorbate, sodium 5,6-benzylidene-L-ascorbate, gallic acid, and caffeic acid, using ESR spectroscopy. Results demonstrated that methionine and methional did not significantly affect the radical intensity of these antioxidants while methionine sulfoxide slightly enhanced the radical intensity of sodium ascorbate and sodium 5,6-benzylidene-L-ascorbate, but not that of gallic acid and caffeic acid. Cysteine, N-acetyl cysteine, and glutathione significantly reduced the radical intensity and cytotoxic activity of these antioxidants except for sodium 5,6-benzylidene-L-ascorbate. The other amino acids were inactive. Furthermore, the study indicates that these antioxidants induce cytotoxicity via their prooxidant action (Satoh and Sakagami 1997c).

Konno (2007) tested eight commercially available natural products for possible effects on the growth of human bladder cancer T24 cells. He found that of these products, only two mushroom extracts, GD- or PL-fractions, induced significant cell death. Furthermore, when nontoxic concentrations of the GD- or PL-fractions were combined with a nontoxic concentration of vitamin C, the combination became highly cytotoxic, resulting in >90% cell death. Maitake mushroom (GD fraction) also induced apoptosis in human prostate cancer PC-3 cells, with >95% cell death at 480 μg/ml (Fullerton

et al. 2000). The same result was obtained with a much lower concentration of GD (30–60 μg/ml) combined with 200 μM ascorbic acid (Fullerton et al. 2000). Li et al. (2010) investigated the effects of theaflavin-3-3′-digallate, a major theaflavin monomer in black tea, in combination with ascorbic acid, and ECGC, the main polyphenol in green tea, on human lung adenocarcinoma SPC-A-1 cellular viability and cell cycle. MTT assay showed that the 50% inhibitory concentrations of theaflavin-3-3′-digallate, EGCG, and ascorbic acid on SPC-A-1 cells were 4.78 μmol/L, 4.90 μmol/L, and 30.62 μmol/L, respectively. Both black tea and green tea polyphenols in combination with ascorbic acid at a molar ratio of 1:6 demonstrated synergistic inhibition of SPC-A-1 cell proliferation (54.4% and 45.5%, respectively), and significantly held SPC-A-1 cells in G_0/G_1 phase, suggesting that the combination with ascorbic acid enhances their anticancer activity (Li et al. 2010).

2.8 Vitamin C with Calcitriol

Calcitriol, the hormonal form of vitamin D_3, sensitizes breast cancer cells to reactive oxygen species (ROS)-dependent cytotoxicity induced by various anticancer modalities. Calcitriol combined with H_2O_2, which is released during IV vitamin C treatment, increased the breast cancer MCF-7 cell death rate by 78% compared to H_2O_2 alone (Weitsman et al. 2005). Some additives, however, actually inhibited the effectiveness of vitamin C. For example, vitamin C lost 95% of its cancer killing power when glutathione was co-injected, and the tumor shrinkage normally seen with vitamin C alone was inhibited (Chen et al. 2011).

2.9 Vitamin C with Vitamin K3 (Menadione)

Combinations of vitamin C with vitamin K3 have been researched as a multipronged strategy against a variety of tumor cells. The precise pathways by which interaction of these nutrients displays anticancer activities are not well established. However, it has been shown that it involves the production of reactive oxygen species (ROS) by redox cycling of vitamin C in association with K3 which may surpass the cancer cellular defense system and induce cell death. Studies have also shown that co-administration of vitamin C and vitamin K3 in a ratio 100:1, respectively, results in a necrotic type of cell death by autoschizis (Verrax et al. 2003). In addition, co-administration of these vitamins reactivated acid and alkaline DNAses inhibited in cancer cells, which was associated with inhibition of tumor growth and metastasis both in vitro and in vivo (Taper 2008).

2.10 Vitamin C with Vitamin E

A combination of vitamin E (alpha-tocopherol acetate) and vitamin C showed radioprotective and pro-apoptotic effects when administered to rats before and after exposure to Y-irradiation (Vasileva et al. 2016). A small clinical trial of vitamin C and E complex showed that short-term supplementation with these nutrients has protective effect against radiation-induced xerostomia in patients with head and neck cancer. The final follow-up showed that the vitamin intake did not affect overall and disease-free survival (Chung et al. 2016).

Overall clinical evidence of anticancer efficacy of vitamin C taken together with vitamin E has brought mixed results, with some studies showing beneficial effects and others not (Coulter et al. 2006; Gaziano et al. 2009). The differences related to the doses and sources of these nutrients used in the studies, populations tested, methods of recoding data, duration of studies, types of cancer, etc. could lead to definite conclusions.

3 Vitamin C in Combination with Multiple Nutrients

3.1 Vitamin C with Lysine, Proline, and Green Tea (EGCG)

Netke et al. investigated the effect of a specific combination of ascorbic acid (100 μM), proline (140 μM), and lysine (400 μM) on proliferation and invasive properties of several human cancer cell lines: breast (MDA-MB-231), colon (HCT116), and skin (melanoma, A2058) (Netke et al. 2003). The nutrient combination showed significant antiproliferative and anti-invasive effects against these cell lines. Addition of 20 μg/ml of EGCG to this nutrient combination further enhanced the antiproliferative effects and inhibition of matrix invasion in these cell lines. For example, Matrigel™ invasion by breast cancer cells and human melanoma cells in the presence of ascorbic acid, proline, lysine, and 20 μg/ml of EGCG was stopped completely (Netke et al. 2003).

3.2 Vitamin C with Carotenoids, Vitamin E, and Retinoic Acid

A study by Prasad et al. indicated that while natural compounds used individually at doses 50 μg/ml of vitamin C, 10 μg/ml carotenoids, 10 μg/ml of alpha-tocopherol succinate, and 7.5 μg/ml retinoic acid did not affect growth of melanoma cells, their combination reduced cell number by

56%. Increase of ascorbate level in this mixture to 100 μg/ml resulted in further reduction of melanoma growth by 87% (Prasad et al. 1994).

4 Green Tea Polyphenol (EGCG) in Combination with Other Natural Compounds

Tea and in particular the polyphenols contained in green tea have potent antioxidant and anti-inflammatory properties contributing to their anticancer and other health benefits. Green tea contains between 30 and 40% of water-extractable polyphenols, among which EGCG has been the most studied and demonstrated the greatest potential for its anticarcinogenic properties. Yet, the other components like epicatechin, epigallocatechin, and epicatechin gallate also help to enhance the actions of EGCG as a part of the green tea extract.

EGCG is important in stimulating the production of the glutathione S-transferase (GST) enzyme that plays an important role in the body's defense against cancer. However, EGCG has multifaceted action in cancer prevention and carcinogenesis. When used alone, EGCG tends to reach higher concentrations only in the digestive system, and its overall bioavailability gradually decreases due to oxidation and metabolism. As such, much less EGCG reaches the blood and other tissues, indicating that the potency of EGCG is lost during its absorption from the digestive tract. For example, in a specialized mouse model of human carcinogenesis, Shimizu et al. showed that the administration of EGCG to mice in their drinking fluid significantly decreased small intestinal tumor formation (Shimizu et al. 2008). Therefore, researchers turned to using combinations with other phytochemicals to enhance the anticancer potency of EGCG.

4.1 EGCG with Resveratrol

Resveratrol is a polyphenol present in grapes, red wine, purple grape juice, peanuts, blueberries, and cranberries. It is believed that plants produce resveratrol to protect them from bacterial and fungal infections. It has been shown that EGCG and resveratrol are important in cancer chemoprevention due to their pro-apoptotic effects (Aggarwal et al. 2004; Manson 2005). As such, in vitro studies by Ahmad et al. (2007) in prostate cancer cells (PC-3) showed that the pro-apoptotic effects of resveratrol and EGCG may be due to modulation of casein kinase 2 (CK2). CK2 is a multifunctional protein kinase involved in cell growth, proliferation, survival of cancer cells (Litchfield 2003), and inhibition of apoptosis (Guo et al. 2001). In one of the in vivo studies evaluating the combination of EGCG with resveratrol, George et al. (2011) concluded that the resveratrol and tea polyphenols act synergistically in inhibiting growth of established skin tumors when compared to any of these antioxidants used alone. As such, they observed lower numbers of tumors and also reduced tumor volume.

4.2 EGCG with Curcumin

Curcumin is the most abundant natural phenol (curcuminoid) present in the Indian curry spice, turmeric, which is obtained from the rhizomes of the *Curcuma longa* plant, and it is used extensively in South Asian cooking and food preservation. In the past few decades, curcumin has been studied for its antioxidant, anti-inflammatory, and immune modulation properties. By acting as a free radical scavenger, curcumin can prevent oxidative DNA damage, which is associated with triggering the cancer process. The anticancer properties of curcumin include various cellular mechanisms such as reduction of cancer cell growth, initiation of apoptosis, inhibition of collagen digesting MMP enzymes, and prevention of angiogenesis (Anand et al. 2008). The anticancer actions of curcumin are thought to relate to its characteristic inhibitory action observed in animal models during cancer initiation steps or in suppressing the promotion and progression various stages of carcinogenesis (Ferreira et al. 2015; Rao et al. 1995).

Studies have shown that curcumin can act in synergy with other polyphenols. For example, Khafif et al. (1998) and his team demonstrated that a combination of EGCG and curcumin inhibited growth of premalignant and malignant oral epithelial cells by different mechanisms, and a combination of these two phytochemicals allowed for reduced dose of each individual compound without compromising anticancer efficacy. Since low bioavailability of individual tea polyphenols has been a limiting factor in their anticancer action, the concomitant administration of curcumin has shown to have a synergistic effect in inhibiting growth of breast cancer cells (Somers-Edgar et al. 2008). Also, enhanced apoptotic effect of this combination was demonstrated in chronic lymphocytic leukemia (Angelo and Kurzrock 2009).

Experimental data from Saha et al. shows that epicatechin acts in synergy with EGCG and curcumin in inhibiting cancer cell growth and inducing apoptosis in lung cancer PC-9 cells (Saha et al. 2010). Also, in vivo studies conducted by Zhou et al. (2013) showed that the combination of EGCG and curcumin reduced tumor growth and tumor burden in a lung cancer xenograft in nude mice model. They also noticed that it protected mice from cancer-induced weight loss, without any adverse side effects.

While exploring the cancer stem cells model in nutraceutical chemoprevention, Chung and Vadgama (2015) showed

that EGCG combined with curcumin suppresses a breast cancer stem cells model which suggests that this approach could be used as a targeted therapy for breast cancer.

4.3 EGCG with Quercetin

Quercetin is an important phytochemical present in fruits and vegetables such as onions, apples, berries, and citrus bioflavonoids complex. It has strong antioxidant and anti-inflammatory properties. Quercetin is also essential in facilitating absorption of vitamin C and preventing its destruction in the body. As an anticancer agent, quercetin selectively inhibited cancer cell proliferation and was shown to be effective in inducing cancer cell apoptosis without harming normal cells (Hirpara et al. 2009; Jagtap et al. 2009). Inhibition of lymphoma cell proliferation by quercetin is thought to be due to arrest in G_1 phase of cell cycle (Lee et al. 2006).

When used alone, green tea has limited anticancer action because of the extensive methylation of EGCG in the body. Wang and his team demonstrated that quercetin can increase cellular uptake of EGCG and inhibit its methylation (Wang et al. 2013). These researchers used two types of prostate cancer cells—PC-3 and LNCaP—to study the effects of a combination of EGCG and quercetin. They found that although simultaneous administration of EGCG and quercetin resulted in decreased absorption of quercetin by the cells, it still led to increased apoptosis and inhibited proliferation of these cancer cells (Wang et al. 2012). Further studies of this combination in prostate cancer xenografts in mice showed that it can inhibit tumor growth by 45%, which was more than achieved with either of these nutrients alone (Wang et al. 2014b).

Focusing on prostate cancer stem cells, Tang and the team showed that EGCG used with quercetin was more potent than used alone. Such a combination decreased the self-renewal capacity of prostate cancer stem cells, inhibited migration and invasion capacity, and also induced cancer cell apoptosis (Tang et al. 2010). In vivo research and clinical evidence show that quercetin can help in increasing the levels of available green tea phenols in the blood (Kale et al. 2010a; Gawande et al. 2008). Therefore, the addition of quercetin can markedly enhance the anticancer activity of green tea extract.

4.4 EGCG with Sulforaphane

Antioxidant properties, including the ability to inhibit tumor growth, induce apoptosis, and facilitate detoxification of pro-carcinogens, are some of the mechanisms by which cruciferous/*Brassica* vegetable extracts act as chemopreventive agents. High intakes of *Brassica* vegetables have been associated with lower risk of cancers, including colon, lung, pancreatic, as well as hormone-dependent breast and prostate cancers. The active phytochemical in these vegetables is a sulfur compound called sulforaphane, which is derived from glucosinolates. Sulforaphane has potent antioxidant and anticancer activity. Studies have reported that when used alone, sulforaphane inhibits cell proliferation and induces apoptosis in leukemia cells (Suppipat et al. 2012).

In vitro studies using the EGCG and sulforaphane combination in colon cancer cells (HT-29 AP-1) showed their synergistic effects resulting in decreased cell viability and decreased cancer cell activity (Nair et al. 2008). Similarly, the EGCG and sulforaphane combination was tested in ovarian cancer cells which were sensitive to paclitaxel (SKOV3-ip1) and resistant to paclitaxel (SKOV3TR-ip2) by Chen et al. They reported that sulforaphane inhibited cell viability in both types of the ovarian cancer cells and EGCG was shown to enhance this effect. This combination also increased apoptosis by downregulating hTERT and Bcl-2 and by inducing DNA damage in paclitaxel-resistant ovarian cancer cells (Chen et al. 2013).

5 EGCG in Combinations with Multiple Nutrients

5.1 EGCG with Genistein and Quercetin

EGCG, genistein and quercetin are predominant phytoestrogens present in Asian diet. Individually they are shown to reduce the risk of several cancers including breast, prostate, and endometrial cancers. Hsieh and Wu tested the combination of these three compounds in prostate cancer (CaP) cells and noted significant synergistic effect in inhibition of cell proliferation. Similar synergistic action was also observed with this combination in enhancing the expression of tumor suppressor gene p53 and the enzyme quinone reductase type 1 which prevents p53 demethylation (Hsieh and Wu 2009).

5.2 EGCG with Phytic Acid and Inositol

Phytic acid and inositol are present in grains, beans, and legumes and have antioxidant activity. Due to their iron-binding capacity, a diet high in phytic acid may lead to iron deficiency. On the other hand, Jariwalla et al. reported that this property of phytic acid is advantageous in decreasing iron-mediated colon cancer in animals and that phytic acid inhibits cell proliferation even after stimulation by carcinogens (Jariwalla et al. 1977). Also Khatiwada et al. used a combination of EGCG, phytic acid, and inositol to

study azoxymethane-induced colon cancer tumors in male rats. According to their results, oral administration of the three components showed greatest reduction in the tumors formed. As such, rats in the control group developed 36 tumors that were significantly larger compared to 3 tumors developed in the group that received green tea, phytic acid, and inositol (Khatiwada et al. 2011).

5.3 EGCG with Resveratrol and Vitamin E

Hsieh and Wu (2008) showed that green tea extract combined with resveratrol and vitamin E isoform (gamma-tocotrienol—GT3) is effective and acts synergistically in suppressing proliferation of estrogen receptor-positive MCF-7 breast cancer cells, modulating gene expression, and increasing antioxidant activity, as compared to each of these three phytochemicals used alone. The results showed that there were approximately 33%, 50%, and 58% inhibition of cell proliferation by $>$ or $=$50 mcM EGCG, $>$ or $=$25 mcM resveratrol, and $>$ or $=$10 mcM GT3, respectively, added as single agents. When a suboptimal dose (10 mcM) of each phytochemical was used, a significant additive effect in suppression of cell proliferation was observed with the combination of resveratrol and GT3, whereas the three phytochemicals added together did not produce more pronounced inhibition of cell proliferation. However, a significant additive effect in reducing cyclin D1 and Bcl-2 expression was found when GT3 was added with either EGCG or resveratrol. Functional synergism among the three phytochemicals was only observed in the induction of quinone reductase NQO1.

5.4 EGCG with Curcumin and Arctigenin

Wang et al. (2014a) showed that a combination of EGCG with curcumin and arctigenin (anti-inflammatory lignan from seeds of *Arctium lappa*) significantly increased antiproliferative effects against breast and prostate cancer cells compared to that achieved with individual compounds. In addition, using these nutrients in a combination had additional benefits such as an increased ratio of Bax to Bcl-2 proteins and decreased activation of NFκB and other pathways involved in the cancer cells migration.

5.5 EGCG and Other Catechins with Quercetin and Sulforaphane

A combination of green tea catechins (EGCG, ECG, and CG), sulforaphane, and quercetin has been shown to be superior in inhibition of cell viability, migration, and MMP-2 and MMP-9 expression than any of the compounds used alone. Moreover, these nutrients complemented each other in inhibiting the progression of pancreatic cancer stem cells by controlling their excessive proliferation and induction of microRNA (miR-let-7), the low level of which has been associated with poor outcome for cancer patients and the inhibition of the K-ras gene (Appari et al. 2014).

5.6 EGCG with Curcumin, Resveratrol, Quercetin, and Cruciferex™

Based on the anticancer, antioxidant properties of several phytochemicals, a unique synergy-based combination was tested by Roomi et al. in multiple cancer cell lines. This phytobiological mixture (PB) includes 400 mg quercetin, 400 mg Cruciferex™ (cabbage, cauliflower, broccoli, and carrot extract), 300 mg curcumin, 50 mg resveratrol, and 300 mg standardized green tea extract.

In vitro testing of PB in head and neck squamous cell carcinoma (HNSCC) cells exhibited a significant dose-dependent toxicity (52%), complete inhibition of MMP-2 and MMP-9 enzymes, and inhibition of cell invasion through Matrigel™. The in vivo studies testing growth of tumor xenografts in nude mice showed that dietary intake of PB (0.5%w/w) inhibited HNSCC tumor growth by 67.6% (Roomi et al. 2015a).

The efficacy of this combination of nutrients in decreasing cell proliferation, inhibition of MMP-2 and MMP-9, and achieving virtually 100% inhibition of cell invasion through Matrigel™ was also confirmed in chondrosarcoma (SW-1353), fibrosarcoma (HT-1080), and melanoma cells (A-2058) (Roomi et al. 2017a, b). Another preliminary study showed that PB can inhibit cell proliferation and induce apoptosis in breast cancer cell lines MDA-MB-231 and MCF-7 (Alqarni et al. 2017).

6 Novel Approach to Cancer Through Synergistic Combination of Vitamin C with EGCG and Other Nutrients

Our research based on a multipronged strategy toward cancer led to the development of a specific nutrient combination (NM) simultaneously targeting key mechanisms of cancer. This particular combination (Table 1) includes vitamin C, EGCG, and several natural compounds aimed at protecting the integrity of the extracellular matrix as a biological barrier against cancer invasion and metastasis (Rath and Pauling 1992).

The micronutrients in this synergistically acting complex have displayed anticancer efficacy in over 50 types of human cancer cells in vitro and in vivo. Its pleiotropic effects

include, among others, inhibition of cancer cell growth, invasion and metastasis, curbing angiogenesis, and stimulation of apoptosis. Individual components of NM showed lower anticancer efficacy than its entire composition (Netke et al. 2003; Roomi et al. 2012c).

6.1 Inhibition of Tumor Growth (Xenografts)

Evaluation of effects of NM on xenografts in murine models demonstrated significant reduction in tumor size and tumor burden in several human and murine cancer cell lines. Examples are presented in Table 2.

6.2 Inhibition of Metastasis and Invasion

The effectiveness of the NM in controlling metastasis and invasive parameters has been confirmed in several studies.

6.2.1 Metastasis

The anti-metastatic efficacy of NM was tested in various mouse models using various routes of nutrient administration (i.e., diet, IP, and IV) and cell introduction, such as by intravenous, subcutaneous, and direct organ injections. The results summarized in Table 3 show strong anti-metastatic effect of this combination.

Table 1 Nutrient composition of NM

Components of NM	Concentrations in 1000 μg/ml solution
Ascorbate	900 μM
Lysine	1100 μM
Proline	1100 μM
Arginine	500 μM
N-acetyl-cysteine	250 μM
EGCG (from green tea)	150 μM
Selenium	85 μM
Copper	7 μM
Manganese	4 μM

6.2.2 Inhibition of MMPs and Cancer Cell Invasion Through Matrigel™

The inhibition of cancer cell invasion by NM can be attributed to its inhibitory effect on the secretion and activity of MMPs and uPA, the enzymes responsible for digestion of the extracellular matrix surrounding cells. It has been shown that uPA and various MMPs, notably the MMP-2 and MMP-9, are elevated in several types of human cancers that have been associated with a poor prognosis (Fingleton 2006; Mannello et al. 2005). Using gelatinase zymography, we evaluated MMP-2 and MMP-9 secretion in many human and nonhuman cancer cell lines, some of which express only MMP-2 or only MMP-9 and some express both (Roomi et al. 2010a, 2015b). We also evaluated the effects of NM on uPA and natural inhibitors of ECM proteolysis, TIMP1, and TIMP2 (Roomi et al. 2013, 2014b). Our study showed a correlation between MMPs activities and the invasive properties of cancer cells (Roomi et al. 2010b). Thus, by inhibiting the ECM proteolytic activity in several different cancer cell lines, the NM inhibits not just multiplication but also the spread of cancer. The results showed that NM had a strong inhibitory effect on MMP secretion in all cancer cell types tested without and under the PMA stimulation. Summary of the anti-invasion potential of NM on select cancer cell lines summarized in Table 4 shows that NM can completely block invasion of various types of cancer cells, but its effective concentrations differ.

6.3 Inhibition of Angiogenesis

Since growth and metastasis of all malignant tumors require an adequate blood supply, targeting inhibition of tumor-induced angiogenesis represents a promising approach in cancer therapy (Yang et al. 2017). Tumor angiogenesis involves interplay of several different factors, including

Table 2 Effect of NM-enriched diet on tumor weight and burden in select xenograft studies

Cancer cell line	Tumor growth inhibition	Tumor burden inhibition	Reference
SK-Hep-1	42% ($p = 0.09$)	36% ($p = 0.005$)	Roomi et al. (2010c)
Colon HCT-116	63% ($p = 0.0002$)	46% ($p = 0.0005$)	Roomi et al. (2005a)
Prostate PC-3	47% ($p < 0.0001$)	53% ($p = 0.0002$)	Roomi et al. (2005b)
Lung A-549	44% ($p = 0.001$)	47% ($p < 0.0001$)	Roomi et al. (2006a)
Melanoma A2058	57% ($p < 0.0001$)	31%	Roomi et al. (2006b)
Cervical HeLa	59% ($P = 0.001$)	N/A	Roomi et al. (2014c)
Head and neck squamous cell carcinoma OHSU-974	47% ($p = 0.0009$)	N/A	Roomi et al. (2012a)
Glioblastoma U-87MG	53% ($p = 0.015$)	48% ($p = 0.010$)	Roomi et al. (2016a)
Breast 4T1	50% ($p = 0.02$)	53.4% ($p =< 0.000$)	Roomi et al. (2014a)
Osteosarcoma MNNG-HOS	53% ($p = 0.0001$)	N/A	Roomi et al. (2006d)
Ovarian ES-2	59.3% (p, 0.0001)	58.7% ($p < 0.0001$)	Roomi et al. (2016b)

Table 3 Effects of NM on organ metastasis tested in different experimental designs

Cancer cell type	Route of cancer cells administration	NM delivery	Inhibition of metastasis in organs	Reference
Melanoma B16FO	Intravenous	Diet 0.5% IV IP	63% to lungs 86% to lungs 86% to lungs	Roomi et al. (2006c)
Melanoma B16FO	Intra-spleen	Diet 0.5%	55% to liver	Roomi et al. (2009)
Melanoma B16FO	Intratesticular	Diet 1%	No peritoneal metastasis in 50% animals, in others only mild compared to control	Roomi et al. (2012b)
Breast cancer 4T1	Subcutaneous	Diet 0.5%	87% to lungs (number of colonies) 60% (by lung weight) Reduced metastasis to liver and spleen	Cha et al. (2013)
Breast cancer 4T1	Orthotopic inoculation into kidney	Diet 0.5%	Reduced metastasis to lungs and spleen compared to control	Roomi et al. (2018)
Ovarian cancer A2780	Intraperitoneal injection	Diet 0.5%	Lung metastasis reduced by 100%	Roomi et al. (2017c)

Table 4 Effective doses of NM resulting in complete inhibition of cancer cell invasion through Matrigel™

Concentration of NM resulting 100% inhibition of cell invasion		
100 mg	500 mcg	1000 mcg
Breast (MBA-MB-231)	Cervical cancer (HeLa)	Bladder cancer (T-24)
Breast (MCF-7 estrogen sensitive) + estradiol	Colon (HCT 116)	Cervical cancer (DoTc2451)
Osteosarcoma (MNNG/U2OS)	Lung carcinoma (A-549)	Fibrosarcoma (HT-1080)
Testicular (NT2/DT)	Pancreas (MIA PACA-2)	Glioma (A-172)
Fanconi Anemia (FAA:P220)	Prostate (LNCaP and DU-145)	Hepatocellular carcinoma (SK-Hep-1)
	Rhabdomyosarcoma	Liposarcoma (SW-872)
	Thyroid (SW 579)	Melanoma (A2058)
	Fanconi anemia (GM13022;FA-A:PD20;)	Ovarian (SK-OV-3)
		Prostate (PC-3)
		Renal carcinoma (786-0)
		Synovial carcinoma
		Chondrosarcoma (SW1353)
		Head and neck (HNSCC)
		Tongue (SC-255)

ECM degradation and rebuilding, which has been associated with activity of MMPs and other proteolytic enzymes. Based on in vivo and in vitro models of angiogenesis, we demonstrated that anti-MMP and uPA efficacy of the NM has been associated with its anti-angiogenic properties (Roomi et al. 2005c, 2007). In addition to its effect on MMPs inhibition, the NM showed its inhibitory effects on the secretion of pro-angiogenic factors such as VEGF, IL-6, IL-8, FGF, TGF-β, and angiopoietin-2 (Roomi et al. 2007). These factors affect the endothelium directly or indirectly by activation of surrounding cells to produce other pro-angiogenic factors or modulation of receptors/receptor activities (Yoshida et al. 1997).

6.4 Pro-apoptotic Effects of NM

Pro-apoptotic effects of NM have been demonstrated in leukemia cancer cell lines showing its stimulatory effect on cancer cell cycle arrest, DNA fragmentation, and increased expression of pro-apoptotic genes (p53, Bax, P21) and inhibition of Bcl2a (Harakeh et al. 2006) (Fig. 1).

In addition, NM pro-apoptotic effects have been documented in vivo such as in cervical HeLa cell xenografts in nude female mice fed NM (Roomi et al. 2014c). The immunohistochemical staining for terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) showed abundant apoptosis in both control and NM-treated tumors; however, more apoptosis occurred in tumors from the NM-treated group, which displayed uniform areas of homogeneous apoptotic cells with peripheral areas of non-apoptotic cells, and some apoptosis was detected in the cells of the fibrous capsule of NM-treated tumors. Control tumors showed high staining for Bcl-2, a pro-survival, anti-apoptotic protein, whereas a reduced level of Bcl-2 was detected in the NM-treated tumors. The NM-treated tumors had vast regions of Bcl-2-free cells, which were clearly nucleated with considerably less Bcl-2 expression.

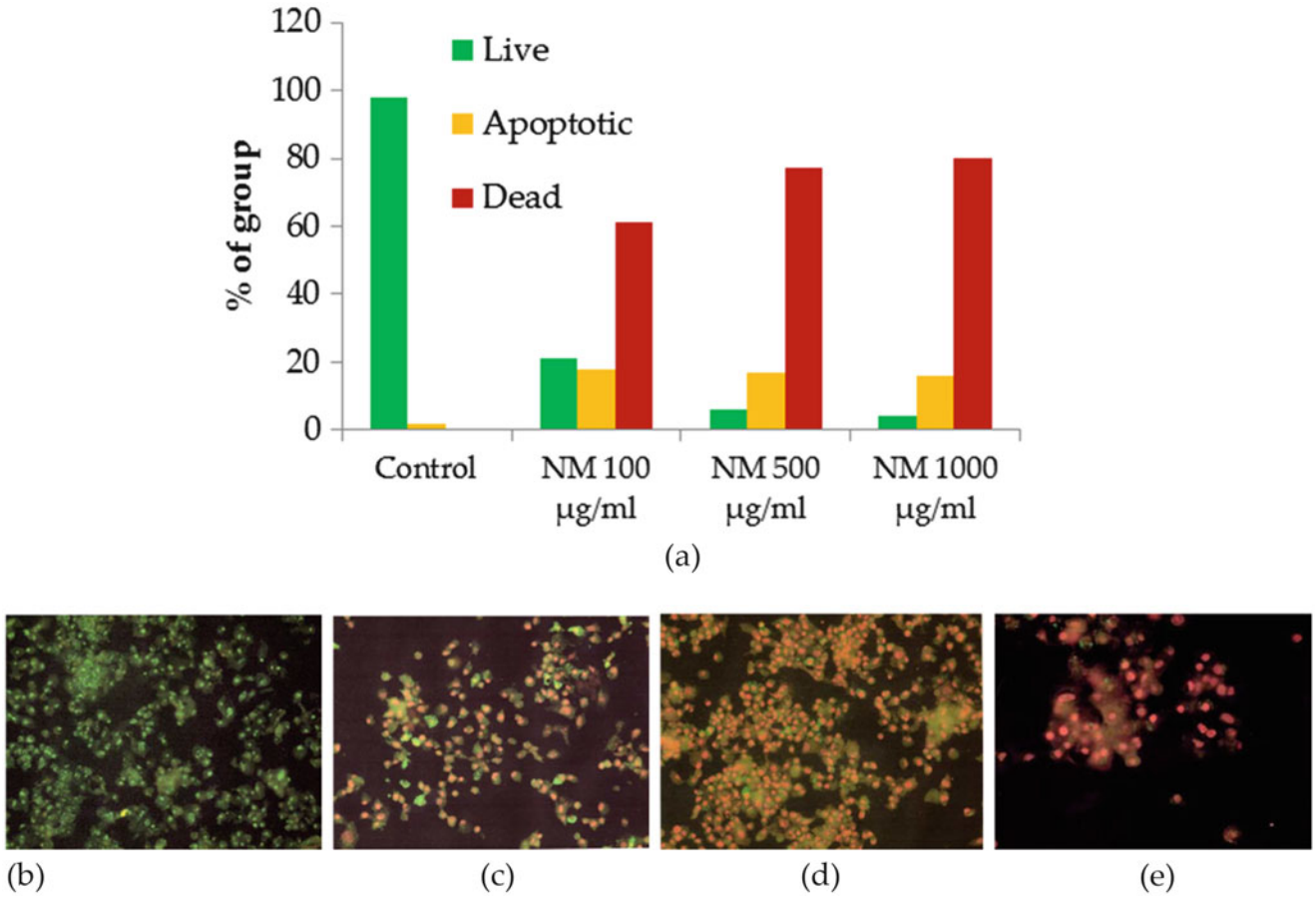


Fig. 1 Effect of NM on apoptosis of HepG2 cells—Green Poly Caspases. Quantitative analysis (**a**) and photomicrographs of HepG2 cells exposed to NM concentrations of 0 (**b**), 100 (**c**), 500 (**d**), and 1000 (**e**) µg/ml (Roomi et al. 2012c)

6.5 Anti-inflammatory Effects of NM

Inflammatory proteins such as Cox-2 contribute to carcinogenesis and promote growth of human tumors and have been found to be elevated in cancer patients (Greenhough et al. 2009; Colotta et al. 2009). We observed that Cox-2 expression in bladder cancer T-24 cells exposed to NM was reduced in a dose-dependent fashion (Roomi et al. 2010d). In addition, in vivo study of the effect of dietary NM supplementation on HeLa cell xenografts in nude female mice showed strong inhibition of the tumor growth with mean tumor weight reduced to 59% ($P = 0.001$) This was accompanied by a lower intensity of staining for Cox-2 and iNOS in tumors developed in the NM group compared to the control group (Roomi et al. 2015c).

6.6 Therapeutic Effects of NM

The therapeutic potential of a nutrient combination in NM compared to green tea extract is exemplified in the study of Kale et al. on the treatment of MNU-induced mammary tumors in Wistar rats (Kale et al. 2010b). Different micronutrient treatment regimens were provided after rats had developed palpable tumors to simulate intervention treatment approaches in clinical settings. The administration of NM resulted in markedly higher (85%) inhibition of tumor growth, compared to green tea extract alone (50%). Moreover, among tumors that developed in NM-treated mice, the majority (90%) were nonmalignant.

7 Concluding Remarks and Future Directions

Nutrients and phytochemicals have proven anticancer properties. However, individual compounds have limited efficacy as compared to select combinations of specific nutrients. Such combinations can overcome some metabolic inadequacies of individual components and through their mutual interactions enhance their therapeutic efficacy. Future research on vitamin C and EGCG in cancer should include its use in combination with other nutrients in targeting selective cancer mechanisms. Our results show that a combination of vitamin C and EGCG with select micronutrients and phytochemicals allows for expanding and simultaneously

Table 5 Experimentally confirmed anticancer effects of natural compounds synergistically combined in nutrient mixture (NM)

Process in cancer	Beneficial effects/mechanisms
Oxidative stress	Scavenging free radicals Reduced oxidative stress
Cancer cell growth and survival	Inhibition of cell proliferation Cell cycle arrest Induction of apoptosis
Tumor growth and metastasis	Inhibition of angiogenesis Inhibition of cancer cells adhesion and invasion Inhibition of proteolytic destruction of connective tissue (MMPs, uPA) Increased synthesis of proteolysis inhibitors TIMP 1 and 2, PAI1 Prevention of MMP dimers formation Tumor encapsulation Inhibition of tumor growth and burden Anti-metastatic effects
Genetic	Inhibition of oncogene expression Induction of tumor suppressor genes
Support systems	Immune system support Anti-inflammatory effects (inhibition of COX2, inducible iNOS) Prevention of chemically induced cancers
Metabolic effects	Modulation of glycolytic and epithelial mesenchymal transition (EMT) genes expression

targeting several mechanisms specific to cancer (Table 5). Moreover, unlike toxic chemotherapy and radiation treatments, the nutrient synergy combination is nontoxic. Studies using the clinically relevant doses of NM have shown a lack of adverse effects on vital organs such as the heart, liver, and kidneys.

Future studies in natural compounds should focus on expanded utilization of their cancer preventive properties through nutrient synergy. Synergistic combinations of nutrients and phytochemicals can offer a safe and effective therapeutic alternative for the current extremely toxic cancer treatments.

Acknowledgments We are grateful to Ms. Cathy Flowers for her valuable comments and edits.

References

Adlercreutz H (1990) Western diet and Western diseases: some hormonal and biochemical mechanisms and associations. Scand J Clin Lab Invest Suppl 20:3–23

Aggarwal BB, Bhardwaj A, Aggarwal RS (2004) Role of resveratrol in prevention and therapy of cancer: preclinical and clinical studies. Anticancer Res 5:2783–2840

Ahmad KA, Harris NH, Johnson AD et al (2007) Protein kinase CK2 modulates apoptosis induced by resveratrol and epigallocatechin-3-gallate in prostate cancer cells. Mol Cancer Ther 6(3):1006–1012

Alqarni AA, Alamoudi AA, Ajabnoor GM et al (2017) Inhibition of proliferation and induction of apoptosis by a novel phytobiological mixture against breast cancer cell lines. Cancer Res 77 (13 Suppl):4208. https://doi.org/10.1158/1538-7445.AM2017-4208

Amin ARMR, Kucuk O, Khuri FR et al (2009) Perspectives for cancer prevention with natural compounds. J Clin Oncol 27 (16):2712–2725

Anand P, Sundaram C, Jhurani S et al (2008) Curcumin and cancer: an "old-age" disease with an "age-old" solution. Cancer Lett 267 (1):133–164

Angelo LS, Kurzrock R (2009) Turmeric and green tea: a recipe for the treatment of B-chronic lymphocytic leukemia. Clin Cancer Res 15 (4):1123–1125

Appari M, Babu KR, Kaczorowski A et al (2014) Sulforaphane, quercetin and catechins complement each other in elimination of advanced pancreatic cancer by miR-let-7 induction and K-ras inhibition. Int J Oncol 45(4):1391–1400

Cha J, Roomi MW, Ivanov V et al (2011) Ascorbate depletion increases growth and metastasis of melanoma cells in vitamin C deficient mice. Exp Oncol 33(4):226–230

Cha J, Roomi W, Ivanov V et al (2013) Ascorbate supplementation inhibits growth and metastasis of B16FO melanoma and 4T1 breast cancer cells in vitamin C-deficient mice. Int J Oncol 42:55–64

Chen P, Stone J, Sullivan G et al (2011) Anti-cancer effect of pharmacologic ascorbate and its interaction with supplementary parenteral glutathione in preclinical cancer models. Free Radic Biol 51 (3):681–687

Chen H, Landen CN, Li Y et al (2013) Epigallocatechin gallate and sulforaphane combination treatment induce apoptosis in paclitaxel-resistant ovarian cancer cells through hTERT and Bcl-2 down-regulation. Exp Cell Res 319(5):697–706

Chen Q, Polireddy K, Chen P et al (2015) The unpaved journey of vitamin C in cancer treatment. Can J Physiol Pharmacol 93 (12):1055–1063

Chung SS, Vadgama JV (2015) Curcumin and epigallocatechin gallate inhibit the cancer stem cell phenotype via down-regulation of STAT3–NFκB signaling. Anticancer Res 35(1):39–46

Chung MK, Kim Do H, Ahn YC et al (2016) Randomized trial of vitamin C/E complex for prevention of radiation-induced xerostomia in patients with head and neck cancer. Otolaryngol Head Neck Surg 155(3):423–430

Colotta F, Allavena P, Sica A et al (2009) Cancer-related inflammation, the seventh hallmark of cancer: links to genetic instability. Carcinogenesis 30(7):1073–1081

Coulter ID, Hardy ML, Morton SC et al (2006) Antioxidants vitamin C and vitamin e for the prevention and treatment of cancer. J Gen Intern Med 21(7):735–744

Dettman I, Meakin C, Allen R (2012) Co-infusing glutathione and vitamin C during cancer treatment: a reply. J Australasian Coll Nutr Environm Med 31(1):8–11

Donaldson MS (2004) Nutrition and cancer: a review of the evidence for an anti-cancer diet. Nutr J 3:19–40

Ferreira LC, Arbab AS, Jardim-Perassi BV et al (2015) Effect of curcumin on pro-angiogenic factors in the xenograft model of breast cancer. Anticancer Agents Med Chem 15(10):1285–1296

Fingleton B (2006) Matrix metalloproteinases: roles in cancer and metastasis. Front Biosci 11:479–491

Fojo T, Mailankody S, Lo A (2014) Unintended consequences of expensive cancer therapeutics—the pursuit of marginal indications and a me-too mentality that stifles innovation and creativity: the John Conley Lecture. JAMA Otolaryngol Head Neck Surg 140 (12):1225–1236

Fullerton SA, Samadi AA, Tortorellis DG et al (2000) Induction of apoptosis in human prostate cancer cells with beta-glucan (Maitake mushroom polysaccharide). Mol Urol 4(1):7–13

Gawande S, Kale A, Kotwal S (2008) Effect of nutrient mixture and black grapes on the pharmacokinetics of orally administered (-)

epigallocatechin-3-gallate from green tea extract: a human study. Phytother Res 22(6):802–808
Gaziano JM, Glynn RJ, Christen WG et al (2009) Vitamins E and C in the prevention of prostate and total cancer in men: the Physicians' Health Study II randomized controlled trial. JAMA 301(1):52–62
George J, Singh M, Srivastava AK et al (2011) Resveratrol and black tea polyphenol combination synergistically suppress mouse skin tumors growth by inhibition of activated MAPKs and p53. PLoS One 6(8): e23395
Gonzalez MJ, Miranda-Massari JR (2014) New insights on Vitamin C and cancer. Springer, New York, p 2014
Greenhough A, Smartt HJ, Moore AE et al (2009) The COX-2/PGE_2 pathway: key roles in the hallmarks of cancer and adaptation to the tumour microenvironment. Carcinogenesis 30(3):377–386
Guo C, Yu S, Davis AT et al (2001) A potential role of nuclear matrix-associated protein kinase CK2 in protection against drug-induced apoptosis in cancer cells. J Biol Chem 276:5992–5999
Harakeh S, Diab-Assaf M, Niedzwiecki A et al (2006) Apoptosis induction by Epican Forte in HTLV-1 positive and negative malignant T-cells. Leuk Res 30(7):869–881
Hirpara KV, Aggarwal P, Mukherjee AJ et al (2009) Quercetin and its derivatives: synthesis, pharmacological uses with special emphasis on anti-tumor properties and prodrug with enhanced bio-availability. Anticancer Agents Med Chem 9(2):138–1361
Hsieh TC, Wu JM (2008) Suppression of cell proliferation and gene expression by combinatorial synergy of EGCG, resveratrol and gamma-tocotrienol in estrogen receptor-positive MCF-7 breast cancer cells. Int J Oncol 33(4):851–859
Hsieh TC, Wu JM (2009) Targeting CWR22Rv1 prostate cancer cell proliferation and gene expression by combinations of the phytochemicals EGCG, genistein and quercetin. Anticancer Res 29 (10):4025–4032
Jacob SW (1983) The current status of MSM in medicine. Am Acad Prev Med
Jagtap S, Meganathan K, Wagh V et al (2009) Chemoprotective mechanism of the natural compounds, epigallocatechin-3-O-gallate, quercetin and curcumin against cancer and cardiovascular diseases. Curr Med Chem 16:1451–1462
Jariwalla RJ, Sabin R, Lawson S et al (1977) Effects of dietary phytic acid on the incidence and growth rate of tumors promoted in Fisher rats by a magnesium supplement. Nutr Res 8:813
Jemal A, Siegel R, Xu J, Ward E (2010) Cancer statistics. CA Cancer J Clin 60(5):277–300
Kale A, Gawande S, Kotwal S et al (2010a) Studies on the effects of oral administration of nutrient mixture, quercetin and red onions on the bioavailability of epigallocatechin gallate from green tea extract. Phytother Res Suppl 1:S48–S55
Kale A, Gawande S, Kotwal S et al (2010b) A combination of green tea extract, specific nutrient mixture and quercetin: an effective intervention treatment for the regression of N-methyl-N-nitrosourea (MNU)-induced mammary tumors in Wistar rats. Onc Letters 1:313–317
Khafif A, Schantz SP, Chou TC et al (1998) Quantitation of chemopreventive synergism between (-)-epigallocatechin-3-gallate and curcumin in normal, premalignant and malignant human oral epithelial cells. Carcinogenesis 19(3):419–424
Khatiwada J, Verghese M, Davis S et al (2011) Green tea, phytic acid, and inositol in combination reduced the incidence of azoxymethane-induced colon tumors in Fisher 344 male rats. J Med Food 14 (11):1313–1320
Kim KN, Pie JE, Park JH et al (2006) Retinoic acid and ascorbic acid act synergistically in inhibiting human breast cancer cell proliferation. J Nutr Biochem 17(7):454–462
Konno S (2007) Effect of various natural products on growth of bladder cancer cells: two promising mushroom extracts. Altern Med Rev 12 (1):63–68
Lee TJ, Kim OH, Kim YH et al (2006) Quercetin arrests G2/M phase and induces caspase-dependent cell death in U937 cells. Cancer Lett 240(2):234–242
Ley BM (1998) The forgotten nutrient MSM: on our way back to health with sulfur in health learning handbooks. BL Publications, California
Li W, Wu J, Tu YJ (2010) Synergistic effects of tea polyphenols and ascorbic acid on human lung adenocarcinoma SPC-A-1 cells. Zhejiang Univ Sci B 11:458–464
Litchfield DW (2003) Protein kinase CK2: structure, regulation and role in cellular decisions of life and death. Biochem J 369:1–15
Mannello F, Tonti G, Papa S (2005) Matrix metalloproteinase inhibitors as anticancer therapeutics. Curr Cancer Drug Targets 5(4):285–298
Manson MM (2005) Inhibition of survival signaling by dietary polyphenols and indole-3-carbinol. Eur J Cancer 41(13):1842–1853
Mayor S (2018) Seven in 10 women with early breast cancer do not need chemotherapy, study finds. BMJ 361:k2473. https://doi.org/10.1136/bmj.k2473
Mehlen P, Puisieux A (2006) Metastasis: a question of life or death. Nat Rev Cancer 6:449–458
Miller A (1990) Diet and cancer: a review. Acta Oncol 29(1):87–95
Mindell EL (1997) The MSM miracle. Enhance your health with organic sulfur. Good Health Guides Keats Publishing, Connecticut
Morgan G, Ward R, Barton M (2004) The contribution of cytotoxic chemotherapy to 5-year survival in adult malignancies. Clin Oncol (R Coll Radiol) 16(8):549–560
Mostafafavi-Pour Z, Ramezani F, Keshavarzi F et al (2017) The role of quercetin and vitamin C in Nrf2-dependent oxidative stress production in breast cancer cells. Oncol Lett 13:1965–1973
Nair S, Hebbar V, Gouxiang S et al (2008) Synergistic effects of a combination of dietary factors sulforaphane and (−) epigallocatechin-3-gallate in HT-29 AP-1 human colon carcinoma cells. Pharm Res 25(2):387–399
Netke SP, Roomi MW, Ivanov V et al (2003) A specific combination of ascorbic acid, lysine, proline and epigallocatechin gallate inhibits proliferation and extracellular matrix invasion of various human cancer cell lines. Res Comm Pharmacol Toxicol 8(1/2):IV37–IV49
Niedzwiecki A, Roomi MW, Kalinovsky T, Rath M (2010) Micronutrient synergy-a new tool in effective control of metastasis and other key mechanisms of cancer. Cancer Metastasis Rev 29(3):529–542
Prasad KN (2004) Multiple dietary antioxidants enhance the efficacy of standard and experimental cancer therapies and decrease their toxicity. Integr Cancer Ther 3(4):310–322
Prasad KN, Hernandez C, Edwards PJ et al (1994) Modification of the effect of tamoxifen, cisplatin, DTIC and interferon alpha 2b on human melanoma cells in culture by mixture of vitamins. Nutr Cancer 22:223–245
Rao CV, Rivenson A, Simi B et al (1995) Chemoprevention of colon carcinogenesis by dietary curcumin, a naturally occurring plant phenolic compound. Cancer Res 55(2):259–266
Rath M, Pauling L (1990) Hypothesis: lipoprotein(a) is a surrogate for ascorbate. Proc Natl Acad Sci U S A 87(16):6204–6207
Rath M, Pauling L (1992) Plasmin-induced proteolysis and the role of apoliporotein (a), lysine and synthetic lysine analogues. J Orthomol Med 7:17–23
Roomi MW, Ivanov V, Kalinovsky T et al (2005a) In vivo antitumor effect of ascorbic acid, lysine, proline and green tea extract on human colon cancer cell HCT 116 xenografts in nude mice: evaluation of tumor growth and immunohistochemistry. Oncol Rep 12 (3):421–425
Roomi MW, Ivanov V, Kalinovsky T et al (2005b) In vivo antitumor effect of ascorbic acid, lysine, proline and green tea extract on human prostate PC-3 xenografts in nude mice: evaluation of tumor growth and immunohistochemistry. In Vivo 19(1):179–184
Roomi MW, Roomi NW, Ivanov V et al (2005c) Inhibitory effect of a mixture containing ascorbic acid, lysine, proline, and green tea

extract on critical parameters in angiogenesis. Oncol Rep 14 (4):807–815
Roomi MW, Ivanov V, Kalinovsky T, Niedzwiecki A, Rath M (2006a) In vivo and in vitro anti-tumor effect of a unique nutrient mixture on lung cancer cell line A-549. Exp Lung Res 32:441–453
Roomi MW, Ivanov V, Kalinovsky T, Niedzwiecki A, Rath M (2006b) In vitro and in vivo antitumor effect of ascorbic acid, lysine, proline and green tea extract on human melanoma cell line A2058. In Vivo 20(1):25–32
Roomi MW, Roomi NW, Ivanov V et al (2006c) Inhibition of pulmonary metastasis of melanoma B16FO cells in C57BL/6 mice by a nutrient mixture consisting of ascorbic acid, lysine, proline, arginine, and green tea extract. Exp Lung Res 32:517–530
Roomi MW, Ivanov V, Kalinovsky T et al (2006d) Effect of ascorbic acid, lysine, proline and green tea extract on human osteosarcoma cell line MNNG-HOS xenographs in nude mice: evaluation of tumor growth and immunohistochemistry. Med Oncol 23:411–417
Roomi MW, Ivanov V, Kalinovsky T et al (2007) A novel nutrient mixture containing ascorbic Acid, lysine, proline, and green tea extract inhibits critical parameters in angiogenesis. In: Losso JN, Shahidi F, Bagchi D (eds) Anti-angiogenic functional and medicinal foods. CRC Press, Taylor & Francis Group, New York, pp 561–580
Roomi MW, Kalinovsky T, Roomi NW et al (2009) A nutrient mixture suppresses hepatic metastasis in athymic nude mice injected with murine B16FO melanoma cells. Biofactors 33:181–189
Roomi MW, Monterrey JC, Kalinovsky T et al (2010a) Comparative effects of EGCG, green tea, and a nutrient mixture on the patterns of MMP-2 and MMP-9 expression in cancer cell lines. Oncol Rep 24:747–757
Roomi MW, Monterrey JC, Kalinovsky T et al (2010b) Inhibition of invasion and MMPs by a nutrient mixture in human cancer cells: a correlation study. Oncol Rep 32:243–248
Roomi MW, Roomi NM, Kalinovsky T et al (2010c) In vivo and in vitro effect of a nutrient mixture on human hepatocarcinoma cell line SK-Hep-1. Exp Oncol 32:84–91
Roomi MW, Kalinovsky T, Niedzwiecki A, Rath M (2010d) Pleiotropic effects of a micronutrient mixture on critical parameters of bladder cancer. In: Nilsson WE (ed) Bladder cancer etymology, diagnosis and treatments. Nova Science Publishers, New York, pp 229–243
Roomi MW, Kalinovsky T, Roomi NW et al (2012a) In vitro and in vivo inhibition of human Fanconi anemia head and neck squamous carcinoma by a novel nutrient mixture. Int J Oncol 41(6):1996–2004
Roomi MW, Kalinovsky T, Roomi NW et al (2012b) Suppression of metastasis of intratesticular inoculation of B16FO melanoma cells by a novel nutrient mixture in male athymic nude mice. Exp Ther Med 4:775–780
Roomi MW, Roomi NW, Kalinovsky T et al (2012c) Micronutrient synergy in the fight against hepatocellular carcinoma. Cancers 4 (2):323–339
Roomi MW, Kalinovsky T, Niedzwiecki A, Rath M (2013) Modulation of u-PA, MMPs and their inhibitors by a novel nutrient mixture in adult human sarcoma cell lines. Int J Oncol 43(1):39–49
Roomi MW, Kalinovsky T, Roomi NW et al (2014a) In vivo and in vitro effects of a nutrient mixture on breast 4T1 cancer progression. Int J Oncol 44(6):1933–1944
Roomi MW, Kalinovsky T, Niedzwiecki A, Rath M (2014b) Modulation of uPA, MMPs and their inhibitors by a novel nutrient mixture in human glioblastoma cell line. Int J Oncol 45:887–894
Roomi MW, Kalinovsky T, Cha J, Roomi NW et al (2014c) Effects of a nutrient mixture on immunohistochemical localization of cancer markers in human cervical cancer HeLa cell tumor xenografts in female nude mice. Exp Ther Med 9(2):294–302
Roomi MW, Kalinovsky T, Roomi NW et al (2015a) In vitro and in vivo inhibition of human Fanconi anemia head and neck squamous carcinoma by a phytonutrient combination. Int J Oncol 46 (5):2261–2266
Roomi MW, Bhanap B, Niedzwiecki A, Rath M (2015b) Suppression of matrix metalloproteinases -2 and -9 in various human cancer cell lines by a nutrient mixture. J Oncobiomark 2(2):17
Roomi MW, Cha J, Kalinovsky T et al (2015c) Effect of nutrient mixture on the localization of extracellular matrix proteins in HeLa human cervical cancer xenograft in female nude mice. J Exp Ther Med 9:294–302
Roomi MW, Kalinovsky T, Rath M, Niedzwiecki A (2016a) A nutrient mixture inhibits glioblastoma xenograft U-87 MG growth in male nude mice. Exp Oncol 38(1):54–56
Roomi MW, Kalinovsky T, Rath M, Niedzwiecki A (2016b) A nutrient mixture modulates ovarian ES-2 cancer progression by inhibiting xenograft tumor growth and Cellular MMP secretion, migration and invasion. Int J Clin Exp Med 9(2):814–822
Roomi MW, Bhanap B, Niedzwiecki A, Rath M (2017a) Anti-cancer potential of a specific mixture of phytonutrients in chondrosarcoma SW-1358 cells. J Cell Med Nat Health, October
Roomi MW, Kalinovsky T, Jariwalla N, et al (2017b) A specific mixture of phytonytrients inhibits cel proliferation, secretion of MMPs and invasion through Matrigel in fibrosarcoma HT-1080 and melanoma A-2058 cells. J Cell Med Nat Health, March
Roomi MW, Kalinovsky T, Niedzwiecki A, Rath M (2017c) A specific mixture of nutrients suppresses ovarian cancer A-2780 tumor incidence, growth and metastasis to the lungs. Nutrients 9:303–314
Roomi MW, Bhanap B, Niedzwiecki A, Rath M (2018) Inhibition of tumor growth and metastasis by a novel nutrient mixture of inoculation of mouse mammary 4T1 carcinoma in kidney of female Balb/c mice. J Cell Med Nat Health, July
Saha A, Kuzuhara T, Echigo N et al (2010) New role of (-)-epicatechin in enhancing the induction of growth inhibition and apoptosis in human lung cancer cells by curcumin. Cancer Prev Res (Phila) 3 (8):953–962
Satoh K, Sakagami H (1997a) Effect of copper and iron ions on cytotoxicity induced by ascorbate, gallate and caffeate. Anticancer Res 17(3C):2181–2184
Satoh K, Sakagami H (1997b) Effect of cysteine N-acetyl-L-cysteine and glutathione on cytotoxic activity of antioxidants. Anticancer Res 17(3C):2175–2179
Satoh K, Kadofuku T, Sakagami H (1997) Copper, but not iron, enhances apoptosis-inducing activity of antioxidants. Anticancer Res 17(4A):2487–2490
Shimizu M, Shirakami Y, Sakai H et al (2008) (-)-Epigallocatechin gallate suppresses azoxymethane-induced colonic premalignant lesions in male C57BL/KsJdb/db mice. Cancer Prev Res (Phila) 1:298–304
Simone CB, Simone NL, Simone V et al (2007) Antioxidants and other nutrients do not interfere with chemotherapy or radiation therapy and can increase kill and increase survival, Part 2. Altern Ther Health Med 13(2):40–47
Somers-Edgar TJ, Scandlyn MJ, Stuart EC et al (2008) The combination of epigallocatechin gallate and curcumin suppresses ER alpha-breast cancer cell growth in vitro and in vivo. Int J Cancer 122 (9):1966–1971
Suppipat K, Park CS, Shen Y et al (2012) Sulforaphane induces cell cycle arrest and apoptosis in acute lymphoblastic leukemia cells. PLoS One 7(12):e51251. https://doi.org/10.1371/journal.pone.0051251
Taketo MM (2011) Reflections on the spread of metastasis to cancer prevention. Cancer Prev Res (Phila) 4(3):324–328
Tang S, Singh C, Nall D et al (2010) The dietary bioflavonoid quercetin synergizes with epigallocathechin gallate (EGCG) to inhibit prostate cancer stem cell characteristics, invasion, migration and epithelial-mesenchymal transition. J Mol Sign 5:14. https://doi.org/10.1186/1750-2187-5-14

Taper H (2008) Altered deoxyribonuclease activity in cancer cells and its role in non toxic adjuvant cancer therapy with mixed vitamins C and K3. Anticancer Res 28(5A):2727–2732

Vasileva IN, Bespalov VG, Baranenko DA (2016) Radioprotective and apoptotic properties of a combination of a-tocopheryl acetate and ascorbic acid. Bull Exp Biol Med 161(2):248–251

Verrax J, Cadrobbi J, Delvaux M et al (2003) The association of vitamins C and K3 kills cancer cells mainly by autoschizis, a novel form of cell death. Basis for their potential use as coadjuvants in anticancer therapy. Eur J Med Chem 38(5):451–457

Wang P, Heber D, Henning SM (2012) Quercetin increased the antiproliferative activity of green tea polyphenol (-)-epigallocatechin gallate in prostate cancer cells. Nutr Cancer 64(4):580–587

Wang P, Heber D, Henning SM (2013) Quercetin increased bioavailability and decreased methylation of green tea polyphenols in vitro and in vivo. Food Funct 3(6):635–642

Wang P, Wang B, Chung S et al (2014a) Increased chemopreventive effect by combining arctigenin, grean tae polyphenol and curcumin in prostate and breast cancer cells. RSC Adv 4(66):35242–35250

Wang P, Vadgama JV, Said JW et al (2014b) Enhanced inhibition of prostate cancer xenograft tumor growth by combining quercetin and green tea. J Nutr Biochem 25(1):73–80

Weitsman GE, Koren R, Zuck E et al (2005) Vitamin D sensitizes breast cancer cells to the action of H2O2: mitochondria as a convergence point in the death pathway. Free Radic Biol Med 39 (2):266–278

Yang W-H, Xu J, Mu J-B, Xie J (2017) Revision of the concept of anti-angiogenesis and its applications in tumor treatment. Chronic Dis Transl Med 3(1):33–40

Yoshida S, Ono M, Shono T et al (1997) Involvement of interleukin-8, vascular endothelial growth factor and basic fibroblast growth factor in tumor necrosis factor α-dependent angiogenesis. Mol Cell Biol 17:4015–4023

Yun J, Mullarky E, Changyuan L et al (2015) Vitamin C selectively kills KRAS and BRAF mutant colorectal cancer cells by targeting GAPDH. Science 350(6266):1391–1396

Zheng QS, Sun XL, Wand CH (2002) Redifferentiation of human gastric cancer cells induced by ascorbic acid and sodium selenite. Biomed Environ Sci 15:223–232

Zhou DH, Wang X, Yang M et al (2013) Combination of low concentration of (-)-epigallocatechin gallate (EGCG) and curcumin strongly suppresses the growth of non-small cell lung cancer in vitro and in vivo through causing cell cycle arrest. Int J Mol Sci 14 (6):12023–12036

Nutraceuticals for Control of Ticks, Fleas, and Other Ectoparasites

Ramesh C. Gupta, Robin B. Doss, Ajay Srivastava, Rajiv Lall, and Anita Sinha

Abstract

The ticks, fleas, mosquitoes, and other ectoparasites, in addition of being nuisance, transmit infectious diseases in companion and livestock animals and wildlife. The use of ectoparasiticides on companion and farm animals appears to be inevitable. Currently, synthetic insecticides of various classes are used to combat ectoparasites on animals. Some of the synthetic insecticides are used as ovicides or larvicides, while others are used as adulticides. But due to their greater toxicity, lack of selective toxicity, and pesticide resistance in insects, their use has been on decline. During the past two decades, the quest for natural products as an alternative to synthetic pesticides has been recognized. This chapter describes some biopesticides which can be used to control ectoparasites in pets and farm animals.

Keywords

Nutraceuticals · Biopesticides · Ectoparasites · Ectoparasiticides · Ovicides · Larvicides · Adulticides

1 Introduction

The blood-sucking ticks, fleas, mosquitoes, biting flies, and other ectoparasites commonly infest pets, livestock, and wildlife. These ectoparasites pose a serious global health concern as they transmit infectious diseases and in some cases cause atopic dermatitis. Therefore, the use of ectoparasiticides on companion and farm animals appears to be inevitable. Currently, synthetic insecticides of various classes are used to combat ectoparasites on animals (Gupta 2006; Marrs, 2012; Marrs and Dewhurst 2012). Some of the synthetic insecticides are used as ovicides or larvicides, while others are used as adulticides. Currently, commonly used ectoparasitic products on pets include Frontline, Advantage, Revolution, Certifect, Activyl, Parastar Plus, Bio Spot Defense, VetGuard, Vectra-3D, and Seresto. These products can have an individual or multiple insecticides (fipronil, imidacloprid, selamectin, amitraz, etofenprox, indoxacarb, dinotefuran, permethrin, cyphenothrin, or spinosad). Some of these products also have piperonyl butoxide to synergize the effect of pyrethrin/pyrethroid and s-methoprene as larvicide.

Currently, the most widely used synthetic insect repellent chemicals are dimethyl phthalate (DMP), N,N-diethyl-m-toluamide (DEET), and picaridin. DEET is recommended by the American Academy of Pediatrics (AAP), the EPA and the CDC for its insect-repellent properties. These chemicals are effective against mosquitoes and other biting insects, but they are known to produce adverse effects, such as contact dermatitis, toxic encephalopathy in children, and skin eruptions (reviewed in Kim et al. 2005; Kitchen et al. 2009).

Due to greater toxicity, lack of selective toxicity, development of insect resistance to synthetic insecticides, and rising costs (WHO 1992; Gupta 2006; Bissinger and Roe 2010; Nicoletti et al. 2016a, b; Lin 2015; Mougabure-Cueto and Picollo 2015), their use has been declining. During the past two decades, the quest for natural products as an ecological alternative that replaces synthetic pesticides has been recognized as a necessity (Berenbaum 1989; Castillo et al. 1998; Purohit et al. 2011; Wink 2012). The botanical pesticides have been used for at least 2000 years in Asia and Middle East (Thacker 2002; Isman and Machial 2006). By now, thousands of plants have been tested for insect repellency and insecticidal effects. Intense research efforts are underway to find plant extracts and phytotoxicants that have repellent, larvicidal, and adulticidal effects against ticks,

R. C. Gupta (✉) · R. B. Doss
Toxicology Department, Breathitt Veterinary Center, Murray State University, Hopkinsville, KY, USA
e-mail: rgupta@murraystate.edu

A. Srivastava · R. Lall · A. Sinha
Vets Plus Inc., Menomonie, WI, USA

R. C. Gupta et al. (eds.), *Nutraceuticals in Veterinary Medicine*, https://doi.org/10.1007/978-3-030-04624-8_43

fleas, lice, flies, mosquitoes, and other ectoparasites. The inhibition of enzymes, such as acetylcholinesterase (AChE), butyrylcholinesterase (BuChE), tyrosinase, α-amylase, endonucleases, cellulase, and others, is used for insect control, altering the central nervous system (CNS), and growth (Sami 2014; Sami et al. 2016; Pulido et al. 2017). Some plant extracts or phytochemicals may also exhibit appetite suppression, reduction of reproductive activity, or direct toxicity in predatory insects (D'Incao et al. 2013). Interestingly, Weldon et al. (2011) also reported that some topically applied natural compounds (present in citrus peel) and monoterpenes, including geraniol, citronellol, citral, carveol, geranyl acetate, α-terpineol, citronellyl acetate, and carvone, are converted into more potent arthropod deterrents when oxidized on the integument of anointed animals.

In developing countries, plant extracts are commonly used to repel or kill ticks, flies, and other ectoparasites. For example, 28 plants from Ethiopia showed promising repellency activities against adult *Rhipicephalus pulchellus* ticks, with *Calpurnia aurea* displaying the highest toxicity toward the ticks (Zoroloni 2007). Currently, in the USA and Europe also, the use of biopesticides is becoming popular (Jaenson et al. 2006; Garboui 2008). This chapter describes some biopesticides which can be used to control ectoparasites in pets and farm animals. A partial list of biopesticides is given in Table 1.

2 Nutraceuticals for Control of Ticks, Fleas, and Other Ectoparasites

2.1 Neem Extract

Neem tree is native to Indian subcontinent, Australia, Africa, and Central and South America. Neem tree extract has been found to exert antidiabetic, antioxidant, anti-inflammatory, immunostimulant, antitumor, antimicrobial (antibacterial, antiviral, and antifungal), antiparasitic (insecticidal and anthelmintic), skin diseases, fever, hepatoprotective, anti-snake venom, and spermicidal activities (Kumar and Navaratnam 2013; Kumar et al. 2016). Ancient Indian texts call it "the curer of all ailments." The National Academy of Sciences published a report in 1992 entitled "Neem: A tree for solving global problems" (NAS 1992). In 2012, the United Nations declared the neem tree as the "Tree of the Twenty-First Century."

The importance of neem products to control arthropods, especially of medical and veterinary significance, has been recognized for more than half-a-century (WHO 1981; Vietmeyer 1992; Kumar and Navaratnam 2013: Nicoletti et al. 2016a, b; Benelli et al. 2015, 2016). Neem products exhibit a wide range of effects including antifeedancy (Tianyun and Mulla 1998; Lucantoni et al. 2006), ovicidal (Tianyun and Mulla 1998; Abdel-Ghaffar et al. 2012), larvicidal (Sinniah et al. 1994; Ziba 1995; Howard et al. 2011; Nicoletti et al. 2012), fecundity suppression (Tianyun and Mulla 1998), insect growth regulation (Mordue and Blackwell 1993; Mordue and Nisbet 2000), and repellency (Sharma and Dhiman 1993; Sharma et al. 1993; Singh et al. 1996). Simmonds et al. (2004) compared antifeedant and insecticidal activity of nimbin and salannin photooxidation products with neem limonoids. Chemical structure of azadirachtin A is shown in Fig. 1.

In a series of publications, Sami and co-workers (Sami and Akhtar 1993; Sami and Shakoori 2007; Sami 2014; Sami et al. 2016) have shown that neem phytoconstituents may produce insecticidal effect by targeting multiple molecules, such as alpha-amylase, acetylcholinesterase, endonucleases, cellulase, and others. Neem extracts have hormones that mimic and interfere with the life cycle of parasites, inhibit their ability to feed, and prevent the eggs from hatching. Abdel-Ghaffar et al. (2012) found a single treatment of head lice with a neem seed extract highly effective.

Different parts (roots, bark, leaves, fruits, and seeds) of neem tree may contain up to 300 phytochemicals. Recently, Benelli et al. (2016) reported that neem oil formulations usually show a range of different azadirachtins amounts, ranging from 1000 to 4000 mg/kg. Insecticidal activity of neem extract and its phytoconstituents is reported in about 100 published papers. Insect growth regulatory activity of neem-borne molecules weakens the cuticle defense system of the young instars causing easy penetration of pathogenic organisms. Emulsified formulations of neem oil showed an excellent larvicidal potential against different mosquito genera, including *Aedes*, *Anopheles*, and *Culex* (reviewed in Benelli et al. 2016). Mosquitocidal activity has been demonstrated for volatile organosulfur compounds from neem seeds (di-n-propyl-disulfide, $LC_{50} = 66$ ppm against third instar larvae of *Aedes aegypti*) (Balandrin et al. 1988).

The use of neem oil as an insecticide, as insect repellent, and in antifeedant activities has been approved by the US EPA (2012). The two most commonly used insecticides are NeemAzal and Fortune AZA, and both are extracts from neem seed kernels. Mosquitocidal effect of neem kernel oil appears to be due to azadirachtin A, salannin, nimbin, and nimbolid (Nicoletti et al. 2012, 2016a, b). Benelli et al. (2013, 2015, 2016) further emphasized that several minor constituents of neem are able to synergize the insecticidal effect of major constituents. Currently, in the USA and other countries, dozens of neem oil-based biopesticides are available on the market. But in the EU community, there is only one product that is margosa extract, which is derived from the extraction with supercritical CO_2 of neem kernels. Bark and roots of a neem tree appear to be excellent sources of bioinsecticides for the control of ectoparasites like fleas and ticks.

It has been suggested that unlike synthetic insecticides, the neem products are less likely to induce insect resistance

Table 1 List of plants with insect repellent and/or insecticidal property

Source	Biopesticide	Bioactivity	References
Allium sativum, *A. Ursinum*, *A. porrum*, *A. cepa* (garlic and onion)	Organosulfur compounds, allicin, and ajoene	Insect repellent effect, insecticidal, tickicide, trypanothione reductase inhibitory, trypanocidal, leishmanicidal, and antioxidative	Nchu (2004), Weiner et al. (2009), McGaw and Eloff (2010), Singh and Singh (2010), Goncharov et al. (2016), Krstin et al. (2018a, b)
Aristeguietia glutinosa	Two labdane diterpenoids	Anti-*Trypanosoma cruzi*	Varela et al. (2012, 2014)
Azadirachta indica (neem tree)	Azadirachtin A, di-n-propyl-disulfide, meliacin, meliacinol, meliatetraolenol, sesquiterpene, salannin, nimbin, and nimbolid	Ovicidal, larvicidal, mosquitocidal, antioxidative, anti-inflammatory, immunostimulant, spermicidal, antibacterial, antiviral, antifungal, and insect repellent	Mulla and Tianyun (1999), Okumu et al. (2007), Kumar and Navaratnam (2013), Benelli et al. (2015, 2016), Kumar et al. (2016), Nicoletti et al. (2016a, b)
BioUD®	2-Undecanone derived from wild tomato plants	Insect repellent	Bissinger et al. (2009)
Calpurnia aurea	–	Tick repellent	Zoroloni (2007)
Castor bean leaf	Trypsin inhibitors	Larvicidal and insecticidal	Rossi et al. (2012)
Cymbopogon nardus, *C. winterianus* (citronella)	Citronella, citronellal, 1,8-cineol, Z-citral, α- citral, and α-pinene	Insect repellent	Ansari and Razdan (1995), Kim et al. (2005), Sakulku et al. (2009), Goodyer et al. (2010), Miro Specos et al. (2010), Maia and Moore (2011)
Eucalyptus globoidea	Globoidnan A	Tick repellent	Mkolo (2008)
Eucalyptus citriodora, *E. globulus* (lemon eucalyptus oil)	*P*-Methane-3,8-diol	Repels mosquitoes, biting flies, and gnats	Trigg (1996), Barasa et al. (2002), Carroll and Loye (2006), and Maia and Moore (2011)
Eugenia caryophyllata (clove oil)	Sesquiterpenes, eugenol, thymol, carvacrol, cinnamaldehyde	Mosquito repellent, insect repellent, acaricidal, ovicidal, larvicidal, antimicrobial, antiviral, antifungal, antioxidative, and anti-inflammatory	El-Hag et al. (1999), Kim et al. (2003), Yang et al. (2003), Jirovetz et al. (2006), Chaieb et al. (2007), Shapiro (2012)
Pelargonium graveolens (geranium oil)	(-)-10-Epi-γ-eudesmol, citronellol, linalool, geraniol, isomenthone, geranyl formate, β-caryophyllene, germacrene D, etc.	Insect repellent, tick repellent	Tabanca et al. (2013)
Lavandula angustifolia (lavender)	Linalool, linalyl acetate, 1,8-cineole, camphor, β-pinene, and terpinene-4-ol	Tick repellent, mosquito repellent, insecticidal	Mkolo (2008), Attia et al. (2016)
Lippia javanica	–	Anti-tick	Nchu (2004)
D-Limonene	D-Limonene	Insecticidal, antioxidative, anti-inflammatory, antibacterial, and antifungal	Sun (2007), Kim et al. (2013), D-Limonene (2017)
Melia azedarach L. (chinaberry tree)	Triterpenes, limonoids, melianol, melianone, vanillin, and vanillic acid	Anti-*Triatoma infestans*, insecticidal, repellency, and antifeedant	Han et al. (1991), Carpinella et al. (2003), Szewczuk et al. (2003), Dadé et al. (2018)
Mentha piperita (peppermint)		Larvicidal, insecticidal, repellent, growth, and reproduction inhibition	Ansari et al. (1999), Kumar et al (2011)
Pinus longifolia (pine oil)	Eugenol, isoeugenol, eugenyl acetate, K-terpineol, K-pinene, B-pinene, cineol, camphor, and caryophyllene	Mosquito repellent and larvicidal (only at high concentration)	Ansari et al. (2005)
Roldana barba-johannis	Sargachromenol, sargahydroquinoic acid, and sargaquinoic acid	Insect growth inhibitory	Céspedes et al. (2004)
Senna italica ssp. *arachoides*	–	Acaricidal effect, tickicide, antifeedant	Thembo (2006)
Tagetes minuta (marigold oil)	Terpene (ocimenone)	Anti-tick, mosquito-larvicidal	Green et al. (1991)
Tanacetum vulgare L.	α-Pinene, β-pinene, pinocamphone, 1,8-cineole, α-thujone, β-thujone, verbenol, and verbenone	Tick repellent	Pålsson et al. (2008)
Thymus vulgaris Thymol	α-Terpinene, carvacrol, thymol, *p*-cymene, linalool, and geraniol	Insect repellent and insecticidal	Reviewed in Maia and Moore (2011)
Tithonia diversifolia (Mexican sunflower)	Sesquiterpene lactones and phenolic compounds	Tyrosine inhibitory, repellent, and insecticidal	Pulido et al. (2017)

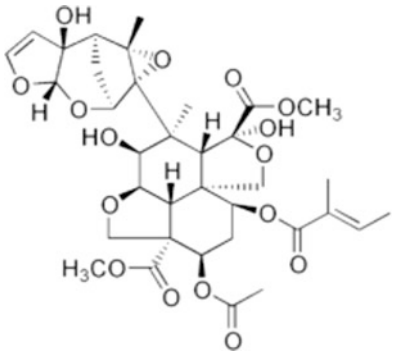


Fig. 1 Chemical structure of azadirachtin A

due to their multiple modes of action (Mulla and Tianyun 1999).

2.2 *Melia azedarach* L.

Melia azedarach L. (also called chinaberry tree) is originated from the Himalaya's region in India. Its phytochemical constituents include triterpene limonoids, melianol, melianone, meliandiol, vanillin, and vanillic acid (Han et al. 1991). Acetonic extract of *M. azedarach* has been demonstrated to exert insecticidal and repellent activities against *Triatoma infestans* (kissing bug) (Dadé et al. 2018), a vector for *Trypanosoma cruzi*, a monoprotozoan parasite responsible for Chagas disease transmission in humans and dogs. In addition to antiparasitic (Valladares et al. 1999), extracts prepared from *Melia azedarach* L. have antibacterial, antiviral, antifungal, and antioxidant properties.

2.3 *Senna italica* ssp. *arachoides*

Thembo (2006) demonstrated that *Senna italica* ssp. *arachoides* ethyl acetate extracts had a concentration-dependent acaricidal effect on *Hyalomma marginatum rufipes*. When *S. italica* ssp. *arachoides* aqueous extracts were fed to guinea pigs and rabbits, the feeding performance of adult *H. m. rufipes* ticks appeared to be impaired.

2.4 Garlic and Onion

The benefits of garlic (*Allium sativum*) and onion (*Allium cepa*) bulbs in health and diseases (including parasitic diseases) have been recognized since ancient times. Chemical analysis of the extracts of these two plants has revealed the presence of several sulfur secondary metabolites (allicin, ajoene, and others) and one (zwiebelane) in the onion (Singh and Singh 2010; Goncharov et al. 2016; Krstin et al. 2018a, b).

Garlic extract has been found to be effective against a host of protozoa, including *Opalina ranarum*, *Balantidium entozoon*, *Entamoeba histolytica*, *Trypanosoma*, *Leishmania*, *Leptomonas*, and *Crithidia* (Greenstock and Larrea 1972: Saleheen et al. 2004; Wabwoba et al. 2010; Sadeghi-Nejad and Saki 2014; Goncharov et al. 2016). Nchu (2004) analyzed the repellent effects of extracts of *Allium species*, as well as the direct toxicity, against adults of *Hyalomma marginatum rufipes*. Findings revealed that acetone extracts of *A. porrum* exhibited high repellency index (65–79.48%) and the dichloromethane extract of *A. sativum* was toxic to 100% of ticks within an hour of exposure.

Recently, Krstin et al. (2018a) evaluated antiparasitic activities of garlic and onion against *Trypanosoma brucei brucei* and *Leishmania tarentolae*. Both garlic and onion extracts inhibited the growth of trypanosomes and leishmanias and killed parasites efficiently. The extracts inhibited trypanothione reductase activity irreversibly in *Trypanosoma brucei* through disulfide bond formation between SH groups of vital redox compounds and sulfur-containing secondary metabolites. Krstin et al. (2018b) reported that dichloromethane extracts from *Allium ursinum* and *Tulbaghia violacea* are capable of inhibiting trypanothione reductase and consequently mediate a growth inhibition of the parasites. The mechanism for antiparasitic activity of garlic and onion appears to be related to the amount and the profile of sulfur-containing compounds. Garlic extract was found to be five times more potent than onion extract for trypanocidal effect due to abundance of organosulfur compounds (especially ajoene) and inhibition of trypanothione reductase activity. These authors and others confirmed the hypothesis that the sulfur-containing compounds produced in the alliinase pathway, such as in garlic and onion, are responsible for the antiparasitic activity (Weiner et al. 2009: Krstin et al. 2018a, b). It needs to be pointed out that the garlic extract also decreased the mitochondrial membrane potential significantly in a dose-dependent manner in trypanosomes (Krstin et al. 2018a) and may lead to apoptosis in protozoa (Ly et al. 2003). It has been reported that the combination of garlic and onion with common trypanocidal and leishmanicidal drugs resulted in a synergistic or additive effect to enhance antiparasitic activity (Krstin et al. 2018a).

2.5 Citronella and Citronellal

Oil of citronella is obtained from a perennial grass *Cymbopogon nardus* or *Citronella winterianus*. It has been used as an insect repellent since 1948. Citronella oil has many phytoconstituents (such as 1,8-cineol, citronellal, Z- and

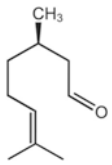


Fig. 2 Chemical structure of citronellal (main component in citronella oil)

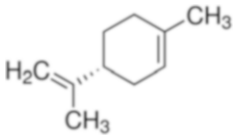


Fig. 3 Structural formula of D-limonene

α-citral, and α-pinene), but citronellal or rhodinal (3,7-dimethyloct-6-en-1-al), which is a monoterpenoid, is the main compound that gives a distinctive aroma for insect repellency. Chemical structure of citronellal is shown in Fig. 2.

Kim et al. (2005) evaluated the actual repellent efficacy of two natural compounds (citronella and citronellal) in vitro and in the field against *Culex pipiens pallens*. Percentage repellency of the three controlled bands (impregnated with 30% citronella extract, 15% citronella extract, and 30% citronellal extract) were calculated at 86%, 73%, and 78%, respectively, in vitro, and 80% on the band impregnated with 30% citronella extract in field. It appears that these two natural aroma chemicals have high repellent efficacy against mosquitoes, fleas, ticks, and other insect vectors. Currently, citronella is one of the most widely used natural repellents on the market used at a concentration of 5–10% (Maia and Moore 2011). Repellent effect of citronella has been reported to be prolonged by delayed release rate using nanoformulation (Sakulku et al. 2009) or microencapsulation (Miro Specos et al. 2010).

As insect repellent, citronellal is commercially available in lemon balm, and citronella is available in sprays, candles, lotions, gels, and towelette wipes. Neither citronella nor citronellal in citronella oil has insecticidal activity. When used according to the label, citronella products are not expected to cause harm to pets, humans, or the environment. Based on laboratory animal studies, oil of citronella poses minimal or no risks to wildlife.

2.6 D-Limonene

D-Limonene (1-methyl-4-(1-methylethenyl) cyclohexane) is a monocyclic monoterpene, and it is obtained from citrus fruit (lemon and orange) peels. Structural formula of D-limonene is shown in Fig. 3.

D-Limonene is commonly used as fragrance in perfumery, aftershave lotions, bath products, personal care products, dietary supplements, chemoprevention, and botanical insecticides (D-Limonene 2017). It exerts antioxidative, anti-inflammatory, antibacterial, and antifungal properties. Additionally, D-limonene increases peristalsis. D-Limonene is listed in the Code of Federal Regulations as generally recognized as safe (GRAS) for a flavoring agent. Currently, a number of D-limonene-containing products as shampoo, soaps, etc. are available as ectoparasiticides for dogs and cats.

D-Limonene is considered to have a fairly low toxicity. The oral LD_{50} for D-limonene in male and female mice is reported to be 5.6 and 6.6 g/kg body wt, respectively, while LD_{50} in male and female rats is reported to be 4.4 and 5.1 g/kg body wt, respectively (Sun 2007). In 1990, the National Toxicology Program (NTP) investigated the toxicity of D-limonene (>99% pure) at doses ranging from 413 to 6600 mg/kg daily administered to rats and mice 5 days/week for 3 weeks. No signs of compound-related toxicity were noted at doses <1650 mg/kg daily. D-Limonene does not pose a mutagenic, carcinogenic, or nephrotoxic risk to humans. D-Limonene applied to the skin may cause irritation, but otherwise appears to be safe for use (Kim et al. 2013).

2.7 Lavender Oil

Lavender oil is an essential oil obtained from *Lavandula angustifolia*. Phytochemical analysis of *Lavandula angustifolia* by GC/MS revealed the presence of linalool (38.57%), followed by linalyl acetate (29.95%), 1,8-cineole (13.66%), camphor (13.13%), β-pinene (3.14%), and terpinene-4-ol (1.54%) (Attia et al. 2016). It has been used in aromatherapy and as insect (mosquitoes, fleas, flies, and other biting insects) repellent for many centuries. Lavender oil exerts analgesic, insecticidal, antiseptic, and calming properties. These properties of lavender are suitable in both preventing insect bites and soothing insect bites and stings. Lavender oil is used to prevent the spread of infection caused by a bug bite and control the itching and inflammation often associated with bug bites. Lavender-containing products are often available in the form of skin lotion, soap, and shampoo. Lavender oil (5%) has been used to treat skin irritation caused by mites laying eggs under the skin and scabies. In case of inflammation caused by mosquito bite, apply a cold compress and the mixture of lavender and tea tree oil, 1–2 drops every hour or until the inflammation subsides.

If lavender oil causes skin irritation at the site of application, then consider mixing it with olive oil (50:50). In high doses, ingestion of lavender oil may cause endocrine disruption by inhibiting the effects of androgen and mimicking the actions of estrogen, thereby producing gynecomastia.

2.8 Peppermint Oil

Peppermint oil is obtained from *Mentha piperita*. Peppermint has natural insect repellent and larvicidal properties (Ansari et al. 1999). In case of mosquito bite itch, the

mixture of peppermint oil (10 drops) and tea tree oil (10 drops) in a carrier oil can relieve the discomfort by soothing and healing effect.

2.9 Pine Oil

Pine oil is obtained from *Pinus longifolia*. Pine has several phytochemicals, including eugenol, isoeugenol, eugenyl acetate, K-terpineol, caryophyllene, K-pinene, B-pinene, B cineole, and camphor (Ansari et al. 2005). Ansari et al. (2005) found that pine oil showed strong repellent action against *An. culicifacies* (malaria vector) and *Cx. quinquefasciatus* (pest mosquito), but larvicidal effect was observed only at very higher doses.

2.10 Lemon Eucalyptus (*Eucalyptus citriodora*) Oil

Eucalyptus citriodora is a lemon-scented gum tree. Eucalyptus oil (an essential oil) is extracted from the leaves and consists of several phytoconstituents, including *p*-methane-3,8-diol and eucamol (Barasa et al. 2002; Maia and Moore 2011). The oil protects the skin from mosquitoes, ticks, and other biting insects, and the effect lasts for several hours. Like citronella, eucalyptus oil needs to be applied regularly to maintain skin protection. Trigg (1996) evaluated eucalyptus-derived insect repellent (PMD), having *p*-methane-3,8-diol as an active ingredient, and compared its repellent effect against DEET. The eucalyptus-based products provided complete protection from insect biting for 6–7.75 h and found to be as effective as DEET. Another product, Mosi-guard Natural (MASTA, London, UK), comprising *p*-methane-3,8-diol and isopulegol and citronella, is available on the market as insect repellent (Carroll and Loye 2006). Insect repellent products derived from eucalyptus are available in the form of spray or lotion.

In laboratory animals, p-methane-3,8-diol showed no adverse effects, except eye irritation. These products pose minimal or no risks to wildlife or the environment.

2.11 Soybean Oil

Soybean oil is an active ingredient in a commercial insect repellent product BiteBlocker™, which has been on the market since 2001. In an experimental study, Campbell and Gries (2012) reported that soybean oil exhibited no repellent effect against *Aedes aegypti* L.

2.12 Cedar Oil

Cedar oil is commonly sprayed (misting) on pet's skin to protect from ectoparasites. Cedar oil kills ticks, fleas, flies, mites, mosquitoes, gnats, chiggers, and other biting insects. It also works as an insect repellent. Cedar oil has lasting parasiticidal and repellent effect and leaves anti-itch healing and a cedar scent on the pet's skin. It is safe to use on puppies and kittens as well. Cedar oil spray can be used on carpet against fleas and ticks. The oil dissolves eggs and larvae and impairs breathing of insects by blocking breathing pores.

2.13 Geranium Oil

Geranium oil is obtained from the leaves and stalks of the *Pelargonium capitatum* or *P. graveolens*. It contains many phytoconstituents, including (-)-10-epi-γ-eudesmol, citronellol, linalool, geraniol, isomenthone, geranyl formate, β-caryophyllene, germacrene D, etc. (Tabanca et al. 2013). Tick repellency is mainly due to (-)-10-epi-γ-eudesmol. It is recommended that to protect dog's skin, mix 20 drops of geranium oil in 3 tablespoons of almond oil, and apply 3–4 drops to sections of the dog's fur, especially the collar area. Using almond oil as a carrier enhances the solution's effectiveness since ticks hate the sulfur odor. Geranium oil may not be safe for cats.

2.14 Clove Oil

Clove oil is extracted from *Eugenia caryophyllata* and consists of several phytoconstituents, including sesquiterpenes, eugenol, thymol, carvacrol, and cinnamaldehyde (Zheng et al. 1992; Jirovetz et al. 2006; Chaieb et al. 2007). Clove oil is reported to repel mosquitoes, ticks, mites, and other biting insects (Kim et al. 2003; Shapiro 2012). Additionally, clove oil exerts an ovicidal and larvicidal effect against *Culex pipiens* and *Pediculus capitis* (Yang et al. 2003) and ticks and mites (El-Hag et al. 1999). The compound eugenol appears to be responsible for insect repellency as well as for acaricidal activity toward *Dermatophagoides farinae* and *D. pteronyssinus* (Perrucci et al. 1995; Kim et al. 2003), in addition to antioxidative, anti-inflammatory, analgesic, and antibacterial activities (Jirovetz et al. 2006; Chaieb et al. 2007). Clove oils should be used on pets with caution, as an overdose may result in toxicity. The clinical signs may include salivation, vomiting, seizure, muscle tremors, and even death. Like humans, animals taking NSAIDs, blood thinners, or medication for diabetes, or cardiac conditions, should not be treated with clove oil. It is noteworthy that dogs and cats can have a much more intense reaction to clove oil than humans.

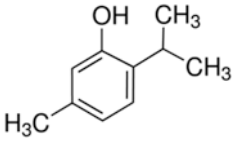


Fig. 4 Structural formula of thymol

2.15 Thyme Oil

Thyme oil is extracted from *Thymus vulgaris*. It contains many phytoconstituents, including α-terpinene, carvacrol, thymol, *p*-cymene, linalool, and geraniol (Maia and Moore 2011). The structural formula of thymol is shown in Fig. 4.

Thymol exhibits insect repellent and insecticidal effects. Mechanism of action of thymol in insects is not well understood. It is suggested that thymol acts upon the neuronal synapses by blocking the production of a neurotransmitter acetylcholine. In *Drosophila melanogaster*, thymol exerts insecticidal effect by binding to GABA receptors and inhibiting the enzyme activity of cytochrome P450 and glutathione-S-transferase. Frequent use of thymol on pets may be needed, as it breaks down by photooxidation. In farm animals, the use of thymol appears to be impractical as they face the sun most of the day. Due to its short life, thymol leaves no residue in food, milk, or meat. Thymol on pets should be used with a caution, as it may be a skin and eye irritant and may cause skin sensitization. Overall, thymol is considered to be safe, as its oral LD_{50} is reported to be 980 mg/kg and dermal LD_{50} >2000 mg/kg body wt in rats.

3 Concluding Remarks and Future Directions

Many blood-sucking ectoparasites bite or sting at human and animal skin; cause inflammation, allergies, and dermatitis; and transmit deadly diseases. Various plant products are available that exert ovicidal, larvicidal, adulticidal, and repellent effects against ticks, fleas, mosquitoes, and other biting ectoparasites via multiple mechanisms. These biopesticides are (1) easily available, (2) are inexpensive, (3) are rapidly biodegradable, (4) leave no insecticide residue in the food/feed, and (5) offer greater safety to nontarget species. Additionally, there is no development of insect resistance to these biopesticides. Some important plant extracts that are commonly used or have potential for ectoparasiticidal or insect repellent effects are discussed in this chapter. The quest for new biopesticides and repellents with greater efficacy and safety will continue.

Acknowledgments The authors would like to thank Ms. Robin B. Doss for her technical assistance in the preparation of this chapter.

References

Abdel-Ghaffar F, Al-Quraishy S, Al-Rasheid KA et al (2012) Efficacy of a single treatment of head lice with a neem seed extract: an *in vivo* and *in vitro* study on nits and motile stages. Parasitol Res 110 (1):277–280

Ansari MA, Razdan RK (1995) Relative efficacy of various oils in repelling mosquitoes. Indian J Malariol 32:104–111

Ansari MA, Vasudevan P, Tandon M et al (1999) Larvicidal and mosquito repellent action of peppermint (*Mentha piperita*) oil. Bioresour Technol 71:267–271

Ansari MA, Mittal PK, Razdan RK et al (2005) Larvicidal and mosquito repellent activities of pine (*Pinus longifolia*, Family: Pinaceae) oil. J Vect Borne Dis 42:95–99

Attia S, Lognay G, Heuskin S et al (2016) Insecticidal activity of *Lavandula angustifolia* Mill against the pea aphid Acyrthosiphon *pisum*. J Entomol Zool Stud 4(1):118–122

Balandrin MFS, Lee SM, Klocke JA (1988) Biologically active volatile organosulfur compounds from seeds of the neem tree, *Azadirachta indica* (*Meliaceae*). J Agric Food Chem 36:1048–1054

Barasa SS, Ndiege IO, Lwande W et al (2002) Repellent activities of stereoisomers of *p*-methane-3,8-diols against *Anopheles gambiae* (Diptera: Culicidae). J Med Entomol 39:736–741

Benelli G, Canale A, Conti B (2013) Eco-friendly control strategies against the Asian tiger mosquito, *Aedes albopictus* (Diptera: Culicidae): repellency and toxic activity of plant essential oils and extracts. Pharmacol Online 47:44–51

Benelli G, Murugan K, Panneerselvam C et al (2015) Old ingredients for a few new recipe? Neem cake, a low-cost botanical by-product in the fight against mosquito-borne diseases. Parasitol Res 114: 391–397

Benelli G, Canale A, Toniolo C et al (2016) Neem (*Azadirachta indica*): towards the ideal insecticide? Nat Prod Res. https://doi.org/10.1080/14786419..2016.1214834

Berenbaum MR (1989) North American ethnobotanicals as source of novel plant-based insecticides. In: Arnason J, Philogene B, Morand P (eds) Insecticides of plant origin. American Chemical Society, Washington, pp 11–24. https://doi.org/10.1021/bk-1989-0387.ch002

Bissinger BW, Roe RM (2010) Tick repellents: past, present, and future. Pest Biochem Physiol 96:63–79

Bissinger BW, Apperson CS, Sonenshine DE et al (2009) Efficacy of the new repellent BioUD® against three species of ixodid ticks. Exp Appl Acarol 48(3):239–250

Campbell C, Gries G (2012) Is soybean oil an effective repellent against *Aedes aegypti*? Canad Entomol 142(4):405–415

Carpinella C, Defagó T, Valladares G et al (2003) Antifeedant and insecticide properties of a limonoids from *Melia azedarach* (*Meliaceae*) with potential use for pest management. J Agr Food Chem 51:369–374

Carroll SP, Loye J (2006) PMD, a registered botanical mosquito repellent with DEET-like efficacy. J Am Mosq Control Assoc 22:507–514

Castillo M, Martinez-Pardo R, Garcera MD et al (1998) Biological activities of natural sesquiterpene lactones and the effect of synthetic sesquiterpene derivatives on insect juvenile hormone biosynthesis. J Agric Food Chem 46:2030–2035

Céspedes CL, Torres P, Marín JC et al (2004) Insect growth inhibition by tocotrienols and hydroquinones from *Roldana barba-johannis*. Phytochemistry 65:1963–1975

Chaieb K, Hajlaoui H, Zmontar T et al (2007) The chemical composition and biological activity of clove essential oil, *Eugenia caryophyllata* (*Syzigium aromaticum* L. Myrtaceae): a short review. Phytother Res 21:501–506

D'Incao MP, Kanak N, Fiuza LM (2013) Phytochemicals taken from plants with potential in management of *Spodoptera frugiperda* (Lepidoptera: Noctuidae). J Biopest 6(2):182–192

Dadé M, Zeinsteger P, Bozzolo F et al (2018) Repellent and lethal activities of extracts from fruits of *Melia azedarach* L. (Chinaberry, *Meliaceae*) against *Triatoma infestans*. Font Vet Sci 5:158. https://doi.org/10.3389/fvets.2018.00158

D-Limonene (2017) National Center for Biotechnology Information. US National Library of Medicine

El-Hag EA, El Nadi AH, Zaiton AA (1999) Toxic and growth retarding effects of three plant extracts on *Culex pipiens* larvae (Diptera: Culicidae). Phytother Res 13:388–392

Garboui SS (2008) Plant-derived chemicals as tick repellents. Doctoral Dissertation, Uppsala University, Uppsala, Sweden

Goncharov N, Orekhov AN, Voitenko N et al (2016) Organosulfur compounds as nutraceuticals. In: Gupta RC (ed) Nutraceuticals: efficacy, safety and toxicity. Academic Press, Amsterdam, pp 555–568

Goodyer LI, Croft AM, Frances SP et al (2010) Expert review of the evidence base for arthropod bite avoidance. J Travel Med 17:182–192

Green M, Singer JM, Sutherland DJ et al (1991) Larvicidal activity of *Tagetus minuta* (Marigold) towards *Aedes aegypti*. J Am Mosq Control Assoc 7:282–286

Greenstock DL, Larrea Q (1972) Garlic as an insecticide. Doubleday Research Association, Braintree, England, p 12

Gupta RC (2006) In: Gupta RC (ed) Toxicology of organophosphate and carbamate compounds. Academic Press/Elsevier, Amsterdam, pp 1–763

Han J, Lin WH, Xu RS et al (1991) Studies on the chemical constituents of *Melia azedarach* Linn. Yao Xue Xue Bao 26(6):426–429

Howard AFV, Adongo EA, Githure JVJ (2011) Effects of a botanical larvicide derived from *Azadirachta indica* (the neem tree) on oviposition behavior in *Anopheles gambiae* s.s. mosquitoes. J Med Plant Res 5:1948–1954

Isman MB, Machial CM (2006) Chapter 2: Pesticides based on plant essential oils: from traditional practice to commercialization. In: Rai M, Carpinella MC (eds) Naturally occurring bioactive compounds. Elsevier, Amsterdam, pp 29–44

Jaenson TGT, Garboui SS, Palsson K (2006) Repellency of oils of lemon eucalyptus, geranium, and lavender and the mosquito repellent MyggA natural to *Ixodes ricinus* (Acari: Ixodidae) in the laboratory and field. J Med Entomol 43:731–736

Jirovetz L, Buchbauer G, Stoilova I et al (2006) Chemical composition and antioxidant properties of clove leaf essential oil. J Agr Food Chem 54:6303–6307

Kim EH, Kim HK, Choi DH et al (2003) Acaricidal activity of clove bud oil compounds against *Tyrophagus putrescentiae* (Acari: Acaridae). Appl Entomol Zool 38:261–266

Kim JK, Kang CS, Lee JK et al (2005) Evaluation of repellency effect of two natural aroma mosquito repellent compounds, citronella and citronellal. Entomol Res 35(2):117–120

Kim YW, Kim MJ, Chung BY et al (2013) Safety evaluation and risk assessment of d-limonene. J Toxicol Environ Health Part B 16 (1):17–38

Kitchen LW, Lawrence KL, Coleman RE (2009) The role of the United States military in the development of vector control products, including insect repellents, insecticides, and bed nets. J Vector Ecol 34:50–61

Krstin S, Sobeh M, Braun MS et al (2018a) Anti-parasitic activities of *Allium sativum* and *Allium cepa* against *Trypanosoma b. brucei* and *Leishmania tarentolae*. Medicine 5:37

Krstin S, Sobeh M, Braun MS et al (2018b) *Tulbaghia violacea* and *Allium ursinum* extracts exhibit anti-parasitic and antimicrobial activities. Molecules 23:313

Kumar VS, Navaratnam V (2013) Neem (*Azadirachta indica*): prehistory to contemporary medicinal uses to humankind. Asian Pac J Trop Biomed 3:505–314

Kumar P, Mishra S, Malik A et al (2011) Insecticidal properties of Mentha species: a review. Ind Crop Prod 34(1):802–817

Kumar D, Rahal A, Malik JK (2016) Neem extract. In: Gupta RC (ed) Nutraceuticals: efficacy, safety and toxicity. Academic Press, Amsterdam, pp 585–597

Lin N (2015) Insecticide resistance in mosquitos: impact, mechanisms, and research directions. Annu Rev Entomol 60:34–41

Lucantoni L, Giusti F, Cristofaro M et al (2006) Effects of neem extract on blood feeding, oviposition and oocyte ultrastructure in *Anopheles stephensi* Liston (Diptera: Culicidae). Tissue Cell 38:361–371

Ly JD, Grubb DR, Lawen A (2003) The mitochondrial membrane potential (deltapsi(m)) in apoptosis: an update. Apoptosis 8:115–128

Maia MF, Moore SJ (2011) Plant-based insect repellents: a review of their efficacy, development and testing. Malaria J 10(Suppl):S11

Marrs TC (2012) Insecticides that interfere with insect growth and development. In: Marrs TC (ed) Mammalian toxicology of insecticides. RSC Publ, Cambridge, pp 221–253

Marrs TC, Dewhurst IC (2012) Toxicology of some insecticides not discussed elsewhere. In: Marrs TC (ed) Mammalian toxicology of insecticides. RSC Publ, Cambridge, pp 288–301

McGaw LJ, Eloff JN (2010) Methods for evaluating efficacy of ethnoveterinary medicinal plants. In: Katerere DR, Luseba D (eds) Ethnoveterinary botanical medicine. Herbal medicines for animal health. CRC Press, Boca Raton, FL, pp 1–24

Miro Specos MM, Garcia JJ, Tornesello J et al (2010) Microencapsulated citronella oil for mosquito repellent finishing of cotton textiles. Trans R Soc Trop Med Hyg 104:653–658

Mkolo NM (2008) Anti-tick properties of some of the traditionally used plant-based products in South Africa. MSc Thesis, University of Limpopo, South Africa.

Mordue (L)AJ, Blackwell A (1993) Azadirachtin; an update. Insect Physiol 39:903–924

Mordue (L)AJ, Nisbet AJ (2000) Azadirachtin from the neem tree *Azadirachta indica*: its actions against insects. Ann Enomol Soc Brasil 29:615–632

Mougabure-Cueto G, Picollo MI (2015) Insecticide resistance in vector Chagas disease; Evolution, mechanisms and management. Acta Trop 149:70–85

Mulla MS, Tianyun S (1999) Activity and biological effects of neem products against arthropods of medical and veterinary importance. J Am Mosq Control Assoc 15(2):133–152

National Academies of Science (1992) Neem, a tree for solving global problems. National Academies Press, Washington, DC

Nchu F (2004) Developing methods for the screening of ethnoveterinary plants for tick control. MSc Thesis, Medical University of South Africa

Nicoletti M, Mariani S, Maccioni O et al (2012) Neem cake: chemical composition and larvicidal activity on Asian tiger mosquito. Parasitol Res 111:205–213

Nicoletti M, Murugan K, Benelli G (2016a) Chapter 11: emerging insect-borne diseases of agricultural, medical and veterinary importance. In: Trdan S (ed) Emerging insect-borne diseases of agriculture, medical and veterinary importance. Intech, Rijeka, pp 219–241

Nicoletti M, Murugan K, Canale A et al (2016b) Neem-borne molecules as eco-friendly control tools against mosquito vectors of economic importance. Curr Org Chem 20(25):2681–2689. https://doi.org/10.2174/1385272820666160218233923

Okumu FO, Knols BGJ, Fillinger U (2007) Larvicidal effects of a neem (*Azadirachta indica*) oil formulation on the malaria vector *Anopheles gambiae*. Malaria J 6:63

Pålsson K, Jaenson TG, Baeckström P et al (2008) Tick repellent substances in the essential oil of *Tencetum vulgare*. J Med Entomol 45(1):88–93

Perrucci S, Macchioni G, Cioni PL et al (1995) Structure/activity relationship of some natural monoterpenes as acaricides against *Psoroptes cuniculi*. J Nat Prod 8:1261–1264

Pulido KDP, Dulcey AJC, Martínez JHI (2017) New caffeic acid derivative from *Tithonia diversifolia* (Hemsl.) A. Gray butanolic extract and its antioxidant activity. Food Chem Toxicol 109:1079–1085

Purohit AM, Rezende AR, Lopez Baldin EL et al (2011) Plant extracts, isolated phytochemicals, and plant-derived agents which are lethal to arthropod vectors of human tropical diseases: a review. Planta Med 77:618–630

Rossi GD, Santos CD, Carvalho GA et al (2012) Biochemical analysis of a castor bean extract and its insecticidal effects against *Spodoptera frugiperda* (smith) (Lepidoptera: noctuidae). Neotrop Entomol 41:503–509

Sadeghi-Nejad B, Saki J (2014) Effect of aqueous *Allium cepa* and *Ixora brachiata* root extract on *Leishmania major* promastigotes. Jundishapur J Nat Pharm Prod 9:e15442

Sakulku U, Nuchuchua O, Uawongyart N et al (2009) Characterization and mosquito repellent activity of citronella oil nanoemulsion. Int J Pharm 372:105–111

Saleheen D, Ali SA, Yasinzai MM (2004) Antileishmanial activity of aqueous onion extract *in vitro*. Fitoterapia 75:9–13

Sami AJ (2014) *Azadirachta indica* derived compounds as inhibitors of digestive alpha-amylase in insect pests: potential biopesticides in insect pest management. Eur J Exp Biol 4:259–264

Sami AJ, Akhtar MW (1993) Purification and characterization of two low-molecular weight endonucleases of *Cellulomonas flavigena*. Enzyme Microb Technol 15:586–592

Sami AJ, Shakoori AR (2007) Extracts of plant leaves have inhibitory effect on the cellulase activity of whole body extracts of insects: a possible recipe for bioinsecticides. Proc Pakistan Congr Zool 27:105–118

Sami AJ, Bilal S, Khalid M et al (2016) Effect of crude neem (*Azadirachta indica*) powder and azadirachtin on the growth and acetylcholinesterase activity of *Tribolium castaneum* (Herbst) (Coleoptera; Tenebrionidae). Pakistan J Zool 48:881–886

Shapiro R (2012) Prevention of vector transmitted diseases with clove oil insect repellent. J Pediatr Nurs 27:346–349

Sharma VP, Dhiman RC (1993) Neem oil as a sand fly (Diptera: Psychodidae) repellent. J Am Mosq Control Assoc 9:364–366

Sharma VP, Ansari MA, Razdan RK (1993) Mosquito repellent action of neem (*Azadirachta indica*) oil. J Am Mosq Control Assoc 9:359–360

Simmonds MS, Jarvis AP, Johnson S et al (2004) Comparison of antifeedant and insecticidal activity of nimbin and salannin photooxidation products with neem (*Azadirachta indica*) limonoids. Pest Manag Sci 60:459–464

Singh YP, Singh RA (2010) *In silico* studies of organosulfur-functional active compounds in garlic. Biofactors 36(4):297–311

Singh N, Mishra AK, Saxena A (1996) Use of neem cream as a mosquito repellent in tribal areas of central India. Indian J Malaria 33:99–102

Sinniah B, Sinniah D, Ibrahim J (1994) Effect of neem oil on mosquito larvae. Mosq Borne Dis Bull 1:90–93

Sun J (2007) D-Limonene: safety and clinical applications. Altern Med Rev 12(3):259–264

Szewczuk VD, Mongelli ER, Pomilio AB (2003) Antiparasitic activity of *Melia azedarach* growing in Argentina. Mol Med Chem 1:54–57

Tabanca N, Wang M, Avonto C et al (2013) Bioactivity-guided investigation of geranium essential oils as natural tick repellents. J Agric Food Chem 61:4101–4107

Thacker JRM (2002) An introduction to arthropods pest control. Cambridge University Press, Cambridge, UK, p 343

Thembo MK (2006) The anti-tick effects of *Senna italica* ssp. *arachoides* extracts on adults *Hyalomma marginatum rufipes*. MSc Thesis, University of Limpopo, South Africa

Tianyun S, Mulla MS (1998) Ovicidal activity of neem products (Azadirachtin) against *Culex tarsalis* and *Culex quinquefasciatus* (Diptera: Culicidae). J Am Mosq Control Assoc 14:204–209

Trigg JK (1996) Evaluation of a Eucalyptus-based repellent against *Anopheles* Spp. in Tanzania. J Am Mosq Control Ass 12 (2):243–246

US EPA (2012) Biopesticide registration document. Cold pressed neem oil. PC Code 025006. Office of Pesticide Programs, Washington, DC

Valladares GR, Ferreyra D, Defago MT et al (1999) Effects of *Melia azedarach* on *Triatoma infestans*. Fitoterapia 70(4):421–424

Varela L, Lavaggi ML, Cabrera M et al (2012) Bioactive-guided identification of labdane diterpenoids from aerial parts of *Aristeguietia glutinosa* Lam. as anti-*Trypanosoma cruzi* agents. Nat Prod Commun 7:1139–1142

Varela J, Serna E, Torres S et al (2014) *In vivo* anti-*Trypanosoma cruzi* activity of hydro-ethanolic extract and isolated active principles from *Aristeguietia glutinosa* and mechanism of action studies. Molecules 19:8488–8502

Vietmeyer N (ed) (1992) Neem: a tree for solving global problems. National Academy Press, Washington, DC

Wabwoba BW, Anjili CO, Ngeiywa MM et al (2010) Experimental chemotherapy with *Allium sativum* (*Liliaceae*) methanolic extract in rodents infected with *Leishmania major* and *Leishmania donovani*. J Vector Borne Dis 47:160–167

Weiner L, Shin I, Shimon LJ et al (2009) Thiol-disulfide organization in alliin lyase (alliinase) from garlic (*Allium sativum*). Protein Sci 18:196–205

Weldon PJ, Carroll JF, Kramer M et al (2011) Anointing chemicals and hematophagous arthropods: responses by ticks and mosquitoes to citrus (Rutaceae) peel exudates and monoterpene components. J Chem Ecol 37(4):348–359

Wink M (2012) Medicinal plants: a source of anti-parasitic secondary metabolites. Molecules 17:12771–12791

World Health Organization (1981) Instructions for determining the susceptibility or resistance of mosquito larvae to insecticides. WHO, Geneva

World Health Organization (1992) Expert Committee on Vector Biology and Control. Vector resistance to pesticides: fifteenth report of the Expert Committee on Vector Biology and Control. WHO Technical Report Series 818, pp 1–62

Yang YC, Lee SH, Lee WJ et al (2003) Ovicidal, and adulticidal effects of *Eugenia caryophyllata* bud and leaf oil compounds on *Pediculus capitis*. J Agric Food Chem 51:4884–4888

Zheng GQ, Kenney PM, Lam LKT (1992) Sesquiterpenes from clove (*Eugenia caryophyllata*). J Nat Prod 55:999–1003

Ziba MM (1995) Preliminary laboratory trial of neem on *Anopheles* and *culex* larvae in Zambia. Cent Afr J Med 41:137–138

Zoroloni A (2007) Evaluation of plants used for the control of animal ectoparasitoses in Southern Ethiopia (Oromiya and Somali regions). MSc Thesis, University of Pretoria, South Africa

Part IV

Nutraceuticals in Specific Animal Species

Nutraceuticals in Cattle Health and Diseases

Begüm Yurdakok-Dikmen and Ayhan Filazi

Abstract

As a result of growing consumer demand for "clean, green, and ethical" products related to public awareness of environmental and health risks of veterinary medicinal products—and the increase in antimicrobial resistance, leading to loss of effectiveness—beef and dairy producers are striving to find effective alternatives. Nutraceuticals provide a valuable tool for prevention and control of diseases in ruminants through their antioxidant, anti-inflammatory, and antimicrobial effects. Nutraceuticals with beneficial effects on the rumen microbiota contribute to increases in productivity and profitability, since the rumen plays an important role in the immune system and nutrition. The beneficial effects are not restricted to cattle health but also impact the environment as a result of their positive impacts on methane emissions. These compounds also have the potential to increase the "healthy fats" in the final products, which are favored for human health. Therefore, nutraceuticals (including probiotics, prebiotics, and synbiotics), dietary lipids, proteins and peptides (including antimicrobial peptides), algae (macroalgae and microalgae), and phytonutraceuticals (tannins, saponins, and essential oils) are valuable tools in cattle health and disease. Meanwhile, many factors affect the efficacy of nutraceuticals, including the source, production technique, and concentration of the compound, along with the physical condition, diet, species, and rumen pH of the animal. To achieve the maximum benefits of nutraceuticals, more studies should be performed to assess their efficacy and toxicity in different ruminant species with different physiological conditions.

Keywords

Cattle · Nutraceuticals · Probiotics · Prebiotics · Synbiotics · Dietary lipids proteins and peptides · Algae · Phytonutraceuticals

1 Introduction

Use of nutraceuticals has received increasing attention for the improvement of animal health, welfare, and productivity in herd health management. For both beef and dairy herd management, preventive medicine is the milestone in the control of risks related to diseases. Preventive medicine requires a well-planned and comprehensive approach. Risk assessment within the locality, disease management plans, vaccination, and biosecurity increase direct costs but are critical for production and efficacy. Avoidance of diseases through immediate accurate diagnosis and early treatment is directly linked to the health of the immune system, where nutrition plays a pivotal role (Ingvartsen and Moyes 2013). An emphasis on nutraceuticals has revealed their potential for support of the immune system.

Nutraceuticals—comprising nutrients, dietary supplements, herbal products, and processed foods (including dietary fiber, probiotics and prebiotics, polyunsaturated fatty acids, antioxidant vitamins, and phytoactive compounds)—induce a wide array of biological process in organisms (Das et al. 2012). Because of the development of resistance to conventional drugs and consumer demand (influenced by social and cultural changes) for more natural/organic animal products, breeders seek alternative and complementary therapeutics in herd medicine. These motives have promoted the value of nutraceuticals, where complex multitarget polypharmacological mechanisms such as activation of antioxidant defense and anti-inflammatory pathways—along with beneficial effects on cells through integrity, survival, proliferation, and differentiation—are exhibited (Dormán et al.

B. Yurdakok-Dikmen (✉) · A. Filazi
Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, Ankara University, Ankara, Turkey

R. C. Gupta et al. (eds.), *Nutraceuticals in Veterinary Medicine*, https://doi.org/10.1007/978-3-030-04624-8_44

2016; Mandel et al. 2005). Nutraceuticals influence the maintenance of gut and metabolic health, while also stimulating immune function, which could eventually prevent complex inflammatory diseases such as mastitis, metritis, or bovine respiratory disease.

Conservation of the great diversity of microorganisms in the rumen is at the core of productivity and emissions of methane gas and ammonia in ruminants. Meanwhile, to reach the increasing demands of the human population, intensive production systems compromising cattle health and welfare have increased metabolic and infectious diseases globally. Therefore, the industry continually seeks alternative strategies for breeding to comply with consumer demands, also bearing in mind animal welfare. Prudent use of nutraceuticals has the potential to help ensure clean, green, and ethical production. Nutraceuticals have the potential to improve nutrient retention and utilization in the rumen, where significant productivity improvements, along with reduction of greenhouse gas emissions, are possible (McGrath et al. 2018). Nutraceuticals derived from various sources (animals, microorganisms, plants, minerals, and algae) exert their effects through various mechanisms such as antimicrobial, anti-inflammatory, and antioxidant effects in ruminants. Meanwhile, there is a lack of species-specific, dose-correlated, and product-specific studies (detailing sources, production techniques, and storage) and physiologically normalized studies (detailing differences in disease and other physiological conditions), making it difficult to adjust use of nutraceuticals appropriately for each breeding facility. This chapter briefly reviews major nutraceutical compounds and their effects on ruminants.

2 Major Classes of Nutraceuticals Used in Cattle

2.1 Probiotics

Probiotics are defined as live, nonpathogenic microorganisms, found in nature and/or in the gastrointestinal (GI) tract of ruminants, which confer beneficial health effects on the host upon ingestion of adequate amounts, mainly by improving the balance of the healthy microflora (beneficial bacteria). Stimulation of healthy microflora improves mucosal immunity, increases digestive capacity, and prevents colonization by harmful bacteria (Uyeno et al. 2015). The mechanisms of action of probiotics are production of antimicrobial substances and inhibitory metabolites (bacteriocins, diacetyl, organic acids), competition with pathogens for gut adhesion sites and nutritional sources, production of nutrients that are beneficial to beneficial bacteria, metabolism/detoxification of undesirable compounds and xenobiotics (by affecting the binding ability of toxins secreted by pathogens), immunomodulation, and production/stimulation of enzymes (Auclair 2001; Retta 2016). GI microbial characteristics differ depending on the region (rumen, large intestine) and host age (preweaned/weaned), including the microbial functions involved in host nutrition in terms of microbial protein synthesis, immunological responses, digestion of polymers, and pathogenesis of harmful bacteria. Therefore, contrary to a common belief, probiotics have greater potential benefits in ruminants than in monogastric animals (Wallace and Newbold 1992).

Probiotics are mainly used in young animals to promote optimal maturation of the rumen microbiota. In adult animals their main use is for reducing the risk of pathogen colonization. In dairy production they are mainly used to increase milk yield and quality, while in beef production their main use is for promotion of weight gain. However, in both dairy and beef production they are also used to promote health by limiting acidosis and increasing feed efficacy (Chaucheyras-Durand and Durand 2010). Their positive effects on dairy production are also related to stabilization of the pH in the rumen, increases in dry matter consumption and energy production/microbial protein, and reduction of the somatic cell count in milk (Maamouri et al. 2014; Suarez and Guevara 2018).

In mammals, Bacteroidetes and Firmicutes are the major bacterial communities in the GI tract. *Fibrobacter*, Archaea, and other protozoan species are also commonly found in the rumen, and *Atopobium*, bifidobacteria, and *Fibrobacter* are also common in the large intestine (Uyeno et al. 2015). Overall, the microorganism composition in the rumen includes bacteria (10^{10}–10^{11}/ml); archaeons such as *Methanobacterium* spp. and *Methanobrevibacter* spp. (10^{8}–10^{9}/ml); protozoans comprising entodiniomorphs, *Holothrix*, and flagellate species (10^{5}–10^{6}/ml); and ruminal fungi such as *Neocallimastix*, *Piromyces*, *Caecomyces*, *Orpinomyces*, and *Anaeromyces* spp. (10^{3}–10^{4}/ml) (Vohra et al. 2016).

Probiotics for ruminants are selected mainly for targeting the rumen, since this complex ecosystem comprising bacteria, anaerobic fungi, and ciliate protozoa is responsible for degradation and fermentation of dietary compounds (Chaucheyras-Durand and Durand 2010). Thus, probiotics used in ruminants are commonly yeast based (*Saccharomyces cerevisiae*) and fungus based (*Aspergillus oryzae*). Meanwhile, other Gram-positive bacteria such as *Lactobacillus* (*L. acidophilus*, *L. brevis*, *L. bulgaricus*, *L. casei*, *L. cellobiosus*, *L. curvatus*, *L. delbrueckii*, *L. fermentum*, *L. lactis*, *L. plantarum*), *Streptococcus* (*S. cremoris*, *S. lactis*, *S. thermophilus*, *S. faecium*), *Leuconostoc mesenteroides*, *Propriobacterium*, *Pediococcus*, *Bifidobacterium* (*B. adolescentis*, *B. infantis*, *B. longum*), *Bacillus* (*B. cereus*, *B. lentus*, *B. natto*), Gram-negative bacteria such as

Bacteriodes (*B. amylophilus*, *B. capillosus*, *B. ruminicola*), yeasts (*Saccharomyces boulardii*, *Torulopsis* candida), and fungi (*Aspergillus niger*) are also used (Chaucheyras-Durand and Durand 2010).

Several modes of action have been described for the effects of yeasts, including increases in the number of total culturable bacteria (through provision of vitamins and dicarboxylic acids, removal of oxygen-favoring anaerobiosis, and a reduction in the ammonium concentration) and the flow of microbial protein, enhancing the amino acid supply to the small intestine and stabilizing the pH through various processes such as competition of lactic acid–producing and/or mannitol-utilizing bacteria (*Megasphaera elsdenii* and *Selenomonas ruminantium*). Moreover, glucans and other macromolecules in the inner part of the yeast cell wall stimulate amplification of host defenses, especially the inflammatory response and the reticuloendothelial system (Auclair 2001; Suarez and Guevara 2018).

Probiotic strains can be administered separately or in combination; mixtures are favored because of their wide range of synergistic effects, which are mainly related to their different mechanisms of action (Chapman et al. 2011; Hatoum et al. 2012). On the other hand, multistrain probiotics may inhibit each other and possible produce antagonistic effects. For instance, *Lactobacillus* within a mixture has been to induce greater efficacy than *Bifidobacterium*; therefore, most mixtures contain *Lactobacillus*. Higher feed conversion efficacy and better growth performance have also been shown in goats fed *L. acidophilus* and *S. cerevisiae* mixtures (Jinturkar et al. 2009) and in lambs fed *Pediococcus acidilactici* and *P. pentosaceus* (Saleem et al. 2017). Commercial cocktails are available that include *Lactobacillus*, *Bacillus*, *A. oryzae* fermentation extract, and cultures of *Saccharomyces* yeast, along with enzymes such as α-amylase, β-glucanases, hemicellulases, and cellulases for ruminants (Chapman et al. 2011). For effective results, specific preliminary studies with the probiotic yeasts or their mixtures are recommended, since the mechanism of action and the success rate depend on the yeast strain, viability, and feed composition (Chiquette 2009).

2.2 Prebiotics

Prebiotics are defined as nondigestible, nonviable ingredients—usually selectively fermented dietary compounds—that confer benefits on host health through the growth and/or activity of gastrointestinal microbiota (Callaway et al. 2012; Roberfroid et al. 2010). Prebiotics that have been confirmed to provide beneficial health effects are mainly nondigestible carbohydrates and oligosaccharides with different molecular structures. Oligosaccharides include fructo-oligosaccharides (FOS; oligofructose and inulin), galacto-oligosaccharides (GOS), transgalacto-oligosaccharides (TOS), and lactulose. These have been found to stimulate the growth of bifidobacteria and lactobacilli, leading to significant changes in the gut microbiota (Gaggìa et al. 2010).

Lactulose—a synthetic disaccharide consisting of galactose and fructose—has been shown to affect the morphology of the intestine through decreases in ileal villus depth and the surface area of lymph follicles from Peyer's patches, with an increase in growth performance in preruminant calves receiving a milk replacer containing *Enterococcus faecium* (Fleige et al. 2007). In another study, a mixture of essential oils (EOs; carvacrol, caryophyllene, p-cymene, cineole, terpinene, and thymol) and prebiotics (arabinogalactans) was tested in newborn calves for promotion of immunity and stimulation of appetite; small amounts (1.25 g per calf daily in a 24:20 milk replacer) were confirmed to induce the aforementioned beneficial effects (Froehlich et al. 2017).

Fiber is defined as the fraction of carbohydrate in the feed that is resistant to digestion by enzymes produced by cattle. Fibers such as cellulose, hemicellulose, lignin, and soluble fibers (fructans, pectins, galactans, and beta-glucans) are mainly found as structural components of cell walls. The digestive actions of rumen microorganisms allow access to the nutritive potential of fibers necessary for proper rumen function and a source of energy (Moreira et al. 2013). As an approximation, it is recommended that 25% of dry matter in feed is neutral dietary fiber, with the other 75% being a forage fiber source. The physical properties of the fiber are important for stimulation of chewing activity (particle size) and establishment of biphasic stratification of ruminal contents. Long forage forms a fiber mat, which floats in the rumen and has a function in particle sorting, by trapping fine particles and slowing their rate of breakdown by reducing ruminal microbial exposure. As the chewing activity increases, salivation also increases, favoring the growth of cellulolytic microbes and production of acetic acid, where a higher acetate to propionate ratio favors synthesis of milk fat (Mirzaei-Aghsaghali and Maheri-Sis 2011; Tackett et al. 1996).

For beef cattle receiving a high-forage diet, where the animals graze or free-choice hay is supplemented, dietary fiber is sufficient. Inadequate fiber can damage the rumen wall, while greater amounts do not guarantee effectiveness, since at a smaller particle size (if it is ground or chopped too finely), rumen health may not be promoted. Promotion of effective fiber supplementation improves overall performance. Effective neutral detergent fibers (cottonseed, Bahia grass, Bermuda grass, corn, soybean, wheat, distiller's grain) in beef cattle feeds can be provided as dried grain, hulls, middlings, cracked corn, normal chop, or fine chop (Parish 2007).

Even though the milk yield is decreased with dry matter intake, with a physically effective neutral detergent fiber

intake of 30–33%, the milk fat yield is increased (3.5%), with greater milk energy efficacy, and the risk of subacute ruminal acidosis is decreased (Zebeli et al. 2008). Prevention of a drop in the milk fat percentage by use of fiber also depends on the physical form of the fiber (the preferred forage mean particle length is ≥0.64 cm) (Woodford et al. 1986) and the humidity (increased fiber results in an increased rectal temperature and increased stress) (Tsai et al. 1967).

2.3 Synbiotics

The synergistic effects of a combination of prebiotics and probiotics, in increasing the survival and deposition of viable beneficial microbiological organisms to retain a healthy natural microbial environment in the rumen, are much greater than those seen with use of probiotics or prebiotics alone (Hamasalim 2016; Radzikowski 2017). A greater improvement in the average daily gain and a greater decrease in fecal *Escherichia coli* were observed in suckling female calves fed a combination of probiotics (1 g of seven bacteria and two yeast strains) and prebiotics (4 g of polysaccharides from *S. cerevisiae* cell walls) (Roodposhti and Dabiri 2012). Moghani suckling lambs supplemented with 3 g of a synbiotic (containing *E. faecium*, a prebiotic derived from chicory, and sea algae as an immune-modulating substance) in their diet had greater body weight, greater body weight gain, increased feed intake, and a better feed conversion ratio. Coliform bacteria were reduced (as were serum cholesterol levels), and lactic acid bacteria were increased, indicating the potential of this supplement as a growth promoter (Moarrab et al. 2016). The effects of synbiotic fermented milk (containing *S. thermophilus*, *L. acidophilus*, and *Bifidobacterium bifidum*)—in synergy with some active ingredients of herbal hydrosols and honey (apple acid)—on sexual activity, semen characteristics, and testosterone levels in Aradhi and Damascus goat bucks were evaluated, and enhanced libido and semen physical characteristics were observed (Al-Sobayil et al. 2008).

2.4 Dietary Lipids

Dietary lipids play important roles in nutrition and biological functions, providing sources of essential fatty acids and energy, and increasing absorption of fat-soluble nutrients. The major lipid constituents in dairy cow nutrition are triglycerides, glycolipids, phospholipids, and free fatty acids (Drackley 2004). The usual ratio in feed is 2–4% lipids, since they directly contribute 50% of the fat in milk. Rumen microorganisms are able to hydrogenate unsaturated fatty acids, including linoleic and linolenic acids, among others; about 90% are hydrogenated into saturated fatty acids (Tiven et al. 2016). As the free fatty acids attach to feed, along with microbial particles, they have a tendency to negatively affect fermentation, especially fermentation of fibrous carbohydrates, and, as a result, the milk production and the fat percentage in the milk decrease (>8%). Their use is supported to increase diet energy density and to increase the forage to concentrate ratio for an optimal milk fat percentage (Palmquist and Conrad 1978; Zhang et al. 2011).

Among dietary lipids, polyunsaturated fatty acids (PUFAs) contain two or more double bonds between carbon atoms and, depending on the position of the methyl group in the carbon chain, are categorized as n-3 (fish, fish oil, nuts) and n-6 (vegetable oils). They can be produced endogenously from linoleic acid (n-6), as the precursor of arachidonic acid and linolenic acid (n-3), and the precursor of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Kadegowda and Yu 2016; Palou and Bonet 2007). As n-3 PUFAs cannot be synthesized by ruminants, these animals have an absolute requirement for some fatty acids in the n-3 and n-6 families, which are considered essential and must be provided in an appropriate ratio. Dairy cows consume a diet predominantly containing PUFAs, where rumen microorganisms split fatty acids from the glycerol backbone to be further fermented into volatile fatty acids. PUFAs provide precursors for prostaglandin synthesis, modulating the key enzymes related to prostaglandin and steroid metabolism, and improving male reproductive performance, as PUFAs are important in sperm membrane integrity. Studies have revealed beneficial effects such as improvements in sperm motility, viability, and testis development, leading to improved fertility parameters in ruminants (Van Tran et al. 2016). The inclusion of PUFAs has also been shown to modulate the immune response through various mechanisms, including reductions in tissue levels of immunosuppressive agents such as arachidonic acid and prostaglandin E_2, which are especially important in reduction of transport stress and feed yard entry stress in ruminants. PUFAs also have the potential to modify the composition of milk fatty acids, while also improving productive performance and metabolic health (Bragaglio et al. 2015; Savoini et al. 2010). The selection of sources for PUFAs is important for relative protection from rumen biodegradation. Reduction of emissions of methane, as a greenhouse gas produced in the rumen, is considered a social and environmental priority. PUFA-enriched diets, such as linseed-rich or fish oil–supplemented diets, have the potential to help mitigate enteric methane production (Savoini et al. 2010). Another important aspect is related to increasing public understanding of the relationships between diet and health regarding consumption of red meat and dietary n-3 intake in humans, as manipulation of beef cattle diets with enriched fatty acids alters the fatty acid composition in beef (Vahmani et al. 2015).

Conjugated linoleic acids (CLAs) are isomers of linoleic acid with isolated double bonds; *cis*-9, *trans*-11-CLA (CLA1) and *trans*-10, *cis*-12-CLA (CLA2) are the most active isomers. *Cis*-9 and *trans*-11 CLA are the intermediates in biohydrogenation of linoleic acid to stearic acid in the rumen by ruminal bacteria; which are the major CLA isomers in milk fat. It may also be synthesized in the mammary gland by the endogenous conversation of transvaccenic acid or linolenic acid by the desaturase enzyme. CLA exhibits anticancer, antioxidant, antiatherosclerotic, and antiobesity effects in human and animals (Kelly 2001). Therefore, an increase in CLA in beef meat and milk has beneficial effects on human health; for that reason, breeders are focusing on increasing the amount of this compound through diet. Feeding CLA to beef cattle for extended periods of time, especially in high-grain diets, has been found to increase the CLA content of meat (Mir et al. 2004).

2.5 Nutraceutical Proteins and Peptides

Biologically active peptides are specific protein fragments derived either through natural enzymatic digestion or through fermentation of food products, promoting body function or condition and enabling prevention and/or treatment of diseases or disorders. Depending on the type and concentration, biological peptides exert different health effects, enhancing the nutrient absorption system, defense system, regulatory system, and nervous system (de Mejia and Dia 2010; Mine and Shahidi 2005). For both beef and dairy cattle, dietary proteins of "bypass" or "undegradable" quality (which escape rumen fermentation), along with other microbial proteins produced through rumen fermentation, are important sources of amino acids. Therefore, besides their nutraceutical effects, these proteins have a major importance in ruminant nutrition. Meanwhile, the source of proteins and the processing procedures are important for the pharmacological effects, along with intactness of certain bioactive protein subunits. Some examples of nutraceutical proteins and peptides (casein phosphopeptides (CPPs), milk whey protein, egg white proteins, soybean proteins, and antimicrobial peptides) used in ruminants are briefly reviewed here.

CPPs are phosphorylated casein-derived peptides. These compounds are able to bind and solubilize macroelements (calcium, magnesium, iron), along with trace elements (zinc, barium, chromium, nickel, cobalt, and selenium). Potential applications of CPPs include prevention of osteoporosis, hypertension, anemia, and dental caries; and humanization of bovine milk (with increased phosphorus levels) (FitzGerald 1998).

Acid, sweet (liquid whey), and casein wheys are highly nutritious co-products of cheese and yogurt production plants. Sweet whey, administered in a concentration of 12–20 L per cow per day, has been found to increase the milk yield, calcium content, and magnesium content, along with the technological properties of milk, in lactating cattle (El-Shewy 2016). As a partial replacement for concentrate (control) feed, 40 L per cow per day of liquid acid whey was found to improve milk fat and protein yields without affecting milk composition (Salem and Fraj 2007). Whey was found to improve the grass and legume silage quality and digestibility, reduced ammonia nitrogen concentrations, and had a favorable effect on growth rates in calves that were fed milk replacers containing up to 89% dried whey (Schingoethe 1976). A whey protein emulsion gel was found to protect unsaturated fatty acids from rumen biohydrogenation, leading to better absorption of these compounds in the small intestine for eventual incorporation into milk fat (Carroll et al. 2006).

Among egg white proteins, ovalbumin, ovotransferrin, ovomucoid, ovomucin, and lysozyme are major groups with great potential for use in the food and pharmaceutical industries. They have antimicrobial, anticancer, antioxidant, metal binding/transporting, and nutrient supplementation effects in organisms (Abeyrathne et al. 2013). The effects of ovalbumin, as a dietary nitrogen source, on peptide concentrations in sheep rumens have been evaluated. In comparison with casein, ovalbumin was found to degrade slowly and therefore did not give rise to significant peptide levels (Broderick and Wallace 1988); this could have been attributable to its potential to stimulate the growth of proteolytic bacteria and other predominant proteolytic species (Tsuda et al. 1991). Another study on the effects of unmarketable egg powder as a protein supplement in preruminant lamb milk replacer reported undesirable effects on the digestive system, indicating that this is unsuitable for lamb feed (Wereme et al. 2016).

Soybean peptides have a great ability to bind to bile acid, reducing cholesterol by restricting reabsorption of the bile acid in the ileum (Ahmad et al. 2014). A slight decrease in low-density lipoprotein (LDL) cholesterol has been reported, but there were no associated effects on high-density lipoprotein (HDL) cholesterol, triglycerides, lipoprotein (a), or blood pressure (Xiao 2008). Black soybean peptides have also been reported to have antidiabetic effects through suppression of hepatic endoplasmic reticulum stress and maintenance of insulin resistance (Ahmad et al. 2014). However, safety concerns regarding their negative impacts on thyroid function (induction of hypothyroidism) and reproductive functions (estrogenic effects), along with an increase in estrogen-related carcinogenesis, restrict their potential use as nutraceuticals in humans (Xiao 2008; Xiao et al. 2004). In both beef and dairy diets, soybean meal is among the most commonly used proteins; as their ruminal bypass content is higher, treated soybeans or soybean meals are favored. Properly treated soybeans are able to increase milk production and improve growth (Radivojević et al. 2011; Ure et al. 2005). Conversely, in one study, no improvement in milk production was observed with feeding of rumen-undegraded protein

from expeller soybean meal or greater amounts of rumen-degraded protein from solvent-extracted soybean meal (Colmenero and Broderick 2006).

Bacterial resistance to many classes of antibiotics is of great concern, given its implications for public health. Use of alternatives such as antimicrobial peptides (AMPs) for adjustment of ruminal fermentation, promotion of feed intake, and secretion of endogenous enzymes with immunological properties are favored, while resistance to these compounds is also expected in near future. AMPs are generally short cationic hydrophobic sequenced peptides (<100 amino acids) within a linear or cyclic structure, which constitute an innate immune defense system and exist in nearly all classes of organisms as a biological weapon (Ageitos et al. 2017). Because of their antiprotozoal and nitrogen-binding affinity, and their effects in promoting optimal ruminal flora, proteins are prevented from undergoing ruminal deamination, to be further used for the animal's own nutrition. This is important especially in dairy production systems, where high levels of energy and protein supply are required (Cheema et al. 2011).

Cattle anionic peptides (BNBD-1–3, 6–11, 13; BNBD-4, 5, 12; tracheal antimicrobial peptide (TAP), lingual antimicrobial peptide (LAP), enteric β-defensin (EBD), indolicidin, dodecapeptide), sheep anionic peptides (sheep BD-1, 2; SMAP 28, 29, 34; OaBac5a, b, 6), goat antimicrobial peptides (BD-1, 2; ChBac5), and several other innate AMPs have been identified in various ruminant tissues, providing antibacterial effects on both Gram-positive and Gram-negative bacteria. Colostrum in ruminants contains numerous cytokines and AMPs, including lactoferrin, defensin, and cathelicidins (Cheema et al. 2011; Wang et al. 2016).

Lactoferrin, as an iron-binding glycoprotein with antimicrobial and antioxidant properties, is approved for decontamination of beef carcasses. Lactoferrin interferes with adhesion/colonization, detaches microorganisms from biological surfaces, inhibits multiplication, neutralizes endotoxins, and boosts the immune system, implying the potential to be used against foodborne pathogens (Acuff 2005). Rectal administration of bovine lactoferrin has been shown to induce antimicrobial and immunomodulatory activities against *E. coli* O157:H7 infections in cattle (Kieckens et al. 2018). Partial antigenic identity has been observed between bovine, goat, and sheep lactoferrin, and, in terms of immunochemical properties, the circular dichroism (CD) spectra were similar (Shimazaki et al. 1991). Lactoferrin also exhibits antiviral, antifungal, and antiparasitic activities (Jenssen and Hancock 2009). Kappacin, a heterogeneous C-terminal fragment of bovine milk kappa-casein casinomacropeptide (CMP), has antibacterial affects, along with biological effects such as suppression of gastric secretion, depression of platelet aggregation, antiviral effects (influenza), inhibition of cholera toxin binding, and immunomodulatory effects (Malkoski et al. 2001). Not just the milk but also the rumen microbiome has been investigated as an underexplored resource for novel antimicrobials. Lynronne-1, Lynronne-2, and Lynronne-3, isolated from bovine rumen, were found to be effective against many clinically important multiresistant pathogens, including methicillin-resistant *Staphylococcus aureus* (MRSA) (Oyama et al. 2017). A large number of AMPs are present in ruminants, which are yet to be exploited for their useful effects. Meanwhile, not only AMPs originating from an animal's own tissues but also other AMPs derived from various different organisms could be potentially used against infectious microbial diseases in ruminants (Cheema et al. 2011). AMPs derived from insects (cecropins and defensins), from amphibians (magainin, limnochariin, hylaranin), and from mammals (defensins and cathelicidins) have potential antibacterial effects, while subtilosin and cathelicidin have potential antiviral effects (Wang et al. 2016).

2.6 Algae

Microalgae (diatoms—Bacillariophyceae, green algae—Chlorophyceae, golden algae—Chrysophyceae, and blue-green algae—Cyanophyceae) and macroalgae (Chlorophyta, Phaeophyta, Chrysophyta, and Rhodophyta) have been studied in both human and animal health and nutrition since ancient times. Depending on the chemical structure and the environmental conditions, they have a wide range of applications, including production of biodiesel from their biolipid oil, fertilizers, feed additives, therapeutic agents, and cosmetics. Through sustainable production (with optimization of water, CO_2, nutrients, heat, light, harvesting systems, and drying systems), various types of algae have become available for animal feed. Numerous bioactive molecules (PUFAs, omega-3 fatty acids such as EPA and DHA, chlorophyll, carotenoids, sterols, etc.), along with high vitamin and mineral content, account for the health-improving properties of algae. Supplementation of dairy cattle feed with algae or algal oil rich in n-3 fatty acid has been reported to positively affect the fatty acid content of milk (Stamey et al. 2012). Extracts of chlorellin isolated from the green microalgae *Chlorella vulgaris* have been shown to induce antibacterial activity against Gram-positive and Gram-negative bacteria (Alwathnani and Perveen 2017; Pratt et al. 1944). Similarly, *Chroococcus dispersus*, with a high chlorellin content, has been reported to have a wide spectrum of antimicrobial activities, including antifungal and antibacterial activities (Ghasemi et al. 2007). Dried spirulina (with a 60% content of rich essential amino acids, though with reduced amounts of methionine, cysteine, and lysine) is highly nutritious and has potential as a replacement in feedlot diets (Nicoletti 2016). Calcium spirulan (Ca-SP)—which is isolated from a marine blue-green alga, *Arthrospira platensis* (previously called *Spirulina platensis*)—has been

found to be active against many types of virus, including measles, influenza, herpes simplex virus (HSV) 1, Coxsackie virus, mumps, and human immunodeficiency virus (HIV) (Hayashi et al. 1996). The carbohydrate fractions of *A. platensis* have been found to be digested efficiently (up to 20%) in comparison with *Chlorella* or *Scenedesmus obliquus*, with an increase in body condition in dairy cows, increases in bodyweight and back fat thickness in weaned lambs, and an increase in bodyweight in lambs being observed. Use of *Schizochytrium* spp., on the other hand, did not have a major effect on carcass weight (Madeira et al. 2017).

Seaweeds—such as subspecies of macroscopic, multicellular, marine algae found in Chlorophyceae (green seaweeds), Rhodophyceae (red seaweeds), and Phaeophyceae (brown seaweeds)—are good sources of some water-soluble vitamins (B_1, B_2, B_{12}, C) and fat-soluble vitamins (β-carotene with vitamin A activity, vitamin E) with antioxidant capacity and have health benefits such as decreasing blood pressure, preventing cardiovascular disease, and reducing the risk of cancer (Škrovánková 2011). Polysaccharides in seaweeds—such as galactans and xylans (in green algae); alginate, laminarin, and sulfated fucose-containing polymers (in brown algae); agars; carrageenans; xylans; sulfated galactans; and porphyrans and minor polysaccharides such as ascophyllan and sugar alcohols—induce prebiotic actions, leading to an increase in productivity and health enhancement in animals (Evans and Critchley 2014).

Seaweeds (*Ulva* spp., *Laminaria ochroleuca*, *Saccharina latissima*, *Gigartina* spp., and *Gracilaria vermiculophylla*) have also been shown to have beneficial effects on gas and methane production and ruminal fermentation parameters in cattle; *Gigartina* presented the lowest value for gas production (Maia et al. 2016). Among seaweeds, brown seaweed *Ascophyllum nodosum* (rockweed) is best known for use in animal feed applications. Commercially available products of brown seaweed have been found to induce beneficial effects on resistance to stressors such as mixing, livestock transportation, exposure to foodborne toxins, and excessive heat or temperature, boosting the immune system, increasing productivity, and reducing the numbers of pathogenic microorganisms (*E. coli* O157:H7 strain, *Salmonella* spp., *Campylobacter* spp., and *Clostridium*) (Allen et al. 2001; Evans and Critchley 2014). The effects of supplementation with brown seaweed by-products (BSB) in Holstein cows were evaluated; BSB did not compromise ruminal fermentation and animal performance at lower levels. Estrogen, triiodothyronine, and thyroxine levels were found to be higher after 3 months of pregnancy, and BSB supplementation did not affect the daily milk yield and composition (Hong et al. 2015).

Research on the effects of phlorotannins isolated from *A. nodosum* on in vitro ruminal digestion of mixed forage or barley grain showed that they reduced methane and total gas production and reduced ruminal fermentation and protein degradation in a dose-dependent manner (Wang et al. 2008). In heat-stressed wether lambs, brown seaweed–treated conserved forage was found to enhance the antioxidant status and immune function (Saker et al. 2004). Mineral levels in brown seaweeds are generally lower than those in green and red seaweeds, while protein levels are higher (Makkar et al. 2016). Mixtures of brown, red, and green seaweeds are also available as feed supplements. As seaweeds are low in cellulose and rich in specific polysaccharides (alginate, laminarin, and fucoidan) and in mannitol, they have an ability to change the rumen microflora substantially, for a high degree of adaptation of the rumen flora (Makkar et al. 2016). However, seaweeds accumulate heavy metals (arsenic), iodine, and other minerals (Bouga and Combet 2015), which requires careful consideration, since this has the potential to be detrimental to both animal and human health.

2.7 Phytonutraceuticals

Secondary metabolites of plant extracts, with various chemical structures and biological activities, are unique resources for pharmaceuticals, food additives, and fine chemicals. They have long been studied in ruminants as alternatives to conventional drug treatments and for their potential to increase productivity. The demand for, and interest in, secondary compounds in plants have increased, with the aims of improving meat and milk quality, and increasing immunity to pathogenic diseases. Major secondary compounds (metabolites in plants) used in farming systems include phenolic compounds (simple phenolics, condensed tannins), saponins, and EOs rich in terpenes (Vasta and Luciano 2011).

Tannins have been considered antinutritive/toxic compounds because of their negative effects on the digestion and absorption of proteins, polysaccharides, and minerals, resulting in reduced animal performance, along with ulcerative, irritative, hepatotoxic, and nephrotoxic effects. The tannins that were used were majorly hydrolyzable tannins, which were further depolymerized in the rumen to gallic acid, with pyrogallol and resorcinol, as metabolized products of gallic acid, being responsible for cellular damage. On the other hand, condensed tannins are not degraded and hence do not cause these toxic effects (Jerónimo et al. 2016). Tannins have several effects on ruminal health, depending on the animal species, its ruminal pH, and the type and concentration of the tannin used. Tannins form indigestible complexes and decrease the rumen turnover rate, which prevents their degradation in the rumen and makes them

eventually available in the intestine. This increases the efficacy of protein digestion and improves animal health (Mueller-Harvey 2006; Naumann et al. 2017). Other major effects of both hydrolyzable and condensed tannins in ruminants are attributed to their anthelmintic effect on larval motility and their inhibition of egg hatching (Naumann et al. 2017; Williams et al. 2014). Monomers of condensed tannins (prodelphinidin, galloyl, procyanidin) were found to affect larval exsheathment of parasitic nematodes in small ruminants (Brunet and Hoste 2006). Tannins also have an effect on lipid metabolism. Depending on the biohydrogenation steps, the microbial species, and the animal's physiology, increases in beneficial fatty acids (such as linolenic acid, vaccenic acid, and rumenic acid) in meat and milk can be achieved (Morales and Ungerfeld 2015). Moreover, condensed tannins form complexes with proteins and polysaccharides in the rations to reduce the digestibility of dry and organic matter for production of metabolic H_2 to be further utilized by methanogens to reduce CO_2 to CH_4 (Piñeiro-Vázquez et al. 2015).

Saponins are amphipathic glycosides with surface active properties, which are widely distributed in nature in marine and plant sources. Saponins produce a variety of biological effects, including hypocholesterolemic, hypolipidemic, immune potentiating, anti-inflammatory, antioxidant, neurohepatoprotective, anticarcinogenic, and antifungal effects (including antibacterial, antifungal, antiprotozoal, antiviral, and antimollusk effects), inhibition of dental caries, and inhibition of platelet aggregation. Because of their detergent properties and pronounced inhibition property of forestomach motility, saponins form a stable foam in the rumen, and some types lead to photosensitization, followed by liver and kidney degeneration, along with other gut problems (Addisu and Assefa 2016; Westendarp 2005). Like tannins, saponins are able to suppress intestinal and ruminal ammonia production through inhibition of proteolytic microorganisms. Moreover, the decrease in methane production is related to defaunation, along with inhibition of methanogenesis or a decrease in the expression of methane-producing genes (Patra and Saxena 2009). Tea saponin has been found to increase the methane yield in lactating dairy cows, presenting a contradiction to the methane reduction effects of saponins (Guyader et al. 2017). A meta-analysis on the methane-mitigating properties of saponin-rich sources showed that the effects were source dependent and concentration dependent (Jayanegara et al. 2014). Among commercially available saponins, *Yucca schidigera* extract has attracted great interest for its manure deodorization and hazardous gas mitigation/methane-decreasing properties (Sun et al. 2017). Yucca extracts were found to be more effective in mitigating methane than tea and *Quillaja saponaria* (Jayanegara et al. 2014). Saponins have also been found to increase the effectiveness of oral vaccines by altering the permeability of the oral mucosa, along with having immune enhancing properties. There are a number of purified or semipurified chemical adjuvants available that are derived from *Q. saponaria*, such as Quil A, Spikoside, and the defined fractions QS-21 and Iscoprep 703. Quil A is widely used as a adjuvant veterinary medicine in equine influenza virus, canine parvovirus, and feline leukemia virus (FeLV) vaccines (Kirk et al. 2004; Spickler and Roth 2003).

EOs are volatile aromatic compounds produced by plants. They are multicomponent chemical combinations with terpenes and terpenoids (20–70%) as their major constituents (Kumari et al. 2014). EOs have antiseptic, antimicrobial, antioxidant, and free radical–screening activities (Ballester-Costa et al. 2017; Patra 2011). The most studied EOs are thyme, oregano, clove, cinnamon, rosemary, dill, eucalyptus, garlic, and anise. Inhibition of methane formation (mainly through reduction of feed degradation) and rumen fermentation–modulating effects have been observed consistently with thyme (thymol), oregano (carvacrol), cinnamon (cinnamaldehyde), and garlic (allicin) (Cobellis et al. 2016). Total volatile fatty acid concentrations were found to be only slightly affected or decreased by EO; the effects were dependent on the rumen pH. Only limited information on the effects of EOs on the performance of ruminants is available; minor changes have been observed to occur in a diet-dependent manner (Patra 2011). A feedlot finishing diet containing thyme and cinnamon EO had beneficial effects on performance and rumen fermentation in Holstein calves consuming a high-concentrate diet, and these EOs could be further adopted as ruminal fermentation modifiers in beef production systems (Vakili et al. 2013). Even though EOs have been shown to be promising feed additives for mitigation of methane and ammonia emission, and have antioxidant, antimicrobial, and immune enhancing effects, their efficacy varies as a result of environmental factors such as light, temperature (considerable loss of activity was recorded with a pelleting temperature of 58 °C in feed), moisture stress, dosage (as adaptation occurs, the need for an increase in dose is inevitable), and the diet of the animal (Cobellis et al. 2016).

3 Concluding Remarks and Future Directions

The ban on antibiotics as growth promoters, along with antimicrobial resistance and demand from consumers for drug-free animals and with food products of high quality, create challenges for breeders to seek alternative methods for control and prevention of pathogens. Nutraceuticals provide

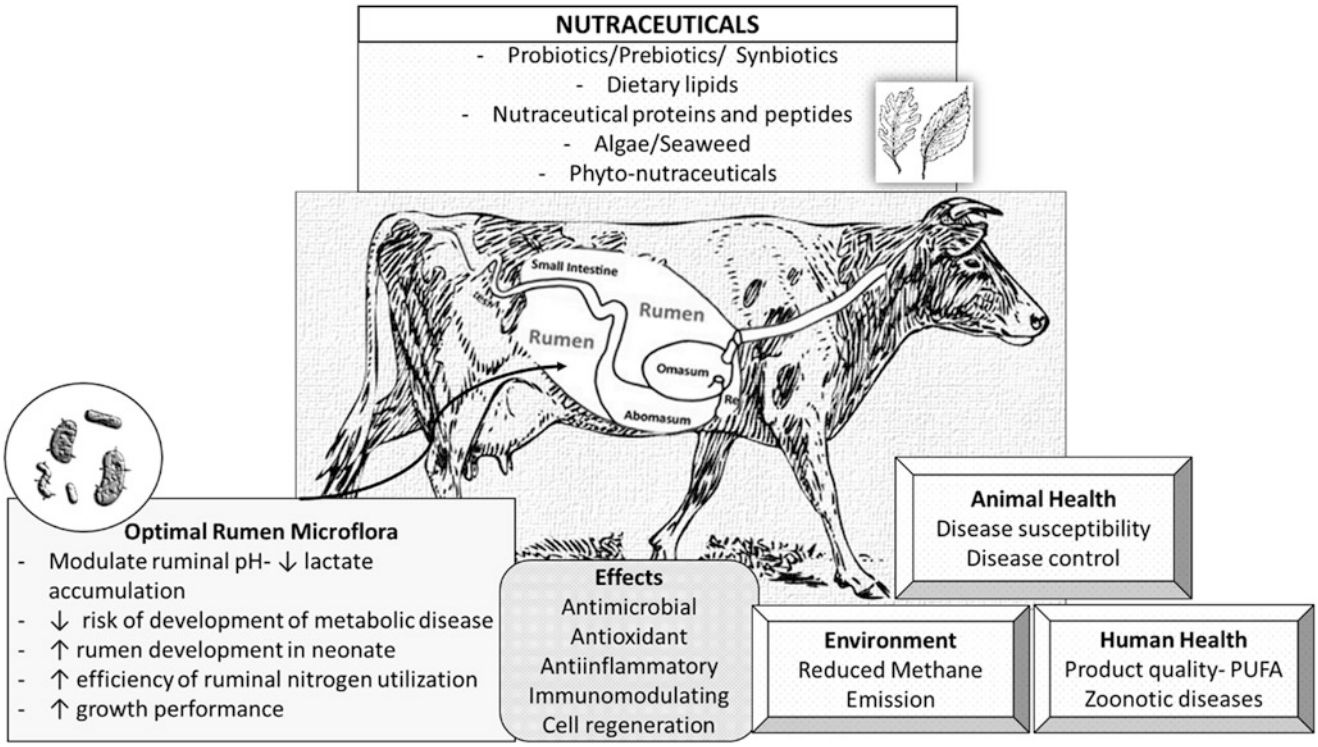


Fig. 1 Effects of nutraceuticals on cattle health

valuable tool in terms of feed additives, mainly focusing on host-protecting functions (antioxidant, anti-inflammatory, antimicrobial, and cell survival effects) to increase productivity. In ruminants, the rumen microbiota plays a fundamental role in the function of the immune system, as well as nutrition; therefore, nutraceuticals are expected to have mainly rumen-modulating effects. Ruminant livestock production also influences the environment through emission of methane and excretion of nitrogen in large quantities, contributing to global methane emissions. Therefore, nutraceuticals not only are important for ruminant health but can also influence public health through inhibition of methane emissions, as well as decreasing pathogenic bacteria in animal-origin food products. Major nutraceutical compounds with potential in ruminants include dietary fiber, probiotics, prebiotics, dietary lipids, proteins and peptides (including antimicrobial peptides), algae (macroalgae and microalgae), and phytonutraceuticals (tannins, saponins, and essential oils) (Fig. 1).

Since many factors affect the efficacy of nutraceuticals—including the source, the technique used for production, and the concentration of the compound, along with the physical condition, diet, species, and rumen pH of the animal—more studies on these natural and potent compounds should be performed to assess their efficacy and toxicity in order to obtain cost-effective benefits.

References

Abeyrathne EDNS, Lee HY, Ahn DU (2013) Egg white proteins and their potential use in food processing or as nutraceutical and pharmaceutical agents—a review. Poult Sci 92(12):3292–3299

Acuff GR (2005) Chemical decontamination strategies for meat. In: Sofos NJ (ed) Improving the safety of fresh meat. Woodhead Publishing, Cambridge, pp 350–363

Addisu S, Assefa A (2016) Role of plant containing saponin on livestock production; a review. Adv Biol Res 10(5):309–314

Ageitos JM, Sánchez-Pérez A, Calo-Mata P et al (2017) Antimicrobial peptides (AMPs): ancient compounds that represent novel weapons in the fight against bacteria. Biochem Pharmacol 133:117–138

Ahmad A, Hayat I, Arif S et al (2014) Mechanisms involved in the therapeutic effects of soybean (*Glycine max*). Int J Food Prop 17 (6):1332–1354

Allen VG, Pond KR, Saker KE et al (2001) Tasco-forage: III influence of a seaweed extract on performance, monocyte immune cell response, and carcass characteristics in feedlot-finished steers. J Anim Sci 79(4):1032–1040

Al-Sobayil KA, Zeitoun MM, Khalil MH et al (2008) Effect of oral administration of a functional synbiotic syrup on libido, semen characteristics, serum testosterone and liver and kidney function of goat's bucks. Asian J Biol Sci 1(1):11–18

Alwathnani H, Perveen K (2017) Antibacterial activity and morphological changes in human pathogenic bacteria caused by *Chlorella vulgaris* extracts. Biomed Res 28(4):1610–1614

Auclair E (2001) Yeast as an example of the mode of action of probiotics in monogastric and ruminant species. In: Brufau J (ed) Feed manufacturing in the Mediterranean region improving safety: from feed to food. CIHEAM, Zaragoza, pp 45–53

Ballester-Costa C, Sendra E, Fernández-López J et al (2017) Assessment of antioxidant and antibacterial properties on meat homogenates of essential oils obtained from four thymus species achieved from organic growth. Foods 6(8):59

Bouga M, Combet E (2015) Emergence of seaweed and seaweed-containing foods in the UK: focus on labeling, iodine content, toxicity and nutrition. Foods 4(2):240–253. https://doi.org/10.3390/foods4020240

Bragaglio A, Braghieri A, Napolitano F et al (2015) Omega-3 supplementation, milk quality and cow immune-competence. Ital J Agron 10(1):9–14

Broderick GA, Wallace RJ (1988) Effects of dietary nitrogen source on concentrations of ammonia, free amino acids and fluorescamine-reactive peptides in the sheep rumen. J Anim Sci 66:2233–2238

Brunet S, Hoste H (2006) Monomers of condensed tannins affect the larval exsheathment of parasitic nematodes of ruminants. J Agric Food Chem 54(20):7481–7487

Callaway TR, Edrington TS, Harvey RB et al (2012) Prebiotics in food animals, a potential to reduce foodborne pathogens and disease. Roman Biotechnol Let 17(6):7808–7816

Carroll SM, DePeters EJ, Rosenberg M (2006) Efficacy of a novel whey protein gel complex to increase the unsaturated fatty acid composition of bovine milk fat. J Dairy Sci 89(2):640–650

Chapman CMC, Gibson GR, Rowland I (2011) Health benefits of probiotics: are mixtures more effective than single strains? Eur J Nutr 50(1):1–17

Chaucheyras-Durand F, Durand H (2010) Probiotics in animal nutrition and health. Benef Microbes 1(1):3–9

Cheema U, Younas M, Sultan J et al (2011) Antimicrobial peptides: an alternative of antibiotics in ruminants. Adv Agric Biotechnol 2:15–21

Chiquette J (2009) Evaluation of the protective effect of probiotics fed to dairy cows during a subacute ruminal acidosis challenge. Anim Feed Sci Technol 153(3–4):278–291

Cobellis G, Trabalza-Marinucci M, Yu Z (2016) Critical evaluation of essential oils as rumen modifiers in ruminant nutrition: a review. Sci Total Environ 545–546:556–568

Colmenero JJO, Broderick GA (2006) Effect of amount and ruminal degradability of soybean meal protein on performance of lactating dairy cows. J Dairy Sci 89(5):1635–1643

Das L, Bhaumik E, Raychaudhuri U et al (2012) Role of nutraceuticals in human health. J Food Sci Technol 49(2):173–183

de Mejia EG, Dia VP (2010) The role of nutraceutical proteins and peptides in apoptosis, angiogenesis, and metastasis of cancer cells. Cancer Metastasis Rev 29(3):511–528

Dormán G, Flachner B, Hajdú I et al (2016) Target identification and polypharmacology of nutraceuticals. In: Gupta RC (e) (ed) Nutraceuticals: efficacy, safety and toxicity. Academic, Amsterdam, pp 263–286

Drackley JK (2004) Overview of fat digestion and metabolism in dairy cows. Illinois Livestock Trail, University of Illinois. http://livestocktrail.illinois.edu/uploads/dairynet/papers/Overview%20of%20Fats%2004.pdf

El-Shewy AA (2016) Whey as a feed ingredient for lactating cattle. Sci Int 4(3):80–85

Evans FD, Critchley AT (2014) Seaweeds for animal production use. J Appl Phycol 26(2):891–899

FitzGerald RJ (1998) Potential uses of caseinophosphopeptides. Int Dairy J 8(5–6):451–457

Fleige S, Preißinger W, Meyer HHD et al (2007) Effect of lactulose on growth performance and intestinal morphology of pre-ruminant calves using a milk replacer containing *Enterococcus faecium*. Animal 1(03):367–373

Froehlich KA, Abdelsalam KW, Chase C et al (2017) Evaluation of essential oils and prebiotics for newborn dairy calves. J Anim Sci 95 (8):3772–3782

Gaggìa F, Mattarelli P, Biavati B (2010) Probiotics and prebiotics in animal feeding for safe food production. Int J Food Microbiol 141: S15–S28

Ghasemi Y, Moradian A, Mohagheghzadeh A et al (2007) Antifungal and antibacterial activity of the microalgae collected from paddy fields of Iran: characterization of antimicrobial activity of *Chroococcus dispersus*. J Biol Sci 7(6):904–910

Guyader J, Eugène M, Doreau M et al (2017) Tea saponin reduced methanogenesis in vitro but increased methane yield in lactating dairy cows. J Dairy Sci 100(3):1845–1855

Hamasalim HJ (2016) Synbiotic as feed additives relating to animal health and performance. Adv Microbiol 6:288–302

Hatoum R, Labrie S, Fliss I (2012) Antimicrobial and probiotic properties of yeasts: from fundamental to novel applications. Front Microbiol 19(3):421

Hayashi T, Hayashi K, Maeda M et al (1996) Calcium spirulan, an inhibitor of enveloped virus replication, from a blue-green alga *Spirulina platensis*. J Nat Prod 59(1):83–87

Hong ZS, Kim EJ, Jin YC et al (2015) Effects of supplementing brown seaweed by-products in the diet of Holstein cows during transition on ruminal fermentation, growth performance and endocrine responses. Asian-Australas J Anim Sci 28(9):1296–1302

Ingvartsen KL, Moyes K (2013) Nutrition, immune function and health of dairy cattle. Animal 7(S1):112–122

Jayanegara A, Wina E, Takahashi J (2014) Meta-analysis on methane mitigating properties of saponin-rich sources in the rumen: influence of addition levels and plant sources. Asian-Australas J Anim Sci 27 (10):1426–1435

Jenssen H, Hancock R (2009) Antimicrobial properties of lactoferrin. Biochimie 91(1):19–29

Jerónimo E, Pinheiro C, Lamy E et al (2016) Tannins in ruminant nutrition—impact on animal performance and quality of edible products. In: Combs CA (ed) Tannins: biochemistry, food sources and nutritional properties. Nova Science, New York, pp 121–168

Jinturkar AS, Gujar BV, Chauhan DS, et al (2009) Effect of feeding probiotics on the growth performance and feed conversion efficiency in goat. Indian Journal of Animal Research 43(1): 49–52

Kadegowda AKG, Yu L (2016) Effects of dietary lipid intake on diabetes functional dietary lipids. In: Sanders TAB (ed.) Functional dietary lipids food formulation, consumer issues and innovation for health (pp 151–176) Amsterdam Woodhead

Kelly GS (2001) Conjugated linoleic acid: a review. Alt Med Rev 6 (4):367–382

Kieckens E, Rybarczyk J, Cox E et al (2018) Antibacterial and immunomodulatory activities of bovine lactoferrin against *Escherichia coli* O157:H7 infections in cattle. Biometals 31 (3):321–330

Kirk DD, Rempel R, Pinkhasov J et al (2004) Application of *Quillaja saponaria* extracts as oral adjuvants for plant-made vaccines. Expert Opin Biol Ther 4(6):947–958

Kumari S, Pundhir S, Priya P, et al (2014) EssOilDB: a database of essential oils reflecting terpene composition and variability in the plant kingdom. Database 2014:bau120

Maamouri O, Selmi H, M'hamdi N (2014) Effects of yeast (*Saccharomyces cerevisiae*) feed supplement on milk production and its composition in Tunisian Holstein Friesian cows. Sci Agric Bohem 45 (3):170–174

Madeira MS, Cardoso C, Lopes PA et al (2017) Microalgae as feed ingredients for livestock production and meat quality: a review. Livest Sci 205:111–121

Maia MRG, Fonseca AJM, Oliveira HM et al (2016) The potential role of seaweeds in the natural manipulation of rumen fermentation and methane production. Sci Rep 6(1):323–321

Makkar HPS, Tran G, Heuzé V et al (2016) Seaweeds for livestock diets: a review. Anim Feed Sci Technol 212:1–17

Malkoski M, Dashper SG, O'Brien-Simpson NM et al (2001) Kappacin, a novel antibacterial peptide from bovine milk. Antimicrob Agents Chemother 45(8):2309–2315

Mandel S, Packer L, Youdim MBH et al (2005) Proceedings from the "Third International Conference on Mechanism of Action of Nutraceuticals". J Nutr Biochem 16(9):513–520

McGrath J, Duval SM, Tamassia LFM et al (2018) Nutritional strategies in ruminants: a lifetime approach. Res Vet Sci 116:28–39

Mine Y, Shahidi F (2005) Nutraceutical proteins and peptides in health and disease: an overview. In: Mine Y, Shahidi F (e) (eds) Nutraceutical proteins and peptides in health and disease. CRC, Boca Raton, pp 3–9

Mir PS, McAllister TA, Scott S et al (2004) Conjugated linoleic acid–enriched beef production. Am J Clin Nutr 79(6S):1207S–1211S

Mirzaei-Aghsaghali A, Maheri-Sis N (2011) Importance of "physically effective fibre" in ruminant nutrition: a review. Ann Biol Res 2 (3):262–270

Moarrab A, Ghoorchi T, Ramezanpour S et al (2016) Effect of synbiotic on performance, intestinal morphology, fecal microbial population and blood metabolites of suckling lambs. Iran J Appl Anim Sci 6 (3):621–628

Morales R, Ungerfeld EM (2015) Use of tannins to improve fatty acids profile of meat and milk quality in ruminants: a review. Chil J Agric Res 75(2):239–248

Moreira LM, Leonel FP, Vieira RAM et al (2013) A new approach about the digestion of fibers by ruminants. R Brasil Saúde Prod Anim 14 (2):382–395

Mueller-Harvey I (2006) Unravelling the conundrum of tannins in animal nutrition and health. J Sci Food Agric 86(13):2010–2037

Naumann HD, Tedeschi LO, Zeller WE et al (2017) The role of condensed tannins in ruminant animal production: advances, limitations and future directions. R Bras Zootec 46(12):929–949

Nicoletti M (2016) Microalgae nutraceuticals. Foods 5(3):54

Oyama LB, Girdwood SE, Cookson AR et al (2017) The rumen microbiome: an underexplored resource for novel antimicrobial discovery. NPJ Biofilms Microbiomes 3(1):33

Palmquist DL, Conrad HR (1978) High fat rations for dairy cows effects on feed intake, milk and fat production, and plasma metabolites. J Dairy Sci 61(7):890–901

Palou A, Bonet ML (2007) Controlling lipogenesis and thermogenesis and the use of ergogenic aids for weight control. In: Henry CJK (ed) Novel food ingredients for weight control. Woodhead Publishing, Cambridge, pp 58–103

Parish J (2007) Effective fiber in beef cattle diets. Cattle Business in Mississippi. https://extension.msstate.edu/sites/default/files/topic-files/cattle-business-mississippi-articles/cattle-business-mississippi-articles-landing-page/mca_mar2007.pdf

Patra AK (2011) Effects of essential oils on rumen fermentation, microbial ecology and ruminant production. Asian J Anim Vet Adv 6 (5):416–428

Patra AK, Saxena J (2009) The effect and mode of action of saponins on the microbial populations and fermentation in the rumen and ruminant production. Nutr Res Rev 22(02):204–219

Piñeiro-Vázquez AT, Canul-Solís JR, Alayón-Gamboa JA et al (2015) Potential of condensed tannins for the reduction of emissions of enteric methane and their effect on ruminant productivity. Arch Med Vet 47:263–272

Pratt R, Daniels TC, Eiler JJ et al (1944) Chlorellin, an antibacterial substance from *Chlorella*. Science 99(1944):351–352

Radivojević M, Grubić G, Šamanc H et al (2011) Heat treated soybeans in the nutrition of high producing dairy cows. Afr J Biotechnol 10 (19):3929–3937

Radzikowski D (2017) Effect of probiotics, prebiotics and synbiotics on the productivity and health of dairy cows and calves. World Sci News 78:193–198

Retta KS (2016) Role of probiotics in rumen fermentation and animal performance: a review. Int J Livest Prod 7(5):24–32

Roberfroid M, Gibson GR, Hoyles L et al (2010) Prebiotic effects: metabolic and health benefits. Br J Nutr 104(S2):S1–S63

Roodposhti P, Dabiri N (2012) Effects of probiotic and prebiotic on average daily gain, fecal shedding of *Escherichia coli*, and immune system status in newborn female calves. Asian-Australas J Anim Sci 25(9):1255–1261

Saker KE, Fike JH, Veit H et al (2004) Brown seaweed (Tasco™) treated conserved forage enhances antioxidant status and immune function in heat-stressed wether lambs. J Anim Physiol Anim Nutr 88(3–4):122–130

Saleem AM, Zanouny AI, Singer AM (2017) Growth performance, nutrients digestibility, and blood metabolites of lambs fed diets supplemented with probiotics during pre- and post-weaning period. Asian-Australas J Anim Sci 30(4):523–530

Salem MB, Fraj M (2007) The effects of feeding liquid acid whey in the diet of lactating dairy cows on milk production and composition. J Cell Anim Biol 1(1):7–10

Savoini G, Agazzi A, Invernizzi G et al (2010) Polyunsaturated fatty acids and choline in dairy goats nutrition: production and health benefits. Small Rumin Res 88(2–3):135–144

Schingoethe DJ (1976) Whey utilization in animal feeding: a summary and evaluation. J Dairy Sci 59(3):556–570

Shimazaki K, Kawano N, Yoo YC (1991) Comparison of bovine, sheep and goat milk lactoferrins in their electrophoretic behavior, conformation, immunochemical properties and lectin reactivity. Comp Biochem Physiol B 98(2–3):417–422

Škrovánková S (2011) Seaweed vitamins as nutraceuticals. Adv Food Nutr Res 64:357–369

Spickler AR, Roth JA (2003) Adjuvants in veterinary vaccines: modes of action and adverse effects. J Vet Int Med 17:273–281

Stamey JA, Shepherd DM, de Veth MJ et al (2012) Use of algae or algal oil rich in n-3 fatty acids as a feed supplement for dairy cattle. J Dairy Sci 95(9):5269–5275

Suarez C, Guevara CA (2018) Probiotic use of yeast *Saccharomyces cerevisiae* in animal feed. Res J Zool 1:1

Sun DS, Jin X, Shi B et al (2017) Effects of *Yucca schidigera* on gas mitigation in livestock production: a review. Braz Arch Biol Technol 60(0):e17160359

Tackett VL, Bertrand JA, Jenkins TC et al (1996) Interaction of dietary fat and acid detergent fiber diets of lactating dairy cows. J Dairy Sci 79(2):270–275

Tiven NC, Siwa IP, Joris L (2016) Effects of *Citrus hystryx* as fat protector on unsaturated fatty acids, cholesterol and chemical composition of lamb meat. J Indones Trop Anim Agric (1):45–49

Tsai YC, Castillo LS, Hardison WA et al (1967) Effect of dietary fiber level on lactating dairy cows in the humid tropics. J Dairy Sci 50 (7):1126–1129

Tsuda T, Sasaki Y, Kawashima R (1991) Physiological aspects of digestion and metabolism in ruminants. In: Tsuda T, Sasaki Y, Kawashima R (eds) Proceedings of the Seventh International Symposium on Ruminant Physiology, San Diego Academic

Ure AL, Dhiman TR, Stern MD et al (2005) Treated extruded soybean meal as a source of fat and protein for dairy cows. Asian-Australas J Anim Sci 18(7):980–989

Uyeno Y, Shigemori S, Shimosato T (2015) Effect of probiotics/prebiotics on cattle health and productivity microbes and environments. Microbes Environ 30(2):126–132

Vahmani P, Mapiye C, Prieto N et al (2015) The scope for manipulating the polyunsaturated fatty acid content of beef: a review. J Anim Sci Biotechnol 6(1):29

Vakili AR, Khorrami B, Mesgaran MD et al (2013) The effects of thyme and cinnamon essential oils on performance, rumen fermentation and blood metabolites in Holstein calves consuming high concentrate diet. Asian-Australas J Anim Sci 26(7):935–944

Van Tran L, Malla BA, Kumar S et al (2016) Polyunsaturated fatty acids in male ruminant reproduction—a review. Asian-Australas J Anim Sci 30(5):622–637

Vasta V, Luciano G (2011) The effects of dietary consumption of plants secondary compounds on small ruminants' products quality. Small Rumin Res 101(1–3):150–159

Vohra A, Syal P, Madan A (2016) Probiotic yeasts in livestock sector. Anim Feed Sci Technol 219:31–47

Wallace RJ, Newbold CJ (1992) Probiotics for ruminants. In: Fuller R (ed) Probiotics. Springer, Dordrecht, pp 317–353

Wang Y, Xu Z, Bach SJ et al (2008) Effects of phlorotannins from *Ascophyllum nodosum* (brown seaweed) on in vitro ruminal digestion of mixed forage or barley grain. Anim Feed Sci Technol 145 (1–4):375–395

Wang S, Zeng X, Yang Q et al (2016) Antimicrobial peptides as potential alternatives to antibiotics in food animal industry. Int J Mol Sci 17(5):603

Wereme D, Grongnet JF, Gelbcke D (2016) Using unmarketable egg powder as protein supplement in pre-ruminant lamb milk replacer. Direct Res J Agric Food Sci 4(9):271–279

Westendarp H (2005) Saponins in nutrition of swine, poultry and ruminants. Dtsch Tierarztl Wochenschr 112(2):65–70

Williams AR, Fryganas C, Ramsay A et al (2014) Direct anthelmintic effects of condensed tannins from diverse plant sources against *Ascaris suum*. PLoS One 9(5):e97053

Woodford JA, Jorgensen NA, Barrington GP (1986) Impact of dietary fiber and physical form on performance of lactating dairy cows. J Dairy Sci 69(4):1035–1047

Xiao CW (2008) Health effects of soy protein and isoflavones in humans. J Nutr 138(6):1244S–1249S

Xiao CW, L'Abbé MR, Gilani GS et al (2004) Dietary soy protein isolate and isoflavones modulate hepatic thyroid hormone receptors in rats. J Nutr 134(4):743–749

Zebeli Q, Dijkstra J, Tafaj M et al (2008) Modeling the adequacy of dietary fiber in dairy cows based on the responses of ruminal pH and milk fat production to composition of the diet. J Dairy Sci 91 (5):2046–2066

Zhang H, Wang Z, Liu G et al (2011) Effect of dietary fat supplementation on milk components and blood parameters of early-lactating cows under heat stress. Slovak J Anim Sci 44(2):52–58

Nutraceuticals in Equine Medicine

A. Sankaranarayanan

Abstract

The use of nutraceuticals in equine medicine is rapidly growing into a global multibillion-dollar industry. With the introduction of Dietary Supplement Health and Education (DSHEA) legislation in 1994, the regulation of nutraceuticals for animals has become rather vague but was partially rescued by the formation of the National Animal Supplements Council (NASC) in 2002. In equine practice, nutraceuticals are commonly used as performance enhancers as well as joint supplements. In both instances, research in humans as well as horses has been of critical importance. Lameness in horses is one of the most important conditions impacting their usefulness, and nutraceuticals are used commonly in its treatment. Products containing glucosamine and chondroitin sulfate have been shown to be effective with some level of scientific support. There are many others, like undenatured type II collagen, manganese, antioxidants, and vitamin C, which have also been employed with varying levels of success. As performance enhancers, L-carnitine, creatine, branched-chain amino acids, and HMB have all been used, with HMB showing promise. In spite of the initial euphoria, it is a challenging time for the nutraceutical industry to generate quality clinical data to support efficacy and safety claims on its products to ensure sustained growth.

Keywords

Veterinary nutraceuticals · Equine health · Equine medicine

1 Introduction

Since the late 1990s, there has been an explosive growth in the nutraceutical industry both in human and animal nutrition, transforming it into a global multibillion-dollar industry. It is therefore no surprise that in equine medicine also, nutraceuticals are growing rapidly in demand both by horse owners and veterinarians as evidenced by the variety and volume of products introduced into the market. Despite this trend, it is ironic that the very definition of nutraceuticals is vague and there is no regulatory definition. The term "nutrient" is defined by the Association of American Feed Control Officials (AAFCO) as "a food constituent in a form and at a level that will help support the life of an animal". The major classes of feed nutrients are proteins, fats, carbohydrates, minerals, and vitamins. AAFCO defines a "drug" as "a substance intended for use in the diagnosis, cure, mitigation, treatment or prevention of disease in man or other animals". The term nutraceutical was coined by DeFelice (1995) as a combination of the words, "nutrient" and "pharmaceutical," and is defined as "food or part of a food that provides medical or health benefits, including prevention and/or treatment of a disease." The North American Veterinary Nutraceutical Council (NAVNC) defines nutraceutical as a substance which is produced in purified or extracted form and administered orally to patients to provide agents required for normal body structure and function and administered with the intent of improving the health and well-being of animals (Crandell and Duren 2001).

DeFelice (1995) was optimistic that the nutraceutical revolution would change the food industry dramatically but it would also depend on the ability of the industry to demonstrate clinical benefits of these products and its ability to patent them. For the sustained growth of the nutraceutical industry, this would be an important factor.

A. Sankaranarayanan (✉)
Vivo Bio Tech Ltd, Hyderabad, Telangana, India
e-mail: sankar@vivobio.com

R. C. Gupta et al. (eds.), *Nutraceuticals in Veterinary Medicine*, https://doi.org/10.1007/978-3-030-04624-8_45

2 Regulatory Scenario

Regulations on nutraceuticals for the use in animals are rather vague and confusing. This is partly due to the very nature of nutraceuticals and their position between nutritional products (animal foods/feeds) and animal drugs/pharmaceuticals. In 1994, the US Congress passed the Dietary Supplement Health Education Act (DSHEA) that was signed into law in October of that year by President Bill Clinton. This led to the creation of dietary supplements, a subset of food, under the Federal Food, Drug, and Cosmetic Act that allowed production and marketing of dietary supplements for human use. While dietary supplements for human consumption were discussed in the Congress, the case of dietary supplements for animals was neither mentioned nor considered. Perhaps at that time, the dietary supplement industry for animals was not well developed, or possibly, the purpose of the DSHEA was specifically to meet the increasing demand for dietary supplements for human use.

The US Food and Drug Administration, Center for Veterinary Medicine (FDA-CVM) is responsible for the regulation of animal food and drugs. It works in close association with agencies like AAFCO and other state authorities in enforcing federal and state laws to ensure that manufacturers of animal feed and drugs comply with regulations. Vitamins, minerals, and feed additives that are generally recognized as safe (GRAS) have been on the market for several years in the category of dietary supplements for animals and are listed in the official publication of the AAFCO.

With the introduction of the DSHEA, ingredients similar to that used in human dietary supplements have been marketed as components of dietary supplements for animals. Many of them contain ingredients that may be unsafe food additives or unapproved new animal drugs. This has led to the publication of a notice by the US FDA in the Federal Register of 1996 clarifying that the DSHEA does not apply to animals. The FDA-CVM's concern was that these animal dietary supplements do not have scientific data to show that they are safe for animals. The result of such reasoning is that products marketed for animals that are similar to human dietary supplements can only legally be placed under one of two categories—animal food or animal drug.

An animal food does not require any pre-market approval by the FDA-CVM, but under the DSHEA it is required to be pure and wholesome and contains no harmful or deleterious ingredients and must be labeled as such. It is only of nutritive value. On the other hand, an animal drug is an article intended for the use in the diagnosis, cure, treatment, or prevention of disease. Under the DSHEA, a new animal drug must have scientific data showing it to be effective and safe as a part of its new animal drug application (NADA). Simply, if a product on the market is not approved for the use in a condition, it shall be considered illegal and subject to regulatory action.

This has led to a complex legal situation as elegantly summarized by the National Animal Supplements Council (NASC), a 501(c)6 not-for-profit organization established as a corporation in Utah with primary offices in Southern California (https://nasc.cc). With the increased demand for animal dietary supplements, this has created an unusual situation for the industry. There are a growing number of ingredients commonly used in human dietary supplements that are not approved for the use in animals. This lack of legal status for dietary supplements for animals has led to imminent proposals by regulatory bodies to remove many of these products from the market. In response to this, the NASC was formed in April 2002.

The NASC surveys revealed that there are over 400 ingredients currently marketed by NASC members that are unapproved for the use in animal feed products. To address this issue, NASC submitted ingredient definition applications in 2002 and 2003, petitioning the FDA-CVM to allow the use of glucosamine and MSM in animal feed as their nutritional requirements had not been established in animals until then. These products are recognized by consumers as being used for purposes other than nutritional, like being used in managing disease conditions such as osteoarthritis. These considerations mandate the industry in complying with the law and market such products as animal drugs. Marketing products as animal drugs requires filing a new animal drug application (NADA) with the FDA-CVM with supporting data on their safety and efficacy. Though this appears to be straightforward, in reality this could be problematic. First, the industry would need to protect its intellectual property rights since considerable investments will have to be made in the product development. However, courts have held that natural substances cannot be protected via intellectual property rights. Secondly, consumers can access human dietary supplements for animal use and may avoid expensive animal products developed and marketed through the NADA process. Obviously, all these make the regulatory position of nutraceuticals for animals rather complex.

3 Nutraceuticals

The US Federal Food, Drug, and Cosmetic Act defines a dietary ingredient as a vitamin, mineral, herb or other botanical, amino acid, or dietary substance for the use by man to supplement the diet by increasing the total dietary intake or a concentrate, metabolite, constituent, extract, or combination of the preceding substances. The term as used covers a broad list of products which are used in human nutrition and have become popular in the equine nutraceutical industry. The

nutraceuticals provide functional benefits by increasing the supply of structural building blocks in the body and thereby mitigating disease signs and/or improving the performance of animals. Most of the scientific data generated on nutraceuticals come from human research, whereas only a limited number of nutraceuticals used in equines have been clinically evaluated. It is also observed that in equine practice, nutraceuticals have been used more commonly as performance enhancers than for treating disease conditions. This chapter shall focus on nutraceuticals in equine medicine that are supported by equine and human clinical research.

4 Nutraceuticals as Joint Supplements

Osteoarthritis is one of the most common causes of lameness in horses. An imbalance in articular cartilage matrix synthesis and degradation is responsible for this joint pathology. The degradation of the articular cartilage ultimately leads to deteriorating compressive and tensile stiffness. Increased levels of interleukin-1β (IL-1β) in the cartilage structures mediate the deterioration of the cartilage via increased nitric oxide (NO) levels and activation of matrix metalloproteinases (MMPs). Conventionally, nonsteroidal anti-inflammatory drugs (NSAIDS), joint injections, and physical therapy are employed in these conditions. However, oral joint supplements are also commonly used by horse owners as simple supplement or replacement for conventional therapies. Hence, it is not surprising that arthritis and joint disease are two of the most common conditions in horses where nutraceuticals are used. A wide range of nutraceutical products are available to treat these conditions. The nutraceuticals are considered to provide structural building blocks and thereby provide functional benefits in such conditions. Providing building blocks can mitigate disease signs and improve performance of the horse. Commonly, joint supplements contain glucosamine, chondroitin sulfate, a combination of glucosamine and chondroitin sulfate, avocado-soybean unsaponifiables, methylsulfonylmethane (MSM), oral hyaluronan gel, green-lipped mussel extract, Epiitalis, etc. However, this chapter shall focus on those with available scientific data to support their activity.

4.1 Glucosamine and Chondroitin Sulfate

Glucosamine is a natural compound found in cartilages. It is harvested from the shells of shellfish or prepared in a laboratory. There are several forms of glucosamine such as glucosamine sulfate, glucosamine hydrochloride, *N*-acetyl glucosamine, etc. Chondroitin sulfate (CS) is a major component of the extracellular matrix (ECM) of connective tissues like the cartilage, bone, skin, ligaments, etc. It is a sulfated glycosaminoglycan (GAG) and is composed of long unbranched polysaccharide chain with a repeating disaccharide structure of *N*-acetylgalactosamine and glucuronic acid. CS is responsible for the resistance, elasticity, and other biomechanical properties of the cartilage. Chondroitin sulfate and glucosamine have been used clinically for joint diseases for almost 50 years. Since they have been investigated together in many clinical and animal studies, they are considered here together for their use as joint supplements.

Several studies in humans have shown that glucosamine decreases pain and improves mobility in those suffering from osteoarthritis. In a double-blind clinical trial (Lopez Vaz 1982), glucosamine (1.5 g) was compared with ibuprofen (1.2 g) daily over a period of 8 weeks. Though the pain score decreased with ibuprofen in the first 2 weeks, glucosamine fared better at the end of 8 weeks. Both drugs were well tolerated.

Glucosamine has been shown to prevent experimentally induced cartilage degradation of equine articular cartilage explants. Cartilage disks harvested from the weight-bearing region of articular surface were cultured. These cartilages were shown to increase the production of NO, release peptidoglycan, and increased MMPs in response to LPS or IL-1 added in the media. Fenton et al. (2000) demonstrated that all these responses were prevented by glucosamine that had been added to the media. The authors postulated that glucosamine may prevent cartilage matrix degradation by inhibition NO production. It is known that the NO levels are increased in synovial fluid of diseased joints and induce chondrocyte apoptosis, inhibition of cell proliferation, and activation of MMPs. Alternatively, glucosamine may simply directly quench the increased NO or act as an antioxidant and prevent MMP-mediated degradation of cartilage matrix. The second mechanism proposed was the direct inhibition of MMPs by glucosamine.

In a study on horses with degenerative joint disease treated with a glucosamine-chondroitin sulfate compound to determine its effectiveness in decreasing lameness, it was found that within 2 weeks of treatment, the lameness grade, flexion test, and stride length were all significantly improved (Hanson et al. 1997). A further significant improvement occurred at 4 weeks of treatment.

It is essential for any therapeutic agent to be absorbed sufficiently so that it reaches its site of action to exert its pharmacological effect. In this context, the bioavailability of any drug is critical for its action. Du et al. (2004) showed that glucosamine hydrochloride and chondroitin sulfate are orally bioavailable in horses by determining the C_{max} and T_{max}. However, the oral bioavailability of CS has been debated for some time due to its large molecular structure. At the same time, however, it is also known that oral administration of CS does provide relief from pain and a slowing of the progression of osteoarthritis in animals. CS may also be

metabolized to unsaturated disaccharides. In vitro studies on Caco-2 cell culture also suggested higher permeability for low-molecular-weight CS. To resolve this issue, Eddington et al. (2001) conducted a study in horses to determine the oral bioavailability of CS by determining the disaccharides formed after oral administration and to determine the influence of its molecular weight. The study found that disaccharides formed specifically following the breakdown of chondroitin sulfate in horse plasma samples after oral dosing of 8.0 and 16.9 kDa molecular weight CS. It was interpreted that CS or fragments of the CS molecule are absorbed after its oral administration. The bioavailability associated with 8.0 kDa CS was higher than the 16.9 kDa CS.

Laverty et al. (2005) examined the concentration of glucosamine in synovial fluid and its pharmacokinetics in serum of horses following clinically relevant oral and iv dose levels. The maximum concentration of glucosamine in serum was ~300 μM following iv dosing and ~6 μM following oral dosing. The corresponding synovial concentrations were 9 μM–15 μM and 0.3 μM–0.7 μM, respectively. Based on these concentrations, the investigators concluded that the therapeutic benefit of dietary glucosamine may be secondary to its effects on non-articular tissues such as the intestinal lining, liver, or kidney. The mean bioavailability of glucosamine was 5.9%, and its mean elimination half-life was 2.82 h. Therefore, even the mechanism of action of glucosamine needs further elucidation.

How do glucosamine and chondroitin sulfate work in joint inflammation and pain? Osteoarthritis is associated with degeneration of the articular cartilage and excess production of proinflammatory cytokines. It is recognized that IL-1β is a proinflammatory cytokine that can induce the catabolic cascade. At physiologically relevant concentrations, glucosamine and chondroitin sulfate have been shown to regulate gene expression and synthesis of nitric oxide (NO) and prostaglandin E_2 and can exert anti-inflammatory properties (Chan et al. 2005). Further, glucosamine can stimulate glycosaminoglycan (GAG) and proteoglycan synthesis and decrease proteoglycan degradation, thereby preserving cartilage.

4.2 Miscellaneous Joint Supplements

Joint supplements containing glucosamine and chondroitin sulfate are also marketed with manganese, various antioxidants including vitamin C, or herbal anti-inflammatory compounds.

Undenatured type II collagen (UC-II), a natural ingredient containing glycosylated undenatured type II collagen (Bagchi et al. 2002), has been shown to modulate joint health both in osteoarthritis (OA) and rheumatoid arthritis (RA). Oral intake of small amounts of glycosylated UC-II (10 mg/day) over 42 days in five female subjects suffering from significant joint pain greatly reduced pain including morning stiffness, stiffness following periods of rest, etc. Similarly, in a placebo-controlled clinical study, Gupta et al. (2009) evaluated undenatured type II collagen (UC-II) against glucosamine and chondroitin in arthritic pain in horses. The horses were evaluated for overall pain, pain upon limb manipulation, pain after physical exertion, and liver and kidney functions. Although the glucosamine- and chondroitin-treated group showed significant reduction in pain, its efficacy was less as compared to UC-II. Clinically, the UC-II-treated animals tolerated the treatment well without any signs of toxicity. All the parameters monitored, i.e., body weight, body temperature, respiration rate, pulse, liver functions (bilirubin, GGT, and ALP), and kidney functions (BUN and creatinine), remained unchanged.

In an experimentally induced equine acute synovitis model, two nutraceutical supplements AT (Cavalor ArtiTec, Nutriquine NV, Belgium) and HP (Hydro-P, Sonac, The Netherlands) were investigated concerning inflammation and biomarkers of cartilage metabolism (Van de Water et al. 2016). Synovitis was induced in the right intercarpal joint by injection of 0.5 ng lipopolysaccharide (LPS) of *E. coli*. Synovial fluid samples were analyzed for total nucleated cell counts (TNCC), total protein (TP), and a comprehensive set of biomarkers of inflammation (PGE_2, IL-6, MMPs, CP-11, and CAG). Both nutraceuticals significantly lowered TP, TNCC, and PGE_2. There were no serious adverse effects.

The market for joint supplements has several other products like New Zealand green-lipped mussel, shark cartilage, a lipid extract from the herb Biota orientalis, etc. For further details on nutraceuticals in equine osteoarthritis, readers are referred to some other publications elsewhere (May et al. 2015; Gupta 2016; Chap. 26).

5 Nutraceuticals as Performance Boosters

The nutraceutical market is replete with nutraceutical products for horses, claiming to delay fatigue, increase stamina, build muscle, reduce recovery time, etc. These so-called ergogenics are supposed to boost horses' exercise performance capacity. The term "ergogenic" is derived from the Greek word "ergo" meaning "work." Unfortunately, many such supplements are not supported by scientific evidence. There are several mechanisms by which various foods or nutritional supplements work to enhance physical performance. However, it is essential to consider the information available on a given supplement and determine the scientific rationale and validity of such claims. Some of the most commonly used nutraceuticals for such purposes are discussed here.

5.1 Carnitine

L-carnitine is a conditionally essential nutrient that plays an important role in mitochondrial β-oxidation. As it is involved with the utilization of fatty acids for energy in the muscle cell, it is believed that supplementation of carnitine would be muscle glycogen-sparing and reduce lactic acid production, thereby improving muscle function and endurance. As a dietary supplement for athletes, L-carnitine has been investigated for its potential to enhance β-oxidation during exercise (Huang and Owen 2012). While some studies have shown a positive impact on VO (2 max) and other performance measures, others have found contradictory results. As L-carnitine is known to enhance vascular endothelial function, it can also improve blood flow to muscle tissues and decrease hypoxic stress and its resulting sequelae. The authors concluded that L-carnitine is regarded as a safe supplement for athletes and has been shown to positively impact the recovery process after exercise. In horses, it has been shown (Zeyner and Harmeyer 1999) that oral supplementation of 5–50 g of L-carnitine/day elevated the carnitine concentration in blood plasma to about twice its basal concentration. However, no clear relationship existed between an orally administered dose of carnitine and the increase of L-carnitine. It is essential to unequivocally demonstrate an increase in muscle carnitine levels following supplementation of carnitine.

5.2 Creatine

Creatine is intricately involved in energy metabolism in the muscles. Creatine is converted into phosphocreatine which anaerobically donates phosphate to adenosine phosphate, creating ATP. Increasing the available ATP can facilitate muscle contraction and therefore may contribute to increased stamina. Inadequate phosphocreatine is considered a major cause of muscle fatigue during intense exercise. On this basis, it is common to supplement the diet of the horse with creatine for improved performance. There are several studies on human volunteers on the effect of creatine supplementation on exercise performance and endurance. In one such study, Becque et al. (2000) investigated the effects of 6 weeks of oral creatine supplementation during a periodized program of arm flexor strength training on arm flexor 1RM, upper arm muscle area, and body composition. It was found that creatine supplementation during arm flexor strength training leads to greater increases in flexor muscular strength, upper arm muscle area, and fat-free mass than strength training alone. However, in horses, creatine supplementation did not appear to provide benefits. In a study on oral creatine supplementation on the performance of quarter horses used in barrel racing, Teixeira et al. (2016) found that supplementation with 28 g of creatine/100 kg body weight, orally for 45 days significantly altered LDH activity, plasma glucose, and PCV. However, there was no change in time scores, heart rate, and plasma lactate; the variables are considered to be performance indicators. The authors concluded that there was no improvement in equine athletic performance due to creatine oral supplementation. The lack of effect of creatine supplementation on equine performance could be due to the poor bioavailability of creatine in horses. In a study undertaken to examine the oral absorption of creatinine in the horse by Sewell and Harris (1995), a single dose of creatine monohydrate only modestly increased the creatine levels. Even after 13 days of feeding 0.15 g/kg body weight of creatine, there was no effect on the muscle content of creatine. Unlike humans, horse has limited ability to absorb creatine when given orally. This is understandable as the horse is normally exposed to very low levels of creatine in its herbivorous diet and as a species does not have ability to orally absorb creatine. This is unlike humans where the diet has animal meat and fish which contain high levels of creatine. Hence, dietary supplements containing creatine are unlikely to improve the performance in horses.

5.3 Amino Acids

Amino acids and protein supplements are considered to have the ability to build muscle mass thereby influencing the performance of the horse. In addition, branched-chain amino acids (BCAA) have the ability to positively impact muscle glycogen content which may have some bearing on fatigue. In male endurance-trained cyclists, treatment with BCAA has shown an interesting muscle glycogen-sparing effect as compared to placebo. It is believed that this glycogen-sparing effect could translate into a delayed muscle fatigue during sustained exhaustive exercise (Blomstrand et al. 1996). In addition, treatment with BCAA may provide carbon intermediates for the citric acid cycle when endogenous carbohydrate reserves are depleted. This could further add to the delay in the onset of fatigue (Wagenmakers 1999).

Central mechanisms of fatigue play an important role in exercise performance. Increase in brain 5-hydroxytryptamine (5-HT) is known to cause a deterioration in sport and exercise performance. Currently, there is evidence that exercise increases the plasma levels of free tryptophan (fTRP) leading to an increase in the ratio fTRP/BCCA, which is associated with increased brain 5-HT and consequently increased fatigue. It has been shown that brain 5-HT levels may be manipulated with drugs and have a predicted effect on brain-mediated exercise fatigue. However, the influence of nutritional manipulations of the fTRP/BCAA ratio on

performance is not well established, partly because of methodological problems. Carbohydrate feeding during prolonged exercise, however, has led to dramatic reductions in fTRP/BCCA and enhanced performance. However, it has been difficult to distinguish between the effects of carbohydrate feedings on direct effect of muscles and central effects (Davis 1995).

Since BCAA and tryptophan compete with each other to enter into the central nervous system (CNS), BCAA supplementation could reduce tryptophan entry and thereby delay the onset of fatigue. Farris et al. (1998) infused horses intravenously with tryptophan to increase its entry into the CNS and predictably decreased the time to fatigue by about 15%. This was taken as evidence that an increase in circulating tryptophan can decrease endurance performance in horses. However, this could not be replicated with oral administration of tryptophan. Again, BCAA supplementation in large quantities did not have expected influence on endurance performance.

Mikulski et al. (2015) investigated the effectiveness of oral BCAA and L-ornithine L-aspartate supplementation (BCAA + OA) in healthy men to reduce plasma ammonia concentration and enhance psychomotor performance during exhaustive exercise. At the end of prolonged exercise, the plasma ammonia concentration was higher in the BCAA + OA group than in the placebo group. Decreases in plasma ammonia during recovery were significantly higher in the BCAA + OA group than in the placebo group. It was concluded that BCAA + OA is a useful way to improve multiple choice reaction time during high-intensity exercise and accelerate the elimination of ammonia at the recovery stage after exercise in healthy young men.

Unfortunately, the beneficial effects of BCAA supplementation on exercise performance could not be demonstrated consistently. In a study on trotter horses (Stefanon et al. 2000), the horses were treated for 4 weeks of supplementation with a BCAA mixture (9.0 g L-leucine, 4.5 g isoleucine, and 4.5 g L-valine) 1 hour before training. BCAA supplementation resulted in higher alpha-ketoisocaproate in supplemented horses. However, BCAA treatment for 4 weeks did not affect any markers of energy metabolism like plasma lactate, glucose, allantoin, or pyruvate concentration. Similarly, in a study (Casini et al. 2000) on the effect of BCAA supplementation on metabolic response to a 1600 m run on a treadmill in standardbred trotters, lactate, ammonia, total protein, urea, uric acid, creatinine, FFA, creatinine kinase, LDH, and AST were monitored. However, none of these parameters showed any statistical difference between groups. The results suggested that BCAA supplementation was not effective in enhancing performance in healthy and well-fed horses.

5.4 HMB (β-Hydroxy β-Methyl Butyrate)

In both horses and humans, nutritional ergogenic aids have been used along with an appropriate training program to improve physical ability. HMB is a metabolite of essential amino acid leucine and is produced endogenously both in animals and man. It is a common dietary supplement in exercise enthusiasts. HMB is involved in cholesterol biosynthesis, and it is believed that during heavy training and exercise, supplementation with HMB may help synthesis of cholesterol in the muscle tissue, mitigate muscle damage, and promote maximal growth and function. In a normal horse diet like alfalfa, HMB is present only in small quantities.

In one study, horses given HMB supplement had lower muscle tissue breakdown as compared to controls (Nissen et al. 1997). In another study conducted to determine the effect of oral supplementation of HMB and γ-Oryzanol (GO) on indices of exercise-induced muscle damage in Thoroughbred race horses (Ostaszewski et al. 2012), it was found that the creatine kinase and lactate levels were lower in the treated group as compared to the controls. It was concluded that HMB and GO supplementation provide prevention of exercise-induced muscle damage. Again, in a meta-analysis of randomized controlled clinical trials, it was found that HMB supplementation reduced LDH and CK serum levels in adults following exercise-induced muscle damage showing its effectiveness as a muscle damage recovery agent (Rahimi et al. 2018). Feeding HMB improves endurance and reduces muscle damage in horses.

6 The Use of Nutritional Supplements in Dressage and Eventing Horses

A survey conducted on the use of nutritional supplements in dressage and eventing horses (Agar et al. 2016) showed that a wide range of supplements was used and most owners and riders perceived that they were important to their horses' health and performance. The main health and performance issues identified for dressage horses were energy/behavior, lameness, and back and muscle problems. For eventing horses, it was stamina and fitness levels, lameness, and energy/behavior. In both disciplines, lameness and/or joint problems were identified as important issues in addition to behavioral problems. The authors concluded that despite the low levels of evidence for most supplements, this study showed that they were widely used across both disciplines. Most owners stated that they felt that the supplements made a marked difference to their horse. Since such food supplements are widely used and their perceived value is great for the horse's health and performance, the veterinary

profession should have an understanding of owners/riders' opinions and concerns.

7 Concluding Remarks and Future Directions

Nutritional and health supplements are frequently used to improve health and/or performance, in addition to prevention of deficiencies of vitamins, minerals, electrolytes etc., in man and animals. The equine nutraceuticals' market has grown greatly in the last 25 years, but there are concerns for its sustained growth. Since the term nutraceuticals was coined, it has not been fully appreciated that the term does not have any regulatory meaning. Unfortunately, this has encouraged only limited attention to the evaluation of safety and efficacy of the products and the quality of manufacture. Until the industry develops capabilities and a capacity to demonstrate the clinical benefits of its products and the ability to manufacture with adherence to quality of its processes, it will continue to be a challenging time for the nutraceutical industry.

References

Agar C, Gemmill R, Hollands T et al (2016) The use of nutritional supplements in dressage and eventing horses. Vet Rec Open 3: e000154

Bagchi D, Misner B, Bagchi M et al (2002) Effects of orally administered undenatured type II collagen against arthritic inflammatory disease: a mechanistic exploration. Int J Clin Pharmacol Res 22:101–110

Becque MD, Lochmann JD, Melrose DR (2000) Effects of oral creatine supplementation on muscular strength and body composition. Med Sci Sports Exer 32:654–658

Blomstrand E, Ek S, Newsholme EA (1996) Influence of ingesting a solution of branched-chain amino acids on plasma and muscle concentrations of amino acids during prolonged submaximal exercise. Nutrition 12:485–490

Casini L, Gatta D, Magni L et al (2000) Effect of prolonged branched-chain amino acid supplementation on metabolic response to anaerobic exercise in Standardbreds. J Equine Vet Sci 20:120–123

Chan PS, Caron JP, Rosa GJM et al (2005) Glucosamine and chondroitin sulphate regulate gene expression and synthesis of nitric oxide and prostaglandin E_2 in articular cartilage explants. Osteoarthr Cartil 13:387–394

Crandell K, Duren S (2001) Nutraceuticals: what are they and do they work? In: Pagan JD (ed) Advances in equine nutrition, vol II. Nottingham University Press, Nottingham, pp 29–36

Davis JM (1995) Carbohydrates, branched-chain amino acids, and endurance: the central fatigue hypothesis. Int J Sport Nutr 5:S29–S38

DeFelice SL (1995) The nutraceutical revolution: Its impact on food industry R&D. Trends Food Sci Technol 6:59–61

Du J, White N, Eddington ND (2004) The bioavailability and pharmacokinetics of glucosamine hydrochloride and chondroitin sulphate after oral and intravenous single dose administration in the horse. Biopharm Drug Dispos 25:109–116

Eddington ND, Du J, White N (2001) Evidence of the oral absorption of chondroitin sulphate as determined by total disaccharide content after oral and intravenous administration to horses. Proc Annu Convention AAEP 47:326–328

Farris JW, Hinchcliff KW, McKeever KH et al (1998) Effect of tryptophan and of glucose on exercise capacity of horses. J Appl Physiol 85:807–816

Fenton JL, Chlebek-Brown KA, Peters TL et al (2000) Glucosamine HCl reduces equine articular cartilage degradation in explant culture. Osteoarthr Cartil 8:258–265

Gupta RC (2016) Nutraceuticals in arthritis. In: Gupta RC (ed) Nutraceuticals: efficacy, safety and toxicity. Academic/Elsevier, Amsterdam, pp 161–176

Gupta RC, Canerdy TD, Skaggs P et al (2009) Therapeutic efficacy of undenatured type-II collagen (UC-II) in comparison to glucosamine and chondroitin in arthritic horses. J Vet Pharmacol Ther 32:577–584

Hanson RR, Smalley LR, Huff GK et al (1997) Oral treatment with a glucosamine-chondroitin sulfate compound for degenerative joint disease in horses: 25 cases. Equine Pract 19:16–21

Huang A, Owen K (2012) Role of supplementary L-carnitine in exercise and exercise recovery. Med Sport Sci 59:135–142

Laverty S, Sandy JD, Celeste C et al (2005) Synovial fluid levels and serum pharmacokinetics in a large animal model following treatment with oral glucosamine at clinically relevant doses. Arthritis Rheum 52:181–191

Lopez Vaz A (1982) Double-blind clinical evaluation of the relative efficacy of ibuprofen and glucosamine sulphate in the management of osteoarthrosis of the knee in out-patients. Curr Med Res Opin 8:145–149

May K, Gupta RC, Miller J et al (2015) Therapeutic efficacy and safety evaluation of a novel chromium supplement (Crominex® +3-) in moderately arthritic horses. Jacobs J Vet Sci Res 2(1):014

Mikulski T, Dabrowski J, Hilgier W et al (2015) Effects of supplementation with branched chain amino acids and ornithine aspartate on plasma ammonia and central fatigue during exercise in healthy men. Folia Neuropathol 53:377–386

Nissen S, Fuller J, Rathmacher J (1997) β-hydroxy β-methylbutyrate (HMB) supplementation in training horses. Metabolic Technologies Bulletin, Ames (Cited by Crandell and Duren 2001)

Ostaszewski P, Kowalska A, Szarska E et al (2012) Effect of β-hydroxy-β-methylbutyrate and γ-oryzanol on blood biochemical markers in exercising Thoroughbred race horses. J Equine Vet Sci 32:542–551

Rahimi MH, Mohammadi H, Eshaghi H et al (2018) The effects of beta-hydroxy-methylbutyrate supplementation on recovery following exercise-induced muscle damage: a systemic review and meta-analysis. J Am Coll Nutr 20:1–10

Sewell DA, Harris RC (1995) Effect of creatine supplementation in the Thoroughbred horse. Equine Vet J 18:239–242

Stefanon B, Bettini C, Guggia P (2000) Administration of branched-chain amino acids to standardbred horses in training. J Equine Vet Sci 20:115–119

Teixeira FA, Araujo AL, Ramalho LO et al (2016) Oral creatine supplementation on performance of Quarter Horses used in barrel racing. J Anim Physiol Anim Nutr 100:513–519

Van de Water E, Oosterlinck M, van Weeren PR et al (2016) The effect of two nutraceuticals on inflammation and biomarkers of cartilage metabolism in equine synovial fluid after experimentally induced acute synovitis. Equine Vet J 48(Suppl 49):7 (Abstract)

Wagenmakers A (1999) Nutritional supplements: effects on exercise performance and metabolism. In: Lamb D, Murray R (eds) Perspectives in exercise science and sports medicine. The metabolic basis of performance in exercise and sport. Indiana Cooper Publishing, Carmel, pp 207–260

Zeyner A, Harmeyer J (1999) Metabolic functions of L-carnitine and its effects as feed additive in horses. A review. Arch Tieremahr 52:115–138

Nutraceuticals for Camelids

Tarun K. Gahlot

Abstract

Nutraceuticals are becoming increasingly popular within the veterinary profession and are popularly used in camelids as dietary supplements for improving health, preventing disease, and providing nutritional supplementation. Camel milk is among such dietary supplement with profound nutraceutical values. About 16 active nutraceuticals have been identified and discussed. These are galactolipid natural emulsifiers; natural tocotrienol antioxidants; oat-derived beta glucans; phospholipids; bioavailable trace and macro minerals; nanotechnology components and simple sugars "oligosaccharides"; natural buffering and yeast cultures; omega-3 fatty acids; prebiotics; vitamin D3, phosphorus, calcium, and yeast cultures; digestible milk proteins; glutamic acid; methylsulfonylmethane (MSM); microscopic toxin binder; nucleotides; and vitamin C. Nutraceutical supplementation improves cartilaginous health within the joint as well as maintains the skin and hair coat quality. These have a profoundly beneficial impact on the health of the body, and antioxidant compounds may help in the prevention of cancer.

Keywords

Camelids · Nutraceuticals · Galactolipid natural emulsifiers · Tocotrienol antioxidants · Oat-derived beta glucans · Phospholipids · Bioavailable trace and macro minerals · Oligosaccharides · Omega-3 fatty acids · Prebiotics · Vit. D3 · Phosphorus · Calcium and yeast cultures · Digestible milk proteins · Glutamic acid · MSM (methylsulfonylmethane) · Microscopic toxin binder · Nucleotides and vitamin C

T. K. Gahlot (✉)
Rajasthan University of Veterinary and Animal Sciences, Bikaner, Rajasthan, India

1 Introduction

Nutraceuticals are becoming increasingly popular within the veterinary profession. An animal's well-being can be improved by a diet containing nutraceuticals. They have been described by the North American Veterinary Nutraceutical Council as a "non-drug substance that is produced in a purified or extracted form and administered orally to provide agents required for normal body structure and function with the intent of improving the health and well-being of animals."

Nutraceuticals are commonly used in a large animal health care. Vitamin and mineral supplements for livestock and horses are used in range, pasture, and equine stable environments. Nutraceutical supplementation improves cartilaginous health within the joint as well as maintains the skin and hair coat quality. These have a profoundly beneficial impact on the health of the body, and antioxidant compounds may help in the prevention of cancer.

Camelids are modified ruminants or "pseudoruminants." Like ruminants, they use foregut fermentation to break down cellulose in fibrous plant species. But in contrast to ruminants, their forestomachs are made up of three compartments rather than the true ruminants' (sheep, goats, cattle, deer) four. The three sections of a camelid forestomach are called C-1, C-2, and C-3; each compartment has a specialized function to perform. Ruminants and camelids dedicate the majority of their digestive capacity to the fermentation of plant fiber. It is important for camelids to eat plenty of fibrous material to sustain normal health, and, as such, the consumption of concentrated hard feeds should always be kept to a minimum. Natural foregut digestion should be maintained because it is crucial for the health and vitality of New World Camelids.

The nature of the feed can have dramatic effects on well-being, as all food goes straight into the fermentation vat of the forestomach where factors like motility, gas eructation, regurgitation, remastication, and re-swallowing are all part of the normal feeding process.

R. C. Gupta et al. (eds.), *Nutraceuticals in Veterinary Medicine*, https://doi.org/10.1007/978-3-030-04624-8_46

Camel milk is among such dietary supplement with profound nutraceutical values. From a medicinal point of view, it has a rich content of protective minerals and proteins that could have a vital role for enhancing immune defense mechanism (Yagil 1982). It contains higher amount of zinc which offer significant role in the development and maintenance of a normally functioning immune system (Hansen et al. 1982). Camel milk has insulin-like activity and regulatory and immunomodulatory functions on β-cells. It contains a good amount of protective proteins, including lysozyme, lactoferrin, lactoperoxidase, peptidoglycan recognition protein (PGRP) enzyme, immunoglobulin G, and secretory immunoglobulin A. These immune factors are present at greater concentrations in camel milk (El Agamy et al. 1992).

Nutraceuticals appear to be of benefit in both the treatment and prevention of disease because they often possess unique chemical actions that are unavailable in pharmaceuticals, e.g., ability of silymarin in milk thistle to protect liver cells from damage and quercetin (found in a variety of plants) to stabilize certain cells of the immune system (mast cells) to avoid allergic reactions.

The effectiveness and dosage of nutraceuticals should be well scrutinized as there is no regulatory agency charged to determine guidelines for nutraceuticals. Some animals may be potentially allergic to the plant or feed source of the nutraceutical product; hence trials of these products should be conducted thoroughly. There are many active nutraceuticals whose composition and mode of action are discussed below in brief.

2 Active Nutraceuticals

1. *Galactolipid Natural Emulsifiers*
   Galactolipids are phosphorus-free glycoglycerolipids in plants and are in fact a combination of sugar and fat molecules which create the suitable medium for the absorption of active ingredients across the gut wall. Galactolipids make up the bulk of photosynthetic membranes. Oxygenic photosynthesis in *Cyanobacteria* and plants depends on the presence of galactolipids. Recently, it has been demonstrated that the galactolipid, 1,2-di-*o*-alpha-linolenoyl-3-*o*-beta-D-galactopyranosyl-sn-glycerol (1), may be important for the anti-inflammatory activity of dog rose (*Rosa canina*), a medicinal plant with documented anti-inflammatory effect in diseases such as arthritis. This galactolipid also occurs in relatively high concentrations in certain legumes (e.g., common bean, pea), leafy vegetables (e.g., kale, leek, parsley, perilla, and spinach), stem vegetables (e.g., asparagus, broccoli, and brussels sprouts), and fruit vegetables (e.g., chili, bell pepper, and pumpkin). Furthermore, compound 1 has been isolated from spinach and several medicinal plants by bioassay-guided fractionation as a galactolipid with possible cancer-preventive effects (Christensen 2009).
2. *Natural Tocotrienol Antioxidants*
   Tocotrienol antioxidants are a natural form of vitamin E. They are 40–60 times more readily bioavailable than other forms of vitamin E and help to maintain the integrity of the cells forming the lining of the gut to aid absorption. Recent mechanistic studies indicate that other forms of vitamin E such as γ-tocopherol (γT), δ-tocopherol (δT), and γ-tocotrienol (γTE) have unique antioxidant and anti-inflammatory properties that are superior to αT in the prevention and therapy against chronic diseases. These vitamin E forms scavenge reactive nitrogen species (ROS), inhibit cyclooxygenase (COX)- and 5-lipoxygenase (5-LOX)-catalyzed eicosanoids, and suppress pro-inflammatory signaling such as NF-κB and STAT3/STAT6. Unlike αT, other vitamin E forms are significantly metabolized to carboxychromanols via cytochrome P-450 (CYP4F2)-initiated side-chain ω-oxidation. Long-chain carboxychromanols, esp. 13′-carboxychromanols, are shown to have stronger anti-inflammatory effects than unmetabolized vitamins and may therefore contribute to beneficial effects of vitamin E forms in vivo (Jiang 2014).
3. *Oat-Derived Beta Glucans*
   Beta glucans have prebiotic properties that support beneficial bacteria in the gut to promote healthy absorption. Six grams of β-glucan from oats added to the AHA Step II diet and moderate physical activity improved lipid profile and caused a decrease in weight and, thus, reduced the risk of cardiovascular events in overweight male individuals with mild to moderate hypercholesterolemia (Reyna-Villasmil et al. 2007).
4. *Phospholipids*
   Phospholipids have emulsifying properties and have been shown to actively influence nutrient digestibility and absorption.
5. *Bioavailable Trace and Macro Minerals*
   Bioavailable trace minerals are essential minerals, provided in a digestible form to supply the needs of productive camelids. The supplementation with high levels of available zinc has shown very positive results, and selenium yeast is proven to be 20 times less toxic than inorganic selenium, which is very beneficial to health.
   Spears (2003) found that absorption of selenium and copper is much lower in ruminants than in nonruminants. The low absorption of these minerals in ruminants is due to modifications that occur in the rumen environment.

Selenium bioavailability is reduced by high dietary sulfur and the presence of cyanogenetic glycosides in certain legumes. Feeding organic selenium from selenomethionine or selenized yeast results in much higher tissue and milk selenium concentrations than are obtained with selenite. High dietary molybdenum in combination with moderate to high dietary sulfur results in the formation of thiomolybdates in the rumen. Thiomolybdates greatly reduce copper absorption, and certain thiomolybdate species can be absorbed and interfere systemically with copper metabolism. Independent of molybdenum, high dietary sulfur reduces copper absorption perhaps via formation of copper sulfide. High dietary iron also reduces copper bioavailability. Dietary factors that affect bioavailability of zinc in ruminants are not well defined. Phytate does not affect zinc absorption in ruminants because microbial phytase in the rumen degrades phytate. Manganese is very poorly absorbed in ruminants, and limited research suggests that high dietary calcium and phosphorus may reduce manganese absorption. Chelated minerals provide bioavailable forms of essential vitamins, copper, manganese, and zinc, which are required by important metabolic processes in a young developing cria.

6. *Nanotechnology Components and Simple Sugars Oligosaccharides*
   Oligosaccharides are used to support the immune system. The harmful organisms being absorbed into the bloodstream are blocked by high levels of macrophagic activity. In conjunction with a natural, high-quality forage diet, exposure to pathogenic molds/fungi will be reduced, and if there is a problem, the active self-defense mechanism will deal with them.
7. *Natural Buffering and Yeast Cultures*
   Natural buffering material and yeast cultures help to stabilize the pH in the foregut and encourage the development of beneficial fiber-digesting bacteria. This is important when feeding cereal-based feeds as starch is converted into lactic acid, which can increase acidity and create an unfavorable environment for the fiber-digesting bacteria.
8. *Omega-3 Fatty Acids*
   Omega-3 fatty acids provide a daily source of essential fat components. Omega-3 fatty acids are widely recognized as anti-inflammatory compounds and balance the pro-inflammatory omega-6 fatty acids in the correct ratio. Omega 3's are also found in large concentrations in spermatozoa and are essential for maintaining cell wall integrity and preventing moisture/oil loss from the skin. Healthy skin means high-quality fiber production.
   Gulliver et al. (2012) found that specific effects of omega-3 polyunsaturated fatty acids (*n*-3) on reproductive success in ruminants have not been examined in detail. There is strong evidence linking consumption of diets high in *n*-3 with reduced circulating peripheral inflammatory markers such as PGF(2α). Inflammatory eicosanoids including PGF(2α), in particular, can significantly affect reproduction outcomes such as the onset of estrus, embryo survival, and parturition. There appears to be an evidence linking *n*-3 supplementation with longer time to estrus and parturition associated with reduced PGF(2α), the effects of *n*-3 on other measurable outcomes of reproductive success, such as pregnancy rate, embryo survival, and intergenerational effects on the health, and production of offspring are largely unknown.
   Increasing omega fatty acids in tissues of the cow has been shown to provide benefits on reproductive performance, immunity and disease resistance, and positive hormonal shifts (Jenkins 2004).
9. *Prebiotics*
   Prebiotics provide specific nourishment for friendly, fiber-digesting bacteria to maintain good digestion and release nutrients for growth, breeding, and performance.
10. *Vitamin D3, Phosphorus, Calcium, and Yeast Cultures*
    Vitamin D3, phosphorus, calcium, and yeast cultures contain high levels of vitamin D3, available phosphorus, and calcium in a very palatable form. All three are crucial for healthy skeletal development, and additional yeast cultures are added to significantly improve the digestibility of phosphorus from normal feed.
11. *Digestible Milk Proteins*
    Milk proteins, particularly caseins, are highly digestible in the intestine and are a high quality source of amino acids. Readily digestible milk proteins provide key amino acids for supporting the growth of all tissues with high protein contents, namely, muscles, ligaments, tendons, hair, and hooves.
12. *Glutamic Acid*
    Glutamic acid is a natural flavor enhancer and an important nutritional support for natural healing and gut health during times of stress.
13. *Methylsulfonylmethane (MSM)*
    MSM is a source of sulfur, which helps to form cross-links with other molecules that maintain the strength and integrity of connective tissues. It is also essential for the manufacture of collagen.
14. *Microscopic Toxin Binder*
    A blend of microscopic adsorbent compounds that aid the natural expulsion of biotoxins, including mycotoxins, found in feed and the environment.
15. *Nucleotides*
    Cell division is the basis of growth and development and involves the splitting of RNA and DNA. Nucleotides provide essential structured units for the production of

RNA and DNA in cells and are very important to sustain normal healthy development.

16. *Vitamin C*
    Vitamin C is required for the formation of collagen in the body that forms the matrix of connective tissues in mammals. Supplementation of vitamin C therefore can help in tissue growth, tissue healing following trauma, and immune system of the camelids.

3 Concluding Remarks and Future Directions

Nutraceutical products are often included in animal feeds but are widely available in many forms, including capsules, tablets, powders, etc. Nutraceuticals are used for the prevention and treatment of common diseases in animals including cardiovascular disease, osteoarthritis, periodontal disease, cognitive dysfunction, and cancer, with their validation through clinical trials. Camelids are used these days as an industry of meat, wool/hair, and milk, in addition to the breeding and sports. Nutraceuticals might play a pivotal role in maintaining health of various body systems of these camelids. The veterinary nutraceuticals can be obtained from vet shops, veterinary practices, pharmacies, and the Internet.

Suggested Readings

Allen JD, Gawthorne JW (1987) Involvement of the solid phase of rumen digesta in the interaction between copper, molybdenum and sulphur in sheep. Br J Nutr 58:265–276

Bremner I, Humphries WR, Phillippo M et al (1987) Iron induced copper deficiency in calves: dose response relationships and interactions with molybdenum and sulphur. Anim Prod 45:403–414

Brower V (1998) Nutraceuticals: poised for a healthy slice of the healthcare market? Nat Biotechnol 16:728–731

Cao J, Henry PR, Guo R et al (2000) Chemical characteristics and relative bioavailability of supplemental organic zinc sources for poultry and ruminants. J Anim Sci 78:2039–2054

Christensen LP (2009) Galactolipids as potential health promoting compounds in vegetable foods. Recent Pat Food Nutr Agric 1 (1):50–58

Cousins RJ, Liuzzi JP, Lichten LA (2006) Mammalian zinc transport, trafficking, and signals. J Biol Chem 281:24085–24089

De Domenico I, Ward DM, Kaplan J (2008) Regulation of iron acquisition and storage: consequences for iron-linked disorders. Nat Rev Mol Cell Biol 9:7281

De Felice SL (2002) FIM rationale and proposed guidelines for the Nutraceutical Research & Education Act-NREA, November 10, 2002. Foundation for Innovation in Medicine. Available at: http://www.fimdefelice.org/archives/arc.researchact.html

El Agamy EI, Ruppanner R, Ismail A et al (1992) Antibacterial and antiviral activity of camel milk protective proteins. J Dairy Res 59:169–175

Elizabeth AC (2002) Over-the-counter products: nonprescription medications, nutraceuticals, and herbal agents. Clin Obstet Gynecol 45(1):89–98

FDA/CFSAN resources page (1994) Food and Drug Administration Web site. Dietary Supplement Health and Education Act of 1994. Available at: http://vm.cfsan.fda.gov/~dms/dietsupp.html

Gulliver CE, Friend MA, King BJ et al (2012) The role of omega-3 polyunsaturated fatty acids in reproduction of sheep and cattle. Anim Reprod Sci 131(1–2):9–22

Hansen M, Fernandes G, Good R (1982) Nutrition and immunity: the influence of diet on autoimmunity and the role of zinc in the immune response. Annu Rev Nutr 2:151–157

Heyland DK (2001) In search of the magic nutraceuticals: problems with current approaches. J Nutr 131(9):2591S–2595S

Hunt JR (2003) Bioavailability of iron, zinc, and other trace minerals from vegetarian diets. Am J Clin Nutr 78(3):633S–639S. https://doi.org/10.1093/ajcn/78.3.633S

Jenkins T (2004) Challenges of meeting cow demands for omega fatty acids. In: Florida ruminant nutrition symposium, pp 52–66

Jiang Q (2014) Natural forms of vitamin E: metabolism, antioxidant and anti-inflammatory activities and the role in disease prevention and therapy. Free Radic Biol Med 72:76–90

Nelson NJ (1999) Purple carrots, margarine laced with wood pulp? Nutraceuticals move into the supermarket. J Natl Cancer Inst 91:755–757

Reyna-Villasmil N, Bermúdez-Pirela VMD et al (2007) Oat-derived β-glucan significantly improves HDLC and diminishes LDLC and Non-HDL cholesterol in overweight individuals with mild hypercholesterolemia. Am J Ther 14(2):203–212

Spears JW (2003) Trace mineral bioavailability in ruminants. J Nutr 133 (Suppl 5):1506S–1509S

Voulgaris D (2011) Alternative therapies in camelids. Faculty publications and other works – large animal clinical sciences. http://trace.tennessee.edu/utk_largpubs/26

Whitman M (2001) Understanding the perceived need for complementary and alternative nutraceuticals: lifestyle issues. Clin J Oncol Nurs 5:190–194

Yagil R (1982) Camels and camel milk. Food and Agriculture Organization, Animal Production. Health Paper 26

Zeisel SH (1999) Regulation of nutraceuticals. Science 285:185–186

Nutraceuticals in Poultry Health and Disease

Ayhan Filazi and Begüm Yurdakok-Dikmen

Abstract

The use of antibiotics at subtherapeutic doses with the aim of accelerating growth in poultry animals has either been prohibited or restricted in many countries, as it causes antibiotic-resistant microorganisms to develop and is a danger to consumers' health. Excluding antibiotics from the diets of poultry animals has caused various performance and health problems; therefore, there is an increasing need for alternative natural substances that can create similar effects. For this purpose, various feed additives have been developed for the improvement of growth and influence health in a positive way. These substances play an important role in the development and continuity of the animals' normal physiological functions and health and in protecting them against infectious diseases. Some of these substances are known as "nutraceuticals." In this section, nutraceuticals such as probiotics, synbiotics, enzymes, organic acids, and phytobiotics and their mechanism of action and possible use in the improvement of growth, immune system, and welfare are discussed.

Keywords

Nutraceuticals · Poultry · Probiotics · Prebiotics · Synbiotics · Organic acids · Enzymes · Phytobiotics

1 Introduction

In poultry farming, the most important targets are accelerating growth and increasing the efficiency of feed. In order to obtain optimal performance in the livestock, many factors, including the animals' genetics, quality of feed, environmental conditions, and diseases, must be considered (Rinttila and Apajalahti 2013). As is the case among all animals, poultry animals' digestive tracts are their most important system for mediating the intake and use of feed. It also bears significance with respect to exposure to environmental pathogens (Yegani and Korver 2008). When the functions of the digestive system are disrupted, digestion and the absorption of feed are disrupted, which endangers the animals' health and performance. Intestinal mucosa functions both as an aid in the absorption of nutrients and as a barrier against negative intestinal content and host interior tissues (Rinttila and Apajalahti 2013). The dynamic balance between the intestines' mucosa layer, epithelium cells, microbiota, and immune cells enables their mucosal functions (Schenk and Mueller 2008). Disruption of this dynamic balance due to a negative composition of feed and/or infectious disease influences the health and performance of poultry animals in a negative way. Antimicrobials have been used at subtherapeutic doses in poultry raising for many years, with the aim of maintaining this balance and improving the animals' development (Filazi et al. 2017). However, since long-term use of antibiotics causes drug-resistant microorganisms to develop, to avoid threats to consumer health and reduce the negative environmental effects, antibiotics have either been prohibited or significantly limited in many developed and developing countries (Filazi et al. 2015). Excluding antibiotics from the diet of poultry animals has caused performance problems in these animals and an increase in incidents of certain poultry animal diseases, such as necrotic enteritis and dysbacteriosis (Huyghebaert et al. 2011). With this in mind, there is an increasing need for alternative natural substances that can create similar effects. In response, various feed and feed additive substances have been developed to help the animals to grow and to have a positive impact on their health. These substances play an important role in changing and maintaining normal physiological functions and animal health and in helping to protect against infectious diseases. Some of these are known as "nutraceutical" (Das et al. 2012).

A. Filazi (✉) · B. Yurdakok-Dikmen
Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, Ankara University, Ankara, Turkey

R. C. Gupta et al. (eds.), *Nutraceuticals in Veterinary Medicine*, https://doi.org/10.1007/978-3-030-04624-8_47

While nutraceuticals are used in humans as an alternative treatment for cancer, diabetes, osteoporosis, and depression, in poultry animals, they are used to regulate the intestinal bacteria population and the immune system and for protecting and treating enteric infections, for correcting intestinal morphology, and for increasing growth performance (Sugiharto 2016).

Generally, it is accepted that the balance between the numbers of useful bacteria and pathogen bacteria (at least 85% of total bacteria are composed of useful bacteria) bears vital importance for the host and that a disruption in this balance damages poultry animals' intestinal health (the morphology of the intestinal wall is disrupted, and immune responses are induced) (Choct 2009). In this case, chickens need more energy and their development slows down. To overcome microbial infections in chickens, inflammatory response is important (Kogut 2013). By contrast, if inflammation cannot be controlled, it can cause intestinal damage and disrupt digestive function (Brisbin et al. 2008). Furthermore, extreme inflammation can disrupt the hosting metabolism (Kogut 2013). In short, intestinal microbiota is important for maintaining immune balance and avoiding inflammation (Lan et al. 2005). Commensal bacteria show anti-inflammatory activities with short-chain fatty acids (SCFAs), which they synthesize to avoid intestinal damage (Brestoff and Artis 2013). With the addition of nutraceuticals to the diet, it is possible to change the population of microorganisms in poultry animals' intestines and to facilitate the growth of useful bacteria (Adil and Magray 2012).

Another effect of nutraceuticals is related to their ability to reduce the undesirable/adverse effects of antimicrobials. Oral or parenteral applications of antibiotics cause a reduction of the population of intestinal microbiota regardless of the site of infection (Sullivan et al. 2001). Thus, since varieties of intestinal bacteria change, irritable intestine syndrome and *Clostridium difficile* infections can be observed (Rashid et al. 2012). Furthermore, considering the undesirable outcomes of systematic antimicrobial use, an intestinal microbiota reservoir of antibiotic-resistant genes can form, and bacteria strains' horizontal gene transfer may increase in the form of carbohydrate composition changes. Then, the population of certain intestinal bacteria increases, and metabolic activities contributing to digestion change can occur in the immune responses of the distal organs. The only way to reduce or eliminate this type of probable negative effect among intestinal microbiota is to use more special products for a specific infection factor (Frei et al. 2015). Within this context, applications such as nutraceuticals are often applied.

The purpose of this section is to discuss those nutraceutical substances used in poultry animals to improve their development, to regulate their immune systems, and to maintain their health. This section also features a discussion of nutraceuticals' probable effect mechanisms.

2 Probiotics

The use of exogenous bacteria (probiotics) to maintain health and avoid disease has been common for years, despite its quite complex outcomes (Huyghebaert et al. 2011). It is well known that the microorganism types used in modern probiotic preparations are quite variable, though mostly *Lactobacillus* (LAB) and *Bifidobacteria* are used. Among these are microorganisms such as *Lactobacillus bulgaricus*, *L. acidophilus*, *L. casei*, *L. helveticus*, *L. lactis*, *L. salivarius*, *L. plantarum*, *Streptococcus thermophilus*, *Enterococcus faecium*, *E. faecalis*, and *Bifidobacterium* spp. (Khaksefidi and Rahimi 2005; Kabir 2009). Some commercial probiotic samples used in poultry are depicted in Table 1.

Due to their beneficial effects, probiotics are commonly used to ward off infection by pathogen microorganisms in various settings, including poultry animals, and to provide a protective and effective means of increasing body weight, regulating feed/food intake and digestibility, improving immune responses, and reducing mortality rates (Armut and Filazi 2012).

Ideal probiotic is not expected to be influenced negatively by normal microflora activities in the digestive system. The beneficial effects in the host environment depend on the quick and easy growth of the probiotic in the environment, be resistant against various pH values and organic acids, regulate intestinal immune system cells, adhere to intestinal cells, and not disrupt intestinal tissues and permeability (Kum and Sekkin 2012; Musa et al. 2009). In this respect, it is important for probiotics to show a direct inhibiting impact on pathogens and/or to have high intestinal colonization resistance. In such cases, probiotics inhibit the effectiveness of pathogen bacteria through the mediation of different mechanisms and reduce the frequency and duration of diseases. They can show their beneficial effects on health via the host's digestive system, immune system, metabolism, and/or mental health. Generally, they reveal their beneficial effects on health as being special for strains and, even if they are from the same type, may not show their effect on one strain but will reveal in another. For this reason, due to similarities in metabolisms, some of their features may be common (Fijan 2014).

The effects of probiotics on poultry animals can be summarized as follows:

1. With their competing and antagonistic effects, they maintain normal intestine microflora against pathogen bacteria.
2. By increasing digestive system activities or bacterial enzyme activities and by reducing ammoniac production, they cause changes in the birds' metabolism.
3. By regulating feed intake, they regulate digestion.
4. By enabling the dissolution of organic substances that bacteria mediate, they contribute to their absorption.

Table 1 Some commercial probiotics samples used in poultry

Some probiotics used in poultry
Bacillus amyloliquefaciens
Bacillus subtilis
Bacillus subtilis + *B. licheniformis*
Bacillus subtilis + *Clostridium butyricum* + *Lactobacillus acidophilus*
Bacillus subtilis + *Saccharomyces cerevisiae*
Bacillus subtilis + *Bifidobacterium*: *animalis*, *bifidum*, *longum* + *Lactobacillus*: *acidophilus*, *casei*, *delbrueckii* subsp. *bulgaricus*, *fermentum*, *plantarum* + *Lactococcus lactis* subsp. *lactis* + *Saccharomyces cerevisiae*+ *Streptococcus thermophilus*
Bacillus: *licheniformis*, *megaterium*, *mesentericus*, *polymyxa*, *subtilis* + *Saccharomyces boulardii* + *Bifidobacterium bifidum* + *Lactobacillus*: *acidophilus*, *bulgaricus*, *plantarum* + *Streptococcus faecium*
Bifidobacterium: *bifidum*, *thermophilus* + *Enterococcus faecium* + *Lactobacillus*: *acidophilus*, *casei*
Bifidobacterium bifidum + *Lactobacillus amylovorus* + *Enterococcus faecium*
Enterococcus faecium
Enterococcus faecium + *Lactobacillus acidophilus*
Enterococcus faecium + *Lactobacillus*: *acidophilus*, *casei*, *plantarum*
Lactobacillus acidophilus + *Streptococcus faecium*
Lactobacillus acidophilus + *Bifidobacterium bifidum* + *Pediococcus faecium*
Lactobacillus: *acidophilus*, *casei*, *plantarum*
Lactobacillus: *paracasei*, *plantarum* + *Lactococcus lactis* + *Saccharomyces cerevisiae*
Lactobacillus: *rhamnosus*, *farciminis*
Lactobacillus salivarius + *Pediococcus parvulus*
Lactobacillus: *plantarum*, *casei* + *Lactococcus lactis* + *Carnobacterium divergens* + *Saccharomyces cerevisiae*
Lactobacillus: *acidophilus*, *casei*, *plantarum*, *lactis* + *Enterococcus faecium* + *Bacillus subtilis*
Lactobacillus fermentum
Lactobacillus: *plantarum*, *delbrueckii* subsp. *bulgaricus*, *acidophilus*, *rhamnosus* + *Bifidobacterium bifidum* + *Streptococcus salivarius* subsp. *thermophilus* + *Enterococcus faecium* + *Aspergillus oryzae* + *Candida pintolepesii*

No trade names given

5. By increasing antibody levels or macrophage activities against pathogen microorganisms or by promoting cytokine secretion, they stimulate the immune system and enable immunomodulation.
6. They inhibit the production of bacterial toxins.
7. By influencing water quality in the intestines in a positive way, they control certain microorganisms, such as protozoon and coccidiosis (Khaksefidi and Rahimi 2005; Lan et al. 2005; Kabir 2009; Brisbin et al. 2010).

It has been stated that, in our time, to enhance the effectiveness of probiotics and to provide a combination of effective features, commercial products with different effect mechanisms comprised of a variety of probiotic combinations could be used. However, we should note that bacterial strains used for probiotic purposes can exhibit differences that are unique for hosts or different types (Musa et al. 2009). The main point to keep in mind with these products is that microorganisms bearing the features of probiotics are not effective against all types of diseases, so to obtain the expected benefits from a special strain for disease, one must know the appropriate dosage (generally 10^9 cfu/kg feed for poultry animals) and period of use. Further, an adjuvant that contains a probiotic microorganism can have side effect risks and prove harmful to some metabolic activities (Mikelsaar 2011).

Our information is still limited as to why bacterial populations become unstable when a single probiotic is added. For this reason, and to strengthen the natural bacteria population and eliminate competitive pathogens, generally complex microbial mixtures are applied. We know that this approach is especially successful in poultry animals. In those animals treated with a mixture of commensal anaerobic bacteria, studies have shown significant reductions in the population of intestinal *Salmonella* (Markowiak and Śliżewska 2018). The most important concern related to probiotics is that of antibiotics resistance in them and the transportation of this property to intestinal bacteria. Competent authorities and industrial institutions have begun to publish reports evaluating the transfer risk of antibiotic resistance to probiotics stating their concerns (Nawaz et al. 2011).

3 Prebiotics

Prebiotics, which are also known as "diet fibers," cause special changes in the composition and/or effectiveness of digestive tract flora and induce beneficial physiological impacts not only in the colon but also throughout the body. They may also reduce the risks associated with various pathogens (Fernandez et al. 2016). A prebiotic substance should stimulate the activity of intestinal bacteria and/or

their development in a selective way and should not be influenced by gastric activities, hydrolyzed by digestive system enzymes, digested in the intestines, or be digested at a significantly low rate (Kolida and Gibson 2011).

Unlike probiotics, prebiotics are nonliving components; host cells do not use them. While probiotics are most effective in the small intestine, prebiotics are most effective in the colon (Kum and Sekkin 2012). Prebiotics bearing these features that are widely used in modern poultry animals include inulin, fructooligosaccharides (FOS), lactulose, oligofructose, galacto-oligosaccharides (GOS), trans-galacto-oligosaccharides (TOS), mannanoligo saccharides (MOS), soy oligosaccharides (SOS), xylo-oligosaccharides (XOS), lactitol, pyrodextrins, and isomalto-oligosaccharides (IMO) (Gibson and Barret 2010; Huyghebaert et al. 2011; Kim et al. 2011; Alloui et al. 2013). Legumes, fruits, and cereals are natural sources of prebiotics. However, the majority of substances used today have been synthesized industrially via chemical and enzymatic methods (Markowiak and Śliżewska 2018).

In the modulation of intestine microbiota, probiotics and prebiotics share common mechanisms (Huyghebaert et al. 2011). Still, the composition and metabolism of prebiotics are significantly different. Prebiotics reveal their osmotic impact when they are not fermented, but they stimulate the activity and/or development of one or limited number of bacteria when they are fermented in a selective way. Then, they increase the formation of SCFAs by reducing the rate of nitrogenous products, reducing the effectiveness of reductant enzymes, and regulating the immune system. As a result of these basic influences, prebiotics increase gas production in the intestines (carbohydrate, methane, and hydrogen) and fecal weight; they help users to avoid constipation, regulate the intestinal habitat, slightly reduce pH levels in the colon, increase the synthesis of proteins providing mineral absorption or increase the ratio of active carrier proteins, and reduce the ratio of toxic, mutagenic, genotoxic metabolites, bile acid, and cancer-developing enzymes (Roberfroid et al. 2010). In addition to the positive effects of prebiotics, it should also be considered that, considering their varying amounts and application periods, different effects can result so that, despite the use of probiotics, in the application of extreme dosages, swelling can occur in the intestines, extreme gas formation can be seen, or diarrhea can be observed, depending on the dose. In short, there can be negative aspects regarding the disposal of specific pathogens, since they cannot show an effect that is as strong as that of antibiotics (Kum and Sekkin 2012).

In poultry animals, prebiotics reduce mortality caused by intestinal pathogens and strengthen hosting defense systems. It is not known exactly how prebiotics achieve this effect. It is likely that this occurs when LAB numbers in the intestines are increased and these bacteria bind to the sites where pathogens will be binding (competitive antagonism) (Alloui et al. 2013). SCFA production caused by prebiotics increases the acidity levels of the intestines, which compresses the pathogens in them. Furthermore, as prebiotics enable the pathogens in the intestine to be removed quickly, they also improve the animal's immune response (Kim et al. 2011). Prebiotics have a direct interaction with the immune cells in the intestines and enable beneficial microorganisms to be colonized more successfully in these places. In this way, they strengthen the immune system in an indirect way (Janardhana et al. 2009).

A study conducted with broiler chickens (Kim et al. 2011) found that the addition of prebiotics to the diet (FOS and MOS used separately) did not change feed consumption, feed efficiency, or death rates, when compared with control groups. However, it did cause an increase in live weights. By contrast, some authors (Biggs et al. 2007; Janardhana et al. 2009) have stated that the addition of prebiotics to the diet revealed no increase in the live weights of broiler chickens. These differences can be explained relative to the types of prebiotics used in each study and the features that are unique to each prebiotic. For example, inulin and FOS are prebiotics preferred for bifidobacteria; for this reason, they avoid the settlement of pathogen bacteria in the hosting intestines, which promotes the growth of bifidobacteria. MOS (for pathogens such as *E. coli* and *Salmonella* spp.) has an effect as a receptor analog; however, it can bind to pathogens and cause their elimination together with the contents of the intestine (Huyghebaert et al. 2011).

Furthermore, some studies have found that prebiotics regulate the barrier features of the intestine's epithelium, which they provide energy for large bowel cells, and that they prevent diarrhea related to antibiotics. They also reduce kidney load by increasing nitrogen extraction from the bowel and reduce low-density lipoprotein and serum triglyceride ratios in the liver while increasing immunoglobulin A production in the immune system. Due to these different effects, prebiotics can be employed in different areas among poultry animals, mainly to increase their feed conversion rate, to create an immunomodulator effect, to improve product quality (such as reducing cholesterol levels in eggs), to regulate intestine microbiota, and to reduce the effectiveness of pathogen microorganisms (Roberfroid et al. 2010).

4 Synbiotics

Synbiotics are defined as a mixture of probiotics and prebiotics which enable for live microbial food/feed additives to remain alive in the digestion tract and which enable their implantation, causing positive influences on the host, which stimulate growth in a selective way and/or which stimulate metabolism of one or limited number of bacteria metabolism

Table 2 Some commercial synbiotic samples used in poultry

Probiotics	Prebiotics
Enterococcus faecium	Fructooligosaccharides
Bifidobacterium animalis, *Pediococcus acidilactici*, *Enterococcus faecium*, *Lactobacillus reuteri*, *Lactobacillus salivarius*	Inulin
Lactobacillus: *acidophilus*, *casei*, *salivarius*, *plantarum*, *rhamnosus*, *brevis*; *Bifidobacterium*: *bifidum*, *lactis*; *Streptococcus thermophilus*	Inulin

No trade names given

(Kolida and Gibson 2011). While prebiotics are selected independently for their complementary effect, probiotics are selected to provide desired beneficial effects in the host. In a synbiotic, a probiotic is chosen to provide beneficial effects in the host, and a prebiotic must be a specific component that stimulates the growth and/or metabolism of a selected probiotic microorganism. In this synergistic effect, the effect of a prebiotic, when taken below the required dosage, is limited, so a low amount of the probiotic is needed. Further, the selected prebiotic should be more interested in the probiotic with which it will be used, and it should be selective in its ability to promote the growth and development of the host. In other words, it is required that the prebiotic support the development of those probiotic microorganisms that are selectively preferred (Kum and Sekkin 2012).

Some authors have argued that probiotics are not activated by prebiotics but that the impact area of probiotics is in the small intestines, while the impact area of prebiotics is in the large intestines. If this is true, we cannot describe a synbiotic impact between the two components but instead must regard this as a synbiotic relationship with their individual mechanisms better described as synergism (Cerezuela et al. 2011). Nonetheless, no matter what kind of relationship there is between them, various studies conducted with animals have shown that combinations of prebiotics and probiotics have beneficial effects in regulating intestine flora, in avoiding septicemia, and in dealing with inflammatory and irritable intestine diseases, colon cancer, bone disease, and certain surgical diseases (Kolida and Gibson 2011). Some commercial synbiotic samples used in poultry today are given in Table 2.

Current data related to the effects of synbiotics on animal health are quite limited. More advanced metagenomic research needs to be conducted in this respect. However, they have been proven to reduce pathogens in the intestines, so probiotics and prebiotics have a synergic effect (Markowiak and Slizewska 2018).

In a study conducted with a probiotic and FOS, researchers found that, when these were used separately, the colonization of *S. enteritidis* in the broiler intestines was reduced, and, when they were combined, they were more effective (Fukata et al. 1999). Furthermore, a combination of *Enterococcus faecium* and FOS has been shown to increase growth in broilers similar to avilamycin, which triggers significant growth in the villus of the small intestines (Awad et al. 2009). In a similar way, when a combination of FOS and *Bacillus subtilis* was applied to 720 broiler chicks, it caused an increase in average daily body weights and improvement in feed efficiency. A reduction in both diarrhea and death rates was also observed in the animals (Li et al. 2008). It has been stated that, when a prebiotic composed of *Bacillus subtilis*, *B. licheniformis*, and *Clostridium butyricum* bacteria and yeast cell wall and xylose oligosaccharides was given to the broilers during their raising period, it increased their body weights, improved feed efficiency, increased breast size, reduced abdominal fat, increased muscle pH, reduced losses during cooking, reduced the formation of malondialdehyde, and reduced the chromium content of leg muscles. In return, this synbiotic both improved the performance of broilers and had a positive effect on meat quality and relevant oxidative features (Cheng et al. 2017). Synbiotics were shown to induce more positive effects on performance parameters than antibiotics; including the reduction of the abdominal fat. As performance parameters are major influence of the industry, synbiotics as antibiotic substitutes are expected to play as a competitive advantage (Toghyani et al. 2011).

5 Exogenous Enzymes

Exogenous enzymes are primarily obtained from *Bacillus subtilis*, *Lactobacillus acidophilus*, and *Streptococcus faecium* bacteria; *Trichoderma longibrachiatum* and *Aspergillus oryzae* fungi; and *Saccharomyces cerevisiae* yeast (Slominski 2011). Various exogenous enzymes, including beta-glucanase, xylanase, amylase, alpha-galactosidase, protease, lipase, and phytase, have been added to poultry animals' feed for many years (Adeola and Cowieson 2011). Exogenous enzymes are significantly important, especially for chickens fed with raw feed materials such as corn and soya, which include anti-nutritive components that make digestion and the absorption of feed in the digestive tract difficult (such as polysaccharides and protease inhibitors) (Yegani and Korver 2008). Further, when these additive substances are inserted into the feed in an extraordinary way to reduce feed costs, they cannot be completely digested or used by the chickens; for this reason, exogenous enzymes may be required (Costa et al. 2008). In such cases, exogenous enzymes address deficiencies in specific endogenous enzymes that are needed to hydrolyze anti-nutritional factors

in the feed and to digest those raw materials that cannot otherwise be digested by chickens (Sugiharto 2016).

When enzyme addition to feed is compared with other animals, it is clear that enzyme additives are preferred primarily among poultry animals. This is because, in poultry animals, the passage speed of feed through the digestive system is short; they do not develop a microbial flora nor produce other enzymes to break down these exogenous enzymes to break down plant cell walls. Similarly, it has been observed that the use of glucanase in feed containing barley and rye and the use of cellulase in rough feed containing high amounts of cellulose are helpful (Slominski 2011).

When xylanase was added to feed, we can observe an increase in the ratios of lactic and organic acids, a reduction in ammoniac production, and a rise in SCFA concentrations (Adeola and Cowieson 2011). Similarly, the intestines of poultry animals fed with a diet containing xylanase display an increase in LAB numbers, coliform, and *Salmonella* (Nian et al. 2011). By contrast, other studies have revealed that the addition of xylanase does not change the numbers of LAB and coliform in the jejunum of poultry animals (Yang et al. 2008). These differences may be attributable to enzyme activity that is influenced by various factors, such as the temperature, moisture, feed composition, age and breed of the animal, and—chiefly—pH level of the intestinal channel (Yang et al. 2008; Adeola and Cowieson 2011).

The combined use of probiotics with enzymes enables the polysaccharides of cereal grains in the cell wall that are not being digested to become splintered (especially wheat and barley), so that intestinal viscosity is increased by the reduction in polysaccharides as the digestibility of nutrients and its metabolic energy values are improved. Further, it has been found that exogenous enzymes reduce the development and fermentation of harmful microorganisms in the intestine and discourage colonization. In this respect, enzymes can be considered an alternative feed additive substance to antibiotics, which are commonly used to regulate growth and development in poultry raising (Kum and Sekkin 2012).

In order for exogenous enzymes to show their effects, chickens must not to be influenced by endogenous digestion enzymes in the digestive tract or at low pH levels (<4) (Sugiharto 2016). To derive the maximum benefit from enzymes, it is recommended that more than one enzyme should be used as a combination since there might be a presence of different types and varieties of anti-nutritive components in feed. In short, the beneficial effects of enzyme combinations are dependent on feed composition (Adeola and Cowieson 2011).

6 Organic Acids

Organic acids are found widely in nature as normal components of plant and animal tissues. They also commonly form in the cecum of poultry animals as a result of the microbial fermentation of carbohydrates (Huyghebaert et al. 2011). Various organic acids have different physical and chemical features that can be used by adding them to feed or drinking water. This can be done either alone or in combination with one another. A combined use of organic acids has been shown to be more effective than using them alone (Menconi et al. 2014).

Certain organic acids (such as formic and propionic acid) have been used for many years to protect feed; they and their salts are referred to as "feed preservative" in European Union countries. Those with short chains (C1–C7) have antimicrobial effects. These include simple monocarboxylic acids, such as formic, acetic, propionic, and butyric acids; carboxylic acids with hydroxyl, such as lactic, malic, tartaric, and citric acids; and carboxylic acids with double bonds, such as fumaric and sorbic acids (Shahidi et al. 2014).

Organic acids have weak acidic features and they can partially become dissociated. Some of them can exist as sodium, potassium, or calcium salts. The advantages of salts are that they are generally odorless and less corrosive, they can dissolve more in water, and they are solid and less volatile than acids during the preparation of feed, so they can be easily processed (Huyghebaert et al. 2011). Organic acids are widely used in poultry animals due to their antimicrobial features, their ability to regulate the intestines, and their positive effects on bowel health, nutrition use, performance, egg efficiency, and quality (Khan and Iqbal 2016).

Some studies have observed a significant increase in crypt depth and villus height, width, and area in the small intestines of broiler chickens whose feed received organic acid additives, either alone or in combination (Adil et al. 2010; Kum et al. 2010; Rodríguez-Lecompte et al. 2012). Similar results have also been obtained related to the salts of organic acids (Paul et al. 2007). Increase in villus height causes bowel epithelium, which serves as a natural barrier against the strengthening of toxic substances and pathogen bacteria in the intestine. In this way, organic acids or their salts reduce the colonization of intestinal pathogens, due to their antimicrobial effects and their ability to increase villus height. In addition, they increase digestion and the absorption of nutrient (Iji and Tivey 1998). In some studies (Adil et al. 2010), when organic acids were added to broiler chickens' diets, there was increase in the LAB numbers in ileum and cecum, a reduction in the numbers of *Enterobacteriaceae* and *Salmonella* spp., and an increase in body weight and feed efficiency. The application of organic acids in poultry animals leads to improvement in performance and health when they are used at both high and low dosages, and they can cause a reduction in the height of the bowel villus and in crypt depth (Smulikowska et al. 2009).

With the addition of organic acids to drinking water, researchers have observed a reduction in pathogens in the stomach/proventriculus and in the water, an improvement in bowel microflora, an increase in feed digestion, and an

acceleration in growth (Hamed and Hassan 2013). Similarly, it has been shown that organic acid mixtures (fumaric acid, calcium format, calcium propionate, potassium sorbate, calcium butyrate, calcium lactate, and hydrogenated vegetable oil) are more effective than polypeptide structured antibiotics as a growth factor for reducing *E. coli* and *Salmonella* spp. in the intestines (Hassan et al. 2010).

The antimicrobial features of organic acids depend on their being transformed from not being dissociated into a dissociated form, their pKa values, and their hydrophobic features. Organic acids pass from the semipermeable membrane of bacteria to the cell cytoplasm in dissociated form in a freeway (Van Immerseel et al. 2006). Thus, in the cell where pH is nearly 7, they depress bacterial enzymes (such as decarboxylases and catalases), and nutrition transport systems are dissociated. As the bacterial cell wall normally has a lipid structure, the hydrophobicity of acid enables it to show its antimicrobial activity. In this way, hydrophobic organic acids bind with lipid materials and disrupt the activities of the bacteria (Huyghebaert et al. 2011).

Not all organic acids are effective on microflora. Each acid has a unique antimicrobial activity spectrum. pKa values of most organic acids with antimicrobial activity vary between 3 and 5. Among these, while lactic acid is more effective on bacteria, acetic, formic, and propionic acid have wider antimicrobial activity, including yeast and fungus. Substances such as sorbic and fumaric acid mainly have antifungal effects (Kum et al. 2010). It has been demonstrated that organic acid salts (such as ammonium formate and calcium propionate) reduced coliform numbers in broilers, but they do not affect clostridiums (Paul et al. 2007). Furthermore, calcium propionate depressed the fungi in feed better than ammonium formate, due to the antifungal impact of propionic acid or propionate (Zha and Cohen 2014). Another study showed that adding potassium diformate to the diet with a ratio of 0.45% significantly reduced mortality caused by necrotic enteritis (Mikkelsen et al. 2009).

Sodium butyrate (in two different forms, coated with herbal oil and not being covered with it) can deter *Salmonella* colonization in the digestive tract of broilers, but only its form covered with herbal oil can reduce *Salmonella* colonization in the liver; thus, as related to *Salmonella*, the form that is covered with herbal oil is more effective than the uncoated form (Fernández-Rubio et al. 2009). With this in mind, we can predict that herbal oils protecting sodium butyrate are more durable against acidic pH, as they allow some part of the butyrate to go further into the bowel. Some authors (Hamed and Hassan 2013) have asserted that most fatty acids with short chains, such as propionic and formic acid, are metabolized and absorbed from the upper digestive tract of poultry animals, so that they change the microflora of hosts in the lower part of digestive tract in a limited way. For this reason, some researchers (Fernández-Rubio et al. 2009; Van Immerseel et al. 2006) have proposed that fatty acids with short chains should be given in a microencapsulation form, in order for them to be effective in the lower parts of digestive tract as well. A protective lipid matrix that is used for microencapsulation enables organic acids to be effective along the whole digestive tract. For example, the addition of an organic acid, prepared in an encapsulation form, to the diet with a ratio of 0.2% caused useful microflora (LAB) in the bowel of poultry animals to increase and the levels of harmful microflora to be reduced (Gheisari et al. 2007).

Among SCFAs, butyrate has the greatest bactericidal effect against acid-sensitive bacteria such as *E.coli* and *Salmonella* (Kwon and Ricke 1998). In in vitro studies, depending on the carbon chain length and pH values of organic acids and on the condition that there is a sufficient amount of acid molecules in the environment not being dissociated and that they remain with bacteria for a long time, it has been suggested that bacteria pass easily from the cell membrane; reduce intracellular pH levels; disrupt the intracellular amino acid metabolism, cytoplasmic membrane structure, proteins, and electron carriage system; and reduce intracellular ATP production and revealed bactericide/static effects in return. Since this effect of organic acids originates from anion accumulation, which occurs during their dissolution, it has no impact on bacteria that are acid-resistant (Patten and Waldroup 1988).

7 Polyunsaturated Fatty Acids

The two most important main types of polyunsaturated fatty acids (PUFAs) are omega-3 and omega-6 fatty acids. Neither of these can be produced by the body; they must be acquired from the diet. For this reason, they are classified, for people, as "essential fatty acids." Examples of omega-3 fatty acids are α-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA). Examples of omega-6 acids include linolenic acid (LA) and arachidonic acid (AA). According to a study (Sioen et al. 2006), adding PUFAs such as omega-3 regularly causes beneficial effects related to physiological functions. Further, the PUFA content in modern diets is low, with respect to omega-3 fatty acid ions. Thus, the omega-6:omega-3 fatty acid ratio is high. This imbalance between omega-3 and omega-6 is an important factor in the pathogenesis of various diseases, such as cancer and inflammatory and autoimmune diseases. By proactively adding omega-3 fatty acids to the diet, the ratio of omega-6:omega-3 can be reduced (Simopoulos 2004). Poultry meat is known to be the main source of PUFA in humans' food (Sioen et al. 2006), and the addition of fish by-products (López-Ferrer et al. 2001a) and flax seed oil (López-Ferrer et al. 2001b) to the feed of poultry animals has increased omega-3 fatty acids in poultry meat (especially ALA).

However, since these products increase the lipid oxidation of meat, they can lead to a reduction in flavor (Bou et al. 2001). In one study (Ponte et al. 2008), researchers demonstrated that feeding free-range chickens green plants could cause an increase in live weights.

It has been found that, in poultry animals, fish oil and corn oil are widely used as omega-3 and -6 sources. Fish oil increases immune responses, and corn oil reduced immune response (Yang and Guo 2006). Furthermore, in broilers whose feed received omega-3 (a tuna oil, sunflower oil, and palm oil combination) additives, increases were seen in spleen weights, infectious bronchitis disease, Newcastle diseases, antibody titers, interleukin-2, and interferon-γ concentrations. Immune responses in poultry animals were enhanced in return (Maroufyan et al. 2012). However, in another study (Al-Khalifa et al. 2012), the opposite was found: the addition of omega-3 in the diet (via fish oil) reduced immune responses in broiler chicken (phagocytosis and lymphocyte proliferation).

Conjugated linoleic acid (CLA) is another PUFA type used to support poultry feed and increase immune responses in chickens (Zhang et al. 2005). When CLA is added to the diet, it promotes the growth of immune tissues such as thymus and bursa in chickens and stimulated T lymphocyte proliferation while increasing antibody production. These studies suggest that unexpected outcomes could result, due to the biological differences (gender, age, and breed), type of essential fatty acid introduced, diet composition, and balance difference between omega-3 and omega-6 fatty acids in the diet with regard to the factors influencing poultry animals' immune response (He et al. 2007).

Finally, omega-3 has shown an antimicrobial effect in an in vitro environment (Kankaanpaa et al. 2001). Still, some researchers argue that omega-3 and CLA do not have much effect on general microbiota in the chicken intestine (Chanuwat et al. 2011).

Information about increases in the performance of poultry animals due to PUFAs is also debatable. While some authors (Roy et al. 2008; Chanuwat et al. 2011) have asserted that the addition of omega-3 (eicosapentaenoic and docosahexaenoic) or CLA to the diet supports the growth of poultry animals, others (Zhang et al. 2005; Cho et al. 2013) have asserted that these do not influence the growth performance. Similarly, different types and sources of PUFAs, the feed composition of animals, and their biological variations can explain these debatable outcomes.

8 Phytobiotics

Phytobiotics are natural bioactive components that can be extracted from various herbal sources such as plants and spices (Windisch et al. 2008). Active components of phytobiotics are generally terpenoids, phenols, glycosides, alkaloids, and similar secondary components (Huyghebaert et al. 2011). Phytobiotics are divided into four classes related to their source and processing features: (1) plants (blooming, nonwoody, and nonpermanent plants), (2) spices (plants with a dense odor or flavor that are generally added to human food), (3) essential oils (volatile lipophilic components), and (4) oleoresins (extracts obtained from non-watery solutions) (Windisch et al. 2008; Yang et al. 2009).

In phytobiotics, the content and chemical composition of the active substance varies according to the plant part that is used (seed, leaf, etc.), geographical sources, and harvesting season (Windisch et al. 2008). Several studies have shown that phytobiotics promote growth and have antimicrobial, antioxidant, and anti-inflammatory properties. However, when we evaluate these studies closely, we can notice that some outcomes are contradictory (Mohammadi Gheisar and Kim 2018).

Some studies found that the addition of a phytogenic mixture to broiler chickens and meat-type duck feed at a ratio of 0.075% led to increases in body weight and feed efficiency (Mohammadi Gheisar et al. 2015a, b). According to the authors, phytobiotics show antimicrobial activity, stimulate the secretion of digestion enzymes, and increase the flavor of feed, which increases their consumption in return. In this way, an increase is seen in poultry animals' growth performance. However, other studies have asserted that the addition of phytobiotics to broiler chickens and laying hens' feed caused a significant reduction in feed intake (Maass et al. 2005; Roth-Maier et al. 2005). There was an increase in trypsin, maltase, and pancreatic amylase activities of broilers (Jang et al. 2007) and in the mucosa secretion of the intestines (Jamroz et al. 2006), with the addition of essential oils to their diet. This reduced the adhesion of pathogens and provided microbial balance in the animals' bowels (Brenes and Roura 2010).

The antimicrobial effects of phytobiotics have been the subject of many studies (Burt 2004; Si et al. 2006; Panghal et al. 2011; Giannenas et al. 2013). Most of them have shown that phenolic components, such as thymol, carvacrol, phenylpropane, limonene, geraniol, and citronellal, are the most effective antimicrobials. Antimicrobial impact of phytobiotics was found to be dependent on the settlement place of the functional hydroxyl and alkyl groups in their structures (Yang et al. 2015). For example, certain terpenes (such as carvacrol and thymol) have similar antimicrobial effects, but their influence against Gram-negative or Gram-positive bacteria differed relative to the settlement place of one or more functional groups in their molecules (Salehi et al. 2018). The Labiatae plant family, which includes plants such as thyme, oregano, and sage, the most well-known phytobiotics, has attracted the most interest in this regard. Hydrophobic essential oils adhere to the cell membranes of pathogens and break up the membrane structures, leading to ion loss in bacteria in return (Burt 2004).

Various substances that are not phenolic but which are obtained from limonene and *Sanguinaria canadensis* also

have antimicrobial effects (Newton et al. 2002; Burt 2004). In some studies of broiler chickens, the effectiveness of essential oils against *E. coli* and *Clostridium perfringens* has been revealed (Jamroz et al. 2006; Mitsch et al. 2004), and certain phytobiotics have also proven effective against *Eimeria* factors (Oviedo-Rondon et al. 2006). Phytobiotics also increased the microbial hygiene of carcass. For example, the addition of oregano essential oil to the diet at a ratio of 0.1% had a beneficial impact on the microbial load of specific pathogens or total live bacteria in broiler carcasses (Aksit et al. 2006). However, current data are significantly limited in revealing phytobiotics as reliable for increasing carcass hygiene.

The antioxidant features of phytobiotics are another characteristic that attracts a great deal of attention. Plants such as rosemary, oregano, and thyme, which are part of Labiatae plant family, and their extracts have high antioxidant effects (Brenes and Roura 2010). This impact has been revealed in meat-type ducks whose diets received thyme additives (Mohammadi Gheisar et al. 2015b) and in broiler chickens to whose diets *Artemisia annua* was added (Cherian et al. 2013). These phytobiotics can have a positive effect on certain antioxidant enzymes, such as glutathione peroxidase and superoxide dismutase, which help to regulate the lipid metabolism in animals in return (Franz et al. 2010). Other studies have shown that plant varieties such as ginger, curcuma, anise, and coriander; spices such as black pepper (*Piper nigrum*), red pepper (*Capsicum annuum* L.), and chili (*Capsicum frutescens*); and plants rich with flavonoids (such as green tea) and anthocyanins (such as various fruits) have antioxidant activities (Nakatani 2000; Wei and Shibamoto 2007; Yatao et al. 2018). The use of most of these plants is limited in animal feeding, due to their sharp odor and taste, which are attributed to their active substances.

While some authors (Abd El-Ghany and Ismail 2014; Diaz-Sanchez et al. 2015) claim that phytobiotics accelerate development in broilers in ways similar to antibiotics, others (Karimi et al. 2010; Al-Mufarrej 2014) claim that they (thyme and black cumin (*Nigella sativa* L.)) do not influence broilers' growth. The type and dose of phytobiotics used in these studies can explain the differences in these diets.

Regarding the effects of phytobiotics used as feed additive substances on the intestinal health and growth performance of poultry animals, these substances and their contents vary depending on biological factors (plant types, places where they are raised, and harvesting conditions), production methods (extraction/distillation, stabilization), and storage conditions (light, temperature, oxygen pressure, and period).

9 Concluding Remarks and Future Directions

Following the prohibition of antibiotics in poultry animal as a growth factor, the need for alternative feed additive substances increased. Feed additive substances, or nutraceuticals, are now being used with the aim of accelerating growth in the poultry sector, avoiding disease, and regulating animals' immune systems. Nutraceuticals provide significant hope in these respects. However, their use in the treatment of diseases and their impact mechanisms remain debatable. In order to feed the world's population, which is constantly increasing, and to produce higher quality and less expensive animal feed, we must increase the varieties and sources of nutraceuticals. Further studies on nutraceuticals are needed among large poultry flocks before they can be used in the commercial poultry industry.

References

Abd El-Ghany WA, Ismail M (2014) Tackling of experimental colisepticaemia in broiler chickens using phytobiotic essential oils and antibiotic alone or in combination. Iran J Vet Res 15(2): 110–115

Adeola O, Cowieson AJ (2011) Board-invited review: opportunities and challenges in using exogenous enzymes to improve nonruminant animal production. J Anim Sci 89:3189–3218

Adil S, Magray SN (2012) Impact and manipulation of gut microflora in poultry: a review. J Anim Vet Adv 11:873–877

Adil S, Banday T, Bhat GA et al (2010) Effect of dietary supplementation of organic acids on performance, intestinal histomorphology, and serum biochemistry of broiler chicken. Vet Med Int 2010: 479485

Aksit M, Goksoy E, Kok F et al (2006) The impacts of organic acid and essential oil supplementations to diets on the microbiological quality of chicken carcasses. Arch Geflügelk 70(4):168–173

Al-Khalifa H, Givens DI, Rymer C et al (2012) Effect of n-3 fatty acids on immune function in broiler chickens. Poult Sci 91(1):74–88

Alloui MN, Szczurek W, Swiatkiewicz S (2013) The usefulness of prebiotics and probiotics in modern poultry nutrition: review. Ann Anim Sci 13(1):17–32

Al-Mufarrej SI (2014) Immune-responsiveness and performance of broiler chickens fed black cumin (*Nigella sativa* L.) powder. J Saudi Soc Agric Sci 13(1):75–80

Armut M, Filazi A (2012) Evaluation of the effects produced by the addition of growth-promoting products to broiler feed. Turk J Vet Anim Sci 36(4):330–337

Awad WA, Ghareeb K, Abdel-Raheem S et al (2009) Effects of dietary inclusion of probiotic and synbiotic on growth performance, organ weights, and intestinal histomorphology of broiler chickens. Poult Sci 88(1):49–56

Biggs P, Parsons CM, Fahey GC (2007) The effects of several oligosaccharides on growth performance, nutrient digestibilities, and caecal microbial populations in young chicks. Poult Sci 86(1): 2327–2336

Bou R, Guardiola F, Grau A et al (2001) Influence of dietary fat source, α-tocopherol, and ascorbic acid supplementation on sensory quality of dark chicken meat. Poult Sci 80(6):800–807

Brenes A, Roura E (2010) Essential oils in poultry nutrition: main effects and modes of action. Anim Feed Sci Technol 158(1–2):1–14

Brestoff JR, Artis D (2013) Commensal bacteria at the interface of host metabolism and the immune system. Nat Immunol 14(7):676–684

Brisbin JT, Gong J, Sharif S (2008) Interactions between commensal bacteria and the gut-associated immune system of the chicken. Anim Health Res Rev 9:101–110

Brisbin JT, Gong J, Parvizi P et al (2010) Effects of lactobacilli on cytokine expression by chicken spleen and caecal tonsil cells. Clin Vaccine Immunol 17(9):1337–1343

Burt S (2004) Essential oils: their antibacterial properties and potential applications in foods—a review. Int J Food Microbiol 94(3): 223–253

Cerezuela R, Meseguer J, Esteban MA (2011) Current knowledge in synbiotic use for fish aquaculture: a review. J Aquac Res Dev S1:008

Chanuwat T, Wongsuthavas S, Smerjai B et al (2011) Effect of supplementation of conjugated linoleic acid in diets on growth performance and total lactic bacteria in small intestine of broiler. J Agric Sci Technol A 1:1141–1143

Cheng Y, Chen Y, Li X et al (2017) Effects of synbiotic supplementation on growth performance, carcass characteristics, meat quality and muscular antioxidant capacity and mineral contents in broilers. J Sci Food Agric 97(11):3699–3705

Cherian G, Orr A, Burke IC et al (2013) Feeding Artemisia annua alters digesta pH and muscle lipid oxidation products in broiler chickens. Poult Sci 92(4):1085–1090

Cho S, Ryu C, Yang J et al (2013) Effect of conjugated linoleic acid feeding on the growth performance and meat fatty acid profiles in broiler: meta-analysis. Asian Australas J Anim Sci 26(7):995–1002

Choct M (2009) Managing gut health through nutrition. Br Poult Sci 50 (1):9–15

Costa FGP, Goulart CC, Figueiredo DF et al (2008) Economic and environmental impact of using exogenous enzymes on poultry feeding. Int J Poult Sci 7(4):311–314

Das L, Bhaumik E, Raychauduri U et al (2012) Role of nutraceuticals in human health. J Food Sci Technol 49(2):173–183

Diaz-Sanchez S, D'Souza D, Biswas D et al (2015) Botanical alternatives to antibiotics for use in organic poultry production. Poult Sci 94(6):1419–1430

Fernández J, Redondo-Blanco S, Gutiérrez-del-Río I et al (2016) Colon microbiota fermentation of dietary prebiotics towards short-chain fatty acids and their roles as anti-inflammatory and antitumour agents: a review. J Funct Foods 25:511–522

Fernández-Rubio C, Ordóñez C, Abad-González J et al (2009) Butyric acid-based feed additives help protect broiler chickens from Salmonella enteritidis infection. Poult Sci 88(5):943–948

Fijan S (2014) Microorganisms with claimed probiotic properties: an overview of recent literature. Int J Environ Res Public Health 11(5):4745–4767

Filazi A, Yurdakok-Dikmen B, Kuzukiran O (2015) Antibiotic resistance in poultry. Turkiye Klinikleri J Vet Sci Pharmacol Toxicol-Special Topics 1(2):42–51

Filazi A, Yurdakok-Dikmen B, Kuzukiran O (2017) The usage of antibacterial agents in poultry. Turkiye Klinikleri J Vet Sci Pharmacol Toxicol-Special Topics 3(3):181–187

Franz C, Baser KHC, Windisch W (2010) Essential oils and aromatic plants in animal feeding – a European perspective. A review. Flavour Frag J 25(5):327–340

Frei R, Akdis M, O'Mahony L (2015) Prebiotics, probiotics, synbiotics, and the immune system: experimental data and clinical evidence. Curr Opin Gastroenterol 31(2):153–158

Fukata T, Sasai K, Miyamoto T et al (1999) Inhibitory effects of competitive exclusion and fructooligosaccharide, singly and in combination, on Salmonella colonization of chicks. J Food Prot 62(3): 229–233

Gheisari AA, Heidari M, Kermanshahi RK, et al (2007) Effect of dietary supplementation of protected organic acids on ileal microflora and protein digestibility in broiler chickens. Proceedings of the 16th European symposium on poultry nutrition, Strasbourg, France, pp 519–522

Giannenas I, Bonos E, Christaki E et al (2013) Essential oils and their applications in animal nutrition. Med Aromatic Plants 2:1–12

Gibson PR, Barrett JS (2010) The concept of small intestinal bacterial overgrowth in relation to functional gastrointestinal disorders. Nutrition 26(11–12):1038–1043

Hamed DM, Hassan AMA (2013) Acids supplementation to drinking water and their effects on Japanese quails experimentally challenged with Salmonella enteritidis. Res Zool 3(1):15–22

Hassan HMA, Mohamed MA, Youssef AW et al (2010) Effect of using organic acids to substitute antibiotic growth promoters on performance and intestinal microflora of broilers. Asian-Australas J Anim Sci 23(10):1348–1353

He XE, Zhang H, Yang X et al (2007) Modulation of immune function by conjugated linoleic acid in chickens. Food Agric Immunol 18 (3–4):169–178

Huyghebaert G, Ducatelle R, Van Immerseel F (2011) An update on alternatives to antimicrobial growth promoters for broilers. Vet J 187(2):182–188

Iji PA, Tivey DR (1998) Natural and synthetic oligosaccharide in broiler chicken diet. Worlds Poult Sci J 54(2):129–143

Jamroz D, Wertelecki T, Houszka M et al (2006) Influence of diet type on the inclusion of plant origin active substances on morphological and histochemical characteristics of the stomach and jejunum walls in chicken. J Anim Physiol Anim Nutr 90(5-6):255–268

Janardhana V, Broadway MM, Bruce MP et al (2009) Prebiotics modulate immune responses in gut-associated lymphoid tissue of chickens. J Nutr 139(7):1404–1409

Jang IS, Ko YH, Kang SY et al (2007) Effect of commercial essential oils on growth performance, digestive enzyme activity and intestinal microflora population in broiler chickens. Anim Feed Sci Technol 134(3-4):304–315

Kabir SML (2009) The role of probiotics in the poultry industry. Int J Mol Sci 10(8):3531–3546

Kankaanpaa PE, Salminen SJ, Isolauri E et al (2001) The influence of polyunsaturated fatty acids on probiotic growth and adhesion. FEMS Microbiol Lett 194(2):149–153

Karimi A, Yan F, Coto C et al (2010) Effect of level and source of oregano leaf in starter diets for broiler chicks. J Appl Poult Res 19(2):137–145

Khaksefidi A, Rahimi S (2005) Effect of probiotic inclusion in the diet of broiler chickens on performance, feed efficiency and carcass quality. Asian-Australas J Anim Sci 18(8):1153–1156

Khan SH, Iqbal J (2016) Recent advances in the role of organic acids in poultry nutrition. J Appl Anim Res 44(1):359–369

Kim G-B, Seo YM, Kim CH et al (2011) Effect of dietary prebiotic supplementation on the performance, intestinal microflora, and immune response of broilers. Poult Sci 90(1):75–82

Kogut MH (2013) The gut microbiota and host innate immunity: regulators of host metabolism and metabolic diseases in poultry? J Appl Poult Res 22(3):637–646

Kolida S, Gibson GR (2011) Synbiotics in health and disease. Annu Rev Food Sci Technol 2:373–393

Kum C, Sekkin S (2012) Alternative approaches to antibiotics. Turkiye Klinikleri J Vet Sci 3(3):84–116

Kum S, Eren U, Onol A et al (2010) Effects of dietary organic acid supplementation on the intestinal mucosa in broilers. Rev Med Vet 161(10):463–468

Kwon YM, Ricke SC (1998) Induction of acid resistance of Salmonella typhimurium by exposure to short chain fatty acids. Appl Environ Microbiol 64(9):3458–3463

Lan Y, Verstegen MWA, Tamminga S et al (2005) The role of the commensal gut microbial community in broiler chickens. Worlds Poult Sci J 61(1):95–104

Li X, Qiang L, Liu, Xu C (2008) Effects of supplementation of fructo-oligosaccharide and/or Bacillus subtilis to diets on performance and on ıntestinal microflora in broilers. Arch Anim Breed 51:64–70

López-Ferrer S, Baucells MD, Barroeta AC et al (2001a) n-3 enrichment of chicken meat. 1. Use of very long-chain fatty acids in chicken diets and their influence on meat quality: fish oil. Poult Sci 80(6): 741–752

López-Ferrer S, Baucells MD, Barroeta AC et al (2001b) n-3 enrichment of chicken meat. 2. Use of precursors of long-chain polyunsaturated fatty acids: linseed oil. Poult Sci 80(6):753–761

Maass N, Bauer J, Paulicks BR et al (2005) Efficiency of Echinacea purpurea on performance and immune status in pigs. J Anim Physiol Anim Nutr 89(7–8):244–252

Markowiak P, Śliżewska K (2018) The role of probiotics, prebiotics and synbiotics in animal nutrition. Gut Pathog 10:21

Maroufyan E, Kasim A, Ebrahimi M et al (2012) Omega-3 polyunsaturated fatty acids enrichment alters performance and immune response in infectious bursal disease challenged broilers. Lipids Health Dis 11:15

Menconi A, Kuttappan VA, Hernandez-Velasco X et al (2014) Evaluation of a commercially available organic acid product on body weight loss, carcass yield, and meat quality during preslaughter feed withdrawal in broiler chickens: a poultry welfare and economic perspective. Poult Sci 93(2):448–455

Mikelsaar M (2011) Human microbial ecology: lactobacilli, probiotics, selective decontamination. Anaerobe 17(6):463–467

Mikkelsen LL, Vidanarachchi JK, Olnood CG et al (2009) Effect of potassium diformate on growth performance and gut microbiota in broiler chickens challenged with necrotic enteritis. Br Poult Sci 50(1):66–75

Mitsch P, Zitterl-Eglseer K, Köhler B et al (2004) The effect of two different blends of essential oil components on the proliferation of *Clostridium perfringens* in the intestines of broiler chickens. Poult Sci 83(4):669–675

Mohammadi Gheisar M, Kim IH (2018) Phytobiotics in poultry and swine nutrition – a review. Ital J Anim Sci 17(1):92–99

Mohammadi Gheisar M, Hosseindoust A, Kim IH (2015a) Evaluating the effect of microencapsulated blends of organic acids and essential oils in broiler chickens diet. J Appl Poult Res 24(1): 511–519

Mohammadi Gheisar M, Im YW, Lee HH et al (2015b) Inclusion of phytogenic blends in different nutrient density diets of meat-type ducks. Poult Sci 94(12):2952–2958

Musa HH, Wu SL, Zhu CH et al (2009) The potential benefits of probiotics in animal production and health. J Anim Vet Adv 8(2): 313–321

Nakatani N (2000) Phenolic antioxidants from herbs and spices. Biofactors 13(1–4):141–146

Nawaz M, Wang J, Zhou A et al (2011) Characterization and transfer of antibiotic resistance in lactic acid bacteria from fermented food products. Curr Microbiol 62(3):1081–1089

Newton SM, Lau C, Gurcha SS et al (2002) The evaluation of forty-three plant species for in vitro antimycobacterial activities; isolation of active constituents from Psoralea corylifolia and Sanguinaria canadensis. J Ethnopharmacol 79(1):57–67

Nian F, Guo YM, Ru YJ et al (2011) Effect of exogenous xylanase supplementation on the performance, net energy and gut microflora of broiler chickens fed wheat-based diets. Asian-Australas J Anim Sci 24(3):400–406

Oviedo-Rondon EO, Hume ME, Hernandez C et al (2006) Intestinal microbial ecology of broilers vaccinated and challenged with mixed Eimeria species, and supplemented with essential oil blends. Poult Sci 85(5):854–860

Panghal M, Kaushal V, Yadav JP (2011) *In vitro* antimicrobial activity of ten medicinal plants against clinical isolates of oral cancer cases. Ann Clin Microbiol Antimicrob 10:21

Patten JD, Waldroup PW (1988) Use of organic acids in broiler diets. Poult Sci 67(8):1178–1182

Paul SK, Halder G, Mondal MK et al (2007) Effect of organic acid salt on the performance and gut health of broiler chicken. J Poult Sci 44(4):389–395

Ponte PI, Rosado CM, Crespo JP et al (2008) Pasture intake improves the performance and meat sensory attributes of free-range broilers. Poult Sci 87(1):71–79

Rashid MU, Weintraub A, Nord CE (2012) Effect of new antimicrobial agents on the ecological balance of human microflora. Anaerobe 18(2):249–253

Rinttila T, Apajalahti J (2013) Intestinal microbiota and metabolites – implications for broiler chicken health and performance. J Appl Poult Res 22(3):647–658

Roberfroid M, Gibson GR, Hoyles L et al (2010) Prebiotic effects: metabolic and health benefits. Br J Nutr 104(Suppl 2):S1–S63

Rodríguez-Lecompte JC, Yitbarek A, Brady J et al (2012) The effect of microbial nutrient interaction on the immune system of young chicks after early probiotic and organic acid administration. J Anim Sci 90(7):2246–2254

Roth-Maier DA, Böhmer BM, Maass N et al (2005) Efficiency of Echinacea purpurea on performance of broilers and layers. Arch Geflügelk 69(3):123–127

Roy R, Singh S, Pujari S (2008) Dietary role of omega-3 polyunsaturated fatty acid (PUFA): a study with growing chicks, *Gallus domesticus*. Int J Poult Sci 7(4):360–367

Salehi B, Mishra AP, Shukla I et al (2018) Thymol, thyme, and other plant sources: health and potential uses. Phytother Res. https://doi.org/10.1002/ptr.6109

Schenk M, Mueller C (2008) The mucosal immune system at the gastrointestinal barrier. Best Pract Res Clin Gastroenterol 22(3):391–409

Shahidi S, Maziar Y, Delaram NZ (2014) Influence of dietary organic acids supplementation on reproductive performance of freshwater Angelfish (Pterophyllum scalare). Glob Vet 13:373–377

Si W, Gong J, Tsao R et al (2006) Antimicrobial activity of essential oils and structurally related synthetic food additives towards selected pathogenic and beneficial gut bacteria. J Appl Microbiol 100: 296–305

Simopoulos AP (2004) Omega-6/omega-3 essential fatty acid ratio and chronic diseases. Food Rev Int 20:77–90

Sioen IA, Pynaert I, Matthys C et al (2006) Dietary intakes and food sources of fatty acids for Belgian women, focused on n-6 and n-3 polyunsaturated fatty acids. Lipids 41:415–422

Slominski BA (2011) Recent advances in enzymes for poultry diets. Poult Sci 90(9):2013–2023

Smulikowska S, Czerwiński J, Mieczkowska A et al (2009) The effect of fat-coated organic acid salts and a feed enzyme on growth performance, nutrient utilization, microflora activity, and morphology of the small intestine in broiler chickens. J Anim Feed Sci 18:478–489

Sugiharto S (2016) Role of nutraceuticals in gut health and growth performance of poultry. J Saudi Soc Agric Sci 15(2):99–111

Sullivan A, Edlund C, Nord CE (2001) Effect of antimicrobial agents on the ecological balance of human microflora. Lancet Infect Dis 1 (2):101–114

Toghyani M, Toghyani M, Tabeidian SA (2011) Effect of probiotic and prebiotic as antibiotic growth promoter substitutions on productive and carcass traits of broiler chicks. International Conference on Food Engineering and Biotechnology, IPCBEE 9:82–86

Van Immerseel F, Russell JB, Flythe MD et al (2006) The use of organic acids to combat Salmonella in poultry: a mechanistic explanation of the efficacy. Avian Pathol 35(3):182–188

Wei A, Shibamoto T (2007) Antioxidant activities and volatile constituents of various essential oils. J Agric Food Chem 55(5): 1737–1742

Windisch W, Schedle K, Plitzner C et al (2008) Use of phytogenic products as feed additives for swine and poultry. J Anim Sci 86(14 Suppl):E140–E148

Yang X, Guo Y (2006) Modulation of intestinal mucosal immunity by dietary polyunsaturated fatty acids in chickens. Food Agric Immunol 17(2):129–137

Yang Y, Iji PA, Kocher A et al (2008) Effects of xylanase on growth and gut development of broiler chickens given a wheat-based diet. Asian-Australas J Anim Sci 21(11):1659–1664

Yang Y, Iji PA, Choct M (2009) Dietary modulation of gut microflora in broiler chickens: a review of the role of six kinds of alternatives to in-feed antibiotics. Worlds Poult Sci J 65(1):97–114

Yang C, Kabir-Chowdhury MA, Hou Y et al (2015) Phytogenic compounds as alternatives to in-feed antibiotics: potentials and challenges in application. Pathogens 4(1):137–156

Yatao X, Saeed M, Kamboh AA et al (2018) The potentially beneficial effects of supplementation with hesperidin in poultry diets. Worlds Poult Sci J 74(2):265–276

Yegani M, Korver DR (2008) Factors affecting intestinal health in poultry. Poult Sci 87(10):2052–2063

Zha C, Cohen AC (2014) Effects of anti-fungal compounds on feeding behavior and nutritional ecology of tobacco budworm and painted lady butterfly larvae. Entomol Ornithol Herpetol 3:120

Zhang H, Guo Y, Yuan J (2005) Conjugated linoleic acid enhanced the immune function in broiler chicks. Br J Nutr 94(5):746–752

Part V

Safety and Toxicity Evaluation of Nutraceuticals and Functional Foods

Safety and Toxicity Evaluation of Nutraceuticals in Animal Models

Nikolay Goncharov, Vladislav Sobolev, Maxim Terpilowski, Ekaterina Korf, and Richard Jenkins

Abstract

Nutraceuticals are derived from various natural sources such as medicinal plants, marine organisms, vegetables, and fruits. Most of them possess antioxidant or anti-inflammatory properties and are claimed to provide protection against many diseases if taken regularly. At the same time, toxicological studies of nutraceuticals have been limited, so the safety of many of them cannot be guaranteed. Animals share many genetic, anatomical, and physiological similarities with humans, and they continue to be widely used in preclinical studies of drugs, in spite of a lack of their validity which is due to the great phenotypic differences. The absence of toxicity in animals provides little probability that adverse reactions will also be absent in humans. There are currently thousands of researchers involved in the development of alternatives to animal use in the life sciences. Statistical machine-learning tools, once developed, might become a powerful means to explain the complex physiological effects of nutraceuticals. The use of different models and algorithms can provide a more scientific basis for risk assessment of nutraceuticals for humans.

Keywords

Preclinical studies · Biomarkers · System analysis · Alternative models · Cytotoxic power

N. Goncharov (✉)
Research Institute of Hygiene, Occupational Pathology and Human Ecology, Leningrad Region, Russia

Sechenov Institute of Evolutionary Physiology and Biochemistry, St. Petersburg, Russia

V. Sobolev
Research Institute of Hygiene, Occupational Pathology and Human Ecology, Leningrad Region, Russia

M. Terpilowski · E. Korf
Sechenov Institute of Evolutionary Physiology and Biochemistry, St. Petersburg, Russia

R. Jenkins
School of Allied Health Sciences, De Montfort University, Leicester, UK

Abbreviations

ADI	Acceptable daily intake
ADRs	Adverse drug reactions
ALA	α-linolenic acid
ARRIVE	Animals in Research: Reporting In Vivo Experiments
BMD	Benchmark dose
BMDL	Benchmark dose lower bound
CDDs	Chlorinated dibenzo-*p*-dioxins
DHA	Docosahexaenoic acid
DNEL	Derived no-effect level
DSHEA	Dietary Supplement Health and Education Act
EDI	Estimated daily intake
EGCG	(−)-epigallocatechin-3-gallate
EPA	Eicosapentaenoic acid
ETs	Ellagitannins
GRAS	Generally recognized as safe
GTE	Green tea extract
HBA	Harm-benefit analysis
LOAEL	Lowest-observed-adverse-effect level
LRs	Likelihood ratios
MOA	Mode of action
MOE	Margin of exposure
NHPs	Nonhuman primates
NOAEL	No-observed-adverse-effect level
OSC	Organosulfur compounds
PARNUTS	Particular nutritional uses
PAs	Pyrrolizidine alkaloids
PFS	Plant food supplement
PODs	Points of departure
RfC	Reference concentration
RfD	Reference dose
ROS	Reactive oxygen species
RPF	Relative potency factors
RYR	Red yeast rice
TEFs	Toxic equivalency factors
TTC	Threshold of toxicological concern

R. C. Gupta et al. (eds.), *Nutraceuticals in Veterinary Medicine*, https://doi.org/10.1007/978-3-030-04624-8_48

1 Introduction

The food products used as nutraceuticals can be categorized as dietary fiber, prebiotics, probiotics, polyunsaturated fatty acids, antioxidants, and other different types and constituents of natural foods. They help in preventing and even combating some health problems such as obesity, cardiovascular diseases, cancer, osteoporosis, arthritis, diabetes, excess cholesterol, etc. (Das et al. 2012; Gupta 2016). Many individuals drink herbal teas for prophylactic purposes or as alternatives to caffeinated beverages. There is also a huge industry of producing food supplements for participation in sports. The perceived advantage of nutraceuticals over medicines is that they are generally regarded as having no side effects, whereas their perceived disadvantage is relatively poor effectiveness, which is claimed to be overcome either by increasing the dosage or by consuming them over longer periods of time. However, such perceptions may be rather deceptive because of our poor understanding of the mechanistic properties of nutraceuticals and their incompetent administration. Far too often, the decision to consume nutraceuticals is based on marketing claims rather than available evidence-based research. In sport activities, it has been shown that with a sufficient calorie intake, nutraceuticals or other supplementation is not necessary to help elite athletes perform better, and many controlled studies have failed to confirm the advertised ergogenic effects of nutritional supplements (Mason and Lavallee 2012; Desbrow et al. 2014).

These days, Americans spend over $28 billion on nutraceuticals and dietary supplements (Ronis et al. 2018). In the USA, dietary supplements are defined and regulated according to the Dietary Supplement Health and Education Act (DSHEA) of 1994 (Mueller 1999). According to the DSHEA, a dietary supplement is a product that contains such dietary ingredients as vitamins, minerals, amino acids, herbs, and botanicals. The term nutraceutical is not defined by US law but is generally understood to refer to a purified product derived from a human food source, which can provide health benefits beyond the basic nutritional value of usual foods. The European legislation also does not mention the term "nutraceuticals," and these could be dealt within the Foods for Particular Nutritional Uses (PARNUTs) regulatory framework (Directive 89/398/EEC 1989). PARNUTs includes foods for special medical purposes or for particular nutritional needs, once their safety and efficacy have been properly assessed by in vitro and in vivo studies. However, in contrast to the manufacture of pharmaceuticals, which requires documented evidence for the effectiveness and safety of drugs, there is no need for demonstrating the efficacy of dietary supplements or nutraceuticals. Consequently, as a rule, they are brought to the market without the support of clinical trials, so there is a lack of studies of adverse effects. A few case reports of symptoms appearing after intake of nutraceuticals do not reveal cause-effect relations. Only accumulation of many cases over time can ultimately establish that intake of a nutraceutical can result in adverse effects, and it has turned out that the risk of adverse effects is rather significant (Ronis et al. 2018).

Plants synthesize many secondary metabolites whose role may be in the defense mechanisms against herbivores and parasites, so consumption of nutraceuticals over a long and even a short period of time should be of great concern. The most popular stimulators of physical or mental adaptation are essentially nutraceuticals—caffeine, nicotine, ethanol and tetrahydrocannabinol—which at the same time can be toxic and not only for addicts. Moreover, numerous human and animal intoxications have been associated with naturally occurring herbal components, including pyrrolizidine alkaloids (PAs), tannins, and safrole (Manteiga et al. 1997). Some toxic impurities are also components of foods being consumpted as a source of nutraceuticals (Shimshoni et al. 2015; Hassanin et al. 2017). Impurities can also be supplied with artificial or not entirely natural additives: many of the resveratrol-containing nutraceuticals are enriched in this compound by adding purified resveratrol that has been extracted from the root of the Japanese knotweed *Polygonum cuspidatum* (Espín et al. 2007).

The most compelling evidence for potential health-beneficial effects of nutraceuticals seems to derive from descriptive and correlative epidemiological data (Sauer and Plauth 2017). On the other hand, the problem of scarce and inconsistent reports on effects of nutraceuticals often lies in the sphere of inadequate testing models or quasi-scientific explanation of experimental data obtained (Goncharov et al. 2016a). The physiological context is of high importance for evaluating effects of diet supplements, some of which were observed in in vitro experiments and must be very cautiously extrapolated to the in vivo context. The in vitro effects of nutraceuticals can be irrelevant in terms of their in vivo properties. Bioavailability, metabolism and tissue distribution are very important factors that need to be established for clear understanding of the biological effects of nutraceuticals. Once distributed within the body, nutraceuticals or their derivatives interact with many different target proteins with very different specificity (Sauer and Plauth 2017). Gut microbiota is one of the principal targets, and sometimes it is a real puzzle as to the true bioactive compounds responsible for the systemic biological activity.

Animals have played a principal role in biomedical experimental research throughout history (Franco 2013). To reduce or avoid unethical procedures, a strategy of 3Rs (reduction, refinement, and replacement) is being applied for animal testing, and alternatives to laboratory use of animals have

been introduced (Doke and Dhawale 2015). Nonetheless, it is imperative to obtain more information on safety and efficacy based on in vivo preclinical and clinical studies, and it is highly important to reach a better understanding of the mechanism of action and bioavailability of these products (Santini et al. 2018).

2 Toxic Actions of Nutraceuticals

The most important dietary phytochemicals are isothiocyanates, glucosinolates, organosulfur compounds (OSC), terpenoids (carotenoids, monoterpenes, and phytosterols), and various groups of polyphenols (anthocyanins, flavones, flavan-3-ols, isoflavones, stilbenoids, ellagic acid (EA), ellagitannins (ETs), etc.) (Ismail et al. 2016). Some of these have a protective effect against toxic compounds, such as carbon tetrachloride, as was shown with green tea extract (GTE) in male hamsters (Elgawish et al. 2015). Also, positive effects of lecithin and/or gallic acid in neurotoxicity induced by aluminum oxide in experimental rats have been demonstrated (Hassanin et al. 2017).

Toxic properties of nutraceuticals are not as extensively studied as those of pharmaceuticals. Indeed, there are little data to suggest that nutraceuticals and dietary supplements are toxic. An interesting variable worth mentioning in this context is the matter of funding for research on their effectiveness and safety. Multiple articles disclose that funding is contributed by the companies which market the product for retail purposes (Vaughan et al. 2014). However, they are not necessarily safe for everybody. Supplements with active ingredients that provide a physiological effect can also cause adverse effects. For example, some metabolites of (−)-epigallocatechin-3-gallate (EGCG) are suspected not to diminish but to enhance oxidative stress and cause liver injury, the mitochondria being a possible target (Mazzanti et al. 2009, 2015; James et al. 2018). Besides GTE and EGCG, a number of herbal medicinal products are associated with a spectrum of hepatotoxicity events, such as Ayurvedic and Chinese herbs, black cohosh, chaparral, germander, greater celandine, Herbalife, Hydroxycut, kava, pennyroyal, skullcap, usnic acid, and PAs (Bunchorntavakul and Reddy 2013). The soy-derived isoflavones genistein and daidzein with its metabolite equol were shown to possess estrogenic properties, including the ability to produce uterine hypertrophy, reduce testis size, inhibit androgen production, etc. (Tan et al. 2006; Akingbemi et al. 2007; Ronis et al. 2014, 2016). There have been case reports of endometriosis in women consuming isoflavone supplements, so there is a risk of estrogen-sensitive cancers in consumers of these products (Mahady et al. 2003).

α-Linolenic acid (ALA) is the precursor of longer-chain omega-3 fatty acids, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA). Although there are limited toxicological data, ALA is considered to be safe as a dietary ingredient. However, it should be noted that ALA like other fatty acids can produce lipid peroxidation products under exposure to air or UV radiation, which may produce adverse effects if not controlled appropriately. Moreover, prostate cancer and macular degeneration risks should be also considered in relation to dietary intake of ALA, although the overall evidence on the association of ALA with those risks remains mixed and inconclusive (Kim et al. 2014). In some experiments, administration of ETs to rats inhibited the initiation and progression of chemically induced colon and esophageal cancers (Stoner et al. 2006). In other experiments no effect was shown on the number or size of adenomas in the small intestine of mice upon administration of either pure EA or diets with ETs (Päivärinta et al. 2006). These contradictory results may be due to different types of tumors but also due to different animal models used. More importantly and less advertised are reports in which ETs were shown to cause liver necrosis and nephrotoxicity in cattle (Filippich et al. 1991; Oelrichs et al. 1994).

There are plenty of plants that contain OSC, the toxicity of which varies in different species and different cells within a body (Munday 2012; Goncharov et al. 2016b). However, if the elevated toxicity is within cancer cells, it would undoubtedly be a positive event for the organism. There is much evidence indicating that they modulate the activity of several metabolizing enzymes that activate (phase 1 enzymes: CYP2E1, CYP1A1, and CYP1A2) or detoxify (phase 2 enzymes: NAD(P)H:quinone oxidoreductase 1, glutathione *S*-transferases, UDP-glucuronosyltransferases, etc.) carcinogens, through inhibition of histone deacetylase activity and inhibition of the formation of DNA adducts in several target tissues (Navarro et al. 2011; Zhang et al. 2013). Ironically, the persistent activation of antioxidant systems via genetic alterations in Nrf2 and Keap1 also contributes to carcinogenesis. Some cancers hijack the Nrf2/Keap1 system with mutation in these genes, which persistently activate antioxidant peroxiredoxin/sulfiredoxin systems in the cancer cells (Toyokuni 2014). Positive effects of OSC in mammals often come from their selective toxic effects on microorganisms. Thus, garlic has been proven to be toxic to a plethora of Gram-positive, Gram-negative, and acid-fast bacteria, including *Salmonella*, *Escherichia coli*, *Pseudomonas*, *Proteus*, *Staphylococcus aureus*, *Klebsiella*, *Micrococcus*, *Bacillus subtilis*, *Clostridium*, *Mycobacterium*, and *Helicobacter* (Bayan et al. 2014).

Red yeast rice (RYR) is a kind of functional food obtained from fermentation with the red yeast *Monascus purpureus*, containing a bioactive component monacolin K (chemically

identical to lovastatin), which has been recognized as a key component in cholesterol reduction. In a study on the safety of RYR, cases of myalgia and/or an increase in creatine phosphokinase were observed, as well as liver injury and gastrointestinal reactions, and, in some cases, hospitalization (Mazzanti et al. 2017). Furthermore, there is another risk factor in RYR—the toxic metabolite mycotoxin citrinin—which is a serious obstacle to the use of RYR as a dietary supplement, since exposure to citrinin can cause nephrotoxicity (Vanacloig-Pedros et al. 2016; Patel 2016).

Some green teas and/or herbal infusions can be contaminated by PAs that are produced by various weed plants and possess acute and chronic toxicity, genotoxic, mutagenic, and carcinogenic properties (Habs et al. 2017). 1,2-Unsaturated PAs should be of special concern because they are genotoxic carcinogens (Chen et al. 2017). Reactive metabolites of PAs are formed in vivo after metabolic activation via CYP3A4 enzymes in the liver and can cause hepatic sinusoidal obstruction syndrome and livestock poisoning (Wiedenfeld and Edgar 2011; Jurgens et al. 2012). Another source of PAs is honey collected from plants producing PAs (Merz and Schrenk 2016).

Selected consumption of Chinese botanical preparations raises concern of exposure to alkenylbenzenes (Ning et al. 2018). Also, methyleugenol and related alkenylbenzenes were found in a series of nutmeg-based plant food supplements (PFS) and pesto sauces, though their toxicities based on estimated daily intake (EDI) and the margin of exposure (MOE) approach are insignificant, and consumption of pesto sauces would only be of concern if consumed on a daily basis over longer periods of time (Al-Malahmeh et al. 2016, 2017). The next section is devoted to these and other indices relevant for estimation of safety and toxicity of nutraceuticals.

3 Safety and Toxicity Studies with Animal Models: Foundations, Problems, and Alternatives

The biological activity of a phytochemical is usually carried out using in vitro and/or in vivo tests. In vitro testing is frequently performed with much higher concentrations of investigated compounds compared to those tested in vivo. Another significant problem for in vitro research is whether testing is necessary, or even possible, for the relevant metabolites which can be found in vivo in varying concentrations in different organs and tissues. On the other hand, extrapolation of the results obtained in an animal model to humans is not entirely adequate due to differences in their physiology, and there are also differences between animal species which may result in contradicting interpretations of experimental data (Espín et al. 2007).

Ideally, a combination of several in vitro models with at least two animal models should be adopted to give a clear understanding of the mechanistic and toxic properties of compounds, including nutraceuticals. People can avoid consuming nutraceuticals as food additives when curing an acute disease, but the question is whether they can be used as therapeutic adjuvants or in a prophylactic manner to avoid diseases. If yes, is it necessary to test them on safety and toxicity? If yes, is it necessary to use animal models? Animal models are used to ascertain the target organs affected by active components, assuming that they are well characterized, their impurities (if any) are also well characterized, and the manufacturing process is standardized (Kruger and Mann 2003). Various animal species—such as fishes, amphibians, birds, mice, rats, hamsters, rabbits, guinea pigs, dogs, cats, and primates—have been and continue to be used in science for a long time. Rodents are the most commonly used laboratory animals, making up nearly 80% of the total of animals used in the EU, followed by cold-blooded animals (9.6%) and birds (6.3%) (European Commission 2010; Franco 2013).

3.1 Foundations

Toxicity studies include (1) repeat-dose general toxicity studies, (2) pharmacokinetic/toxicokinetic studies, (3) reproductive toxicology, (4) genotoxicity studies, (5) carcinogenicity studies, and (6) special pharmacology studies, which can include in vitro and in silico studies and thus provide explanations for toxic findings in animal studies (Kruger and Mann 2003). To assess the toxic potential of a chemical, toxicologists have to assess the acute, subacute, subchronic and chronic toxicity. For acute toxicity studies, a dose is administered to animals and symptoms observed over 14 days. In subchronic studies, which are the most popular among toxicologists and adequate for nutraceuticals, animals receive several doses of a chemical over 90 days. At the end, animals are sacrificed and samples are taken for hematological and biochemical analyses, organ weights, and histopathological examination. The usual way of evaluation of genotoxicity potential is through a micronucleus test, and a negative control should be incorporated. Points of departure (PODs) in toxicological studies are defined as the points on a dose-response curve established from experimental data corresponding to an estimated lowest-observed-adverse-effect level (LOAEL), no-observed-adverse-effect level (NOAEL), or statistical benchmark dose (BMD) (Augustin et al. 2013). They mark the beginning of extrapolation to toxicological reference dose (RfD) or reference concentration (RfC). In the EU, RfD may be called derived no-effect level (DNEL). The US EPA defines RfD or RfC as follows (ChemSafetyPRO 2018):

- Reference dose (RfD) is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily oral or dermal exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. Its unit is usually mg/kg bw/day or mg/kg.
- Reference concentration (RfC) is an estimate (with uncertainty spanning perhaps an order of magnitude) of a continuous inhalation exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. Its unit is usually mg/L or ppm.

In the USA, many nutraceuticals have been classified as "generally recognized as safe" (GRAS), with acceptable NOAEL (Augustin et al. 2013). In the design of animal studies, an important parameter that must be considered is the margin of exposure (MOE) between the NOAEL and the anticipated or estimated level of daily intake (EDI) by humans. For a food additive, an acceptable daily intake (ADI) is usually derived by applying a safety factor of 100-fold to the NOAEL determined in animal studies. If the EDI falls below the ADI, then exposure is considered to be safe. This concept is applicable for nutraceuticals consumed by humans in amounts not exceeding 1.5 g/day (equal to around 25 mg/kg/day), since this level of intake can be supported by applying a 100-fold safety factor to a NOAEL in a rat model of testing (about 5% of the rat's diet) (Kruger and Mann 2003). The concept of the 100-fold safety factor should not be applied for drugs or nutrients, since effects found in animal studies would not be a toxicological response to the active ingredients but may be due to their physiologic or pharmacologic activities.

In this context an example of inadequate animal studies should be mentioned, in which hepatotoxic potential of GTE and EGCG was registered. A very high single dose of EGCG (500–750 mg/kg) intragastrically to C57BL/6J mice daily on an empty stomach for 3 days caused hepatic inflammation, necrosis and hemorrhage. Hepatotoxicity was associated with increased oxidative stress and decreased superoxide dismutase and glutathione peroxidase levels (James et al. 2018). For comparison, in our studies of possible effects of GTE on endurance and fatigue in a rat model of forced swimming, GTE was administered in quantity equaling an EGCG dosage of 6 mg/kg (two orders of magnitude less than that used by James et al. 2018) for 2–3 weeks twice daily after taking of food, and no hepatotoxicity was found; moreover, GTE was shown to increase the swimming duration, as compared with control animals that consumed water (Novozhilov et al. 2015). Later studies confirmed those results, and additionally it was found that administration of GTE increased endurance due to involvement of slow-twitch muscles whose adaptation is associated with enhanced expression of genes responsible for the subtle regulation of calcium balance in the muscles (Korf et al. 2017). Unfortunately, there are lots of examples of how experiments with animals serve to justify the obviously prejudiced position of researchers, irrespective whether this position boils down to praising or mocking nutraceuticals. Some other problems are discussed in the next section.

3.2 Problems and Alternatives to Animal-Based Studies

A list of methodological problems concerning animal-based research were devised in 2004 (Pound et al. 2004): (1) variations in drug dosing schedules and regimens, (2) variability in the way animals are selected for study, (3) choice of comparison therapy (none, placebo, vehicle), (4) small experimental groups and simplistic statistical analysis, (5) nuances in laboratory technique that may influence results, (6) selection of a variety of outcome measures and their relevance to the human clinical condition, and (7) length of follow-up before determination of disease outcome and its relevance to disease latency in humans. Several years later, the ARRIVE (Animals in Research: Reporting In Vivo Experiments) guidelines were designed to improve the standard of reporting of research using animals (Kilkenny et al. 2010). They consist of 20 items on the minimum information that all scientific publications reporting research with animals should include. If this is done, there is a good reason to combine many studies which adhere to specific criteria in systematic reviews and/or meta-analyses, in order to reach more reliable conclusions regarding experimental research with animals. At the same time, there are claims that any animal model fails as a predictive modality for human response to drugs and disease.

The problem is not an absence of side effects, as many side effects from drugs were observed in animal models, but there were no predictive value for humans. Therefore, systematic reviews and meta-analyses of animal-based research by any means cannot be good tools for trying to reach conclusions regarding human interventions (Greek and Menache 2013). According to some analytical studies, the correlation of adverse effects found in animal models for those observed in humans was estimated to be 63% in non-rodent experiments and 43% in experiments with rodents (Olson et al. 2000). The basis of many publications of this kind is that animals are of no help at all in experimental medicine, because regardless of the way a problem is approached, animal and humans will always differ in complex (Greek and Menache 2013). However, in this and similar publications, the real potential and perspective development of science is not fully taken into account.

We cannot completely abandon animal models just now; the affirmative view is a populistic claim, often from people having little or no competence and responsibility. Yet we must think about the elaboration of algorithms for system analysis and integrative approaches, in order to minimize the use of animals and to maximize the scientific benefit they can bring to human society. It is particularly important with regard to searching for new integrative biomarkers of various pathological state and invariant functional changes—irrelevant of species membership, in different animals and humans—induced by different chemicals or their complex mixtures (Goncharov et al. 2017b). Biomarkers are used not only as diagnostic or prognostic indicators but also to formulate theories of pathogenesis (Ghezzi et al. 2018). Two problems were identified in the use of biomarkers in mechanistic studies: the first arises in the case of multifactorial diseases, where different combinations of multiple causes result in patient heterogeneity; the second arises when a pathogenic mediator(s) is (are) difficult to measure. The latter applies in the case of the oxidative stress theory of disease, where the causal components are ROS which have very short half-lives; it is usual to measure the traces left by the reaction of ROS with biological molecules, rather than the ROS themselves. Different facets of biomarkers and their different values and meanings in multifactorial diseases and system medicine have recently been discussed (Ghezzi et al. 2018; Goncharov et al. 2017b). This seems to be especially helpful and promising for preclinical and clinical studies of nutraceuticals, most of which are involved in redox regulations and used in relatively low quantities. Subchronic toxicological studies of very low concentrations of aliphatic hydrocarbons with metabolomic and chemometric techniques revealed novel metabolic markers of the toxic effect: a ratio of concentrations of pyrophosphate and oxalate in the blood plasma of rats was found to be the most sensitive marker, termed the "pyrophosphate index." This finding helped us to put forward a hypothesis on the redox balance violation as the leading pathogenetic mechanism of neuropathies associated with hydrocarbons chronic intoxication, irrespective of animal species; the hypothesis needs to be tested with several other species of animals (Ukolov et al. 2017).

Combinatorial biomarkers are considered more specific and sensitive than single markers in medical diagnostics and prediction, yet even detection of these combinatorial biomarkers requires deep computational analysis. The principles of analytic combinatorics, together with linear and kernel ridge regression, helped to identify several strongly correlated combinatorial biomarkers of muscle damage with high prediction accuracy scores, which could also be a common platform for animal and human studies (Terpilowski et al. 2018). Metabolomic studies with application of chemometrics and other computational methodology often reveal that not minor and exotic but very simple biochemical parameters and their derivatives could be very sensitive biomarkers with high prognostic value. For example, albumin is among the few selected biomarkers that provide insight into the risk of death from all causes (Fischer et al. 2014; Hui et al. 2014). Albumin is a major plasma protein and contributes greatly to the plasma protein-SH pool. Homocysteinylation of albumin at Cys34 makes it increasingly susceptible to oxidative degradation (Prakash et al. 2008). The future search for new properties of albumin from different species under the influence of nutraceuticals is considered to be very important, via in vivo, in vitro and in silico studies (Goncharov et al. 2017a, c).

Toxic equivalency factors (TEFs) and MOE approaches are used for plant food supplements (PFS) containing different toxic substances, such as alkenylbenzenes (Al-Malahmeh et al. 2017). Initially, an interim concept of TEFs was proposed for chlorinated dibenzo-*p*-dioxins (CDDs), and in acute experiments with rats, an assumption was proved that interactions of four homologous CDDs were strictly additive (Stahl et al. 1992). For nutraceuticals or their toxic ingredients such as PAs, risk assessment is usually estimated after (sub)chronic application to animals, and the interim relative potency factors (RPF) conception was proposed as the dose metric having a higher potential for toxicological risk assessment, as compared with TEFs (Merz and Schrenk 2016). This novel approach was built on the foundation of predicted human exposures to metabolites in various compartments (such as food and water), the threshold of toxicological concern (TTC) and the concept of comparative toxicity (Terry et al. 2015). The ultimate aim was to address the human safety of the metabolites with the minimum number of in vivo studies while at the same time ensuring that human safety would be considered addressed on a global regulatory scale. The third component, comparative toxicity, was primarily designed to determine whether the metabolites had the same or similar toxicity profiles to their parent molecule and also to one another. The ultimate goal was to establish whether the metabolites had the potential to cause key effects—such as cancer and developmental toxicity, based on mode-of-action (MOA) studies—and to develop an RPF compared to the parent molecule. The MOA for the primary effects of the parent molecule was elucidated in detail, and a series of in silico, in vitro, and/or in vivo experiments were conducted on the environmental metabolites to assess relative potency of their toxicity profiles when compared to the parent. This strategy of comparative assessment—utilizing MOA data, relative potency, hazard characterization, readacross, predicted exposure and TTC—provided a robust database, which minimized animal use yet comprehensively assessed the hazard and human risk presented by these metabolites (Terry et al. 2015).

Animal and two-dimensional cell culture models have had a profound impact on medical research, despite inherent flaws and differences when compared with in vivo and clinical observations. Three-dimensional (3D) tissue models are a natural progression and extension of existing techniques that seek to plug the gaps and mitigate the drawbacks of two-dimensional and animal technologies (Konar et al. 2016). Most exogenous compounds, the more so nutraceuticals, have multiple cellular and molecular targets with low levels of specificity and different levels of toxicity to many of them. For example, some thiols and disulfides are used as nutraceuticals in complex cardiovascular or cancer therapy (Cerella et al. 2011; Ried and Fakler 2014) and, at certain concentrations, they can be toxic to endothelial cells (Prokofieva and Goncharov 2014) and red blood cells (Munday 2012; Alzoubi et al. 2015; Mindukshev et al. 2016), with ROS being mediators of their toxic effects. Development of a methodology for cytophysiological screening of various substances, including pharmaceuticals and nutraceuticals, is an urgent need of our days. Intuitively, there should be a strong correlation between cellular responses in vitro and the final efficacy of the therapy, though this correlation has yet to be proved.

3.3 Cytotoxic Power

The development of tools for interventions aimed at improving human health requires a comprehensive knowledge of the cellular reactions to positive or negative stimuli, correlating these in vitro and ex vivo obtained data with final in vivo therapeutic or toxic effects (Mindukshev et al. 2016). One of these tools could be the conception of "cytotoxic power," the initial version of which was published recently (Goncharov et al. 2018). The currently existing terms "apoptotic index" and "apoptotic potential" are insufficient for adequate qualitative evaluation and analysis of the proapoptotic activity of chemical agents within the wide range of therapeutic or toxic doses, so a new concept of cytotoxic power was introduced as an alternative to these terms related to apoptosis and other types of cell death. This concept is being further developed these days, and the essence of it is as follows: In in vitro experiments, the final calculation of the effective values is made on the basis of quantitative ratios of a substance and cells studied. The cytotoxic power (P) of the substance can be quantitatively represented as the product of the amount of substance (S) in moles acting on a certain number of cells (n) and the intensity of development of cytotoxic processes (I) in the same number of cells per unit time (t):

$$P = \frac{IS}{n^2 t}$$

The concept and corresponding algorithm to calculate the power makes it possible to perform a valid transformation of the quantum dependence into a graded one, based on the multimarker search strategy and, along with relatively universal and non-specific cytotoxicity indicators for a given tissue, to identify and use tissue-specific indicators (markers)—components of the so-called phenom. With each type of cell death, markers can be identified, the concentrations of which gradually increase or decrease before the death of the cell. The level of expression of each marker depends on the dose (amount) of the cytotoxic substance. Importantly, we observe expression by living cells, no matter what percentage of dead cells are registered; moreover, we can additionally observe the expression by cells in the stage of early apoptosis or autophagy, since these are also kinds of living cells. Thus, the intensity of signal development reflects an increase in the number of discrete, functionally coupled and relatively stable though transient intracellular structures that are constituents of the cell's phenom; we call them "phens," F, then $I = F/t$. The conventional unit for measurement of the cytotoxic power of a substance is "molphen"—the amount of nanomols that changes the intensity of expression of phenotypic markers (constituents of phens) by 100 arbitrary units per hour from the moment of exposure to 1000 cells. This definition, not claiming completeness, emphasizes the fractional number of phens in relation to the number of cells, as well as the different initial state of the cells, which explains the different rates of cell death in the population. The concept of cytotoxic power can help to integrate the data obtained with various cells in in vitro studies with those obtained in vivo.

4 Unresolved Issues

Animals are used in research when there is a need to find out what happens in the whole living body, which is far more complex than the sum of its parts. It is difficult, and in most cases simply not yet possible, to replace the use of living animals in research with alternative methods. There are four main reasons why animals are used in research (Anon 2018a): (1) to advance scientific understanding; (2) as models to study disease; (3) to develop and test potential forms of treatment; and (4) to protect the safety of people, animals and the environment. At the same time, animal models have shown very low potential to significantly contribute toward the development of human clinical interventions (Knight 2008). While the presence of toxicity can add considerable evidential weight for human risk, the likelihood ratios are extremely inconsistent, varying by over two orders of magnitude for different classes of compounds and their effects (Bailey et al. 2014). Questions remain about the adequacy of existing guidelines and whether researchers, review boards

and funders have fully implemented the "3Rs" (Ferdowsian and Beck 2011). Researchers have acknowledged the central role of the 3Rs and the need for more transparency regarding animal use in biomedical studies through the Basel Declaration (Anon 2018b). The harm-benefit analysis (HBA) is the cornerstone of animal research regulation and is considered to be a key ethical safeguard for animals (Pound and Nicol 2018). On the other hand, the emphasis on practical benefits has the drawback of driving researchers into speculation on the societal benefit of their research and, therefore, into promising too much, thereby leading to a loss of trust and credibility (Grimm et al. 2017; Eggel and Grimm 2018). Thus, the concepts of benefit and benefit assessment require a reevaluation in a spirit that embraces the value of knowledge per se. The generation of scientific knowledge has been utilized to great benefit for humans, animals and the environment. The HBA, as it currently stands, tends to turn this idea upside down and implies that research is of value only if the resulting findings bring about immediate societal benefit (Grimm et al. 2017).

In the search for mechanistic properties of nutraceuticals, it often seems useless to artificially break down effects to single molecule-protein interactions just to satisfy the "mechanistic research paradigm" (Weinberg 2010). Moreover, it may happen that the emerging properties of additive, synergistic, or inhibitory molecular interactions and resulting effects of nutraceuticals lead to an overall beneficial physiological output that cannot be traced back to a single molecular event. Nowadays, omics-based methodologies and adequate data analyses allow for efficient determination of systemic effects (Sauer and Luge 2015; Goncharov et al. 2015). "Big data" analysis, i.e., statistical machine-learning tools, will become increasingly important to complex biological phenomena and causality structures to develop appropriate computational models for prediction (Sauer and Plauth 2017). For example, new approaches based on nonlinear state space reconstruction were developed to differentiate causality from correlation and to efficiently analyze weakly connected dynamic biological systems (Sugihara et al. 2012). Omics-based biology should be more often consulted to solve complex biological problems. One of them is epigenetic mechanisms and their modulation by nutraceuticals, which have been shown to target enzymes involved in epigenetic gene regulation (Gerhauser 2018). Another complex problem is that the gut microbiota and microbial metabolites might be important mediators of diet-epigenome interactions. Only a few microbial metabolites, including folate, phenolic acids, *S*-(−)equol, urolithins, isothiocyanates, and short- and long-chain fatty acids, have been tested with respect to their potential to influence epigenetic mechanisms (Gerhauser 2018).

The concepts described in this chapter can help to greatly enhance the prognostic value of in vitro research to finally reject—who knows?—the in vivo studies at all. The methodology of safety and toxicity studies should be biased to in vitro and in silico models, the more so when nutraceuticals are the object of research. Both ethical and financial considerations favor these scientific endeavors. Animals should not be sacrificed for the sake of healthy people seeking to be even healthier. Future directions of nutraceutical testing are certainly bound to the development of a methodology of cytophysiological screening in vitro, together with in silico simulations of molecular interactions and computational algorithms for enhancing prognostic value of research. These should be indispensable supplements to the methodology of investigating natural supplements, i.e., nutraceuticals. These supplements, taken together, would greatly add to human health and our associated knowledge.

Acknowledgment This work has been supported by the Russian Foundation for Basic Research Grant 18-015-00304 and by the Russian FASO Programme AAAA-A18-118012290142-9.

References

Akingbemi BT, Braden TD, Kemppainen BW et al (2007) Exposure to phytoestrogens in the perinatal period affects androgen secretion by testicular Leydig cells in the adult rat. Endocrinology 148:4475–4488

Al-Malahmeh AJ, Al-Ajlouni AM, Wesseling S et al (2016) Determination and risk assessment of naturally occurring genotoxic and carcinogenic alkenylbenzenes in basil-containing sauce of pesto. Toxicol Rep 4:1–8

Al-Malahmeh AJ, Alajlouni AM, Ning J et al (2017) Determination and risk assessment of naturally occurring genotoxic and carcinogenic alkenylbenzenes in nutmeg-based plant food supplements. J Appl Toxicol 37(10):1254–1264

Alzoubi K, Calabrò S, Faggio C et al (2015) Stimulation of suicidal erythrocyte death by sulforaphane. Basic Clin Pharmacol Toxicol 116(3):229–235

Anon (2018a.) http://www.animalresearch.info/en/designing-research/why-animals-are-used/. Accessed Jun 2018

Anon (2018b) 5th international conference of the Basel Declaration Society openness and transparency: building trust in animal research, 14th–15th Feb 2018. http://www.basel-declaration.org/

Augustin MA, Sanguansri L, Lockett T (2013) Nano- and microencapsulated systems for enhancing the delivery of resveratrol. Ann N YAcad Sci 1290:107–112

Bailey J, Thew M, Balls M (2014) An analysis of the use of animal models in predicting human toxicology and drug safety. Altern Lab Anim 42(3):181–199

Bayan L, Koulivand PH, Gorji A (2014) Garlic: a review of potential therapeutic effects. Avicenna J Phytomed 4(1):1–14

Bunchorntavakul C, Reddy KR (2013) Review article: herbal and dietary supplement hepatotoxicity. Aliment Pharmacol Ther 37(1):3–17

Cerella C, Dicato M, Jacob C et al (2011) Chemical properties and mechanisms determining the anti-cancer action of garlic-derived organic sulfur compounds. Anti Cancer Agents Med Chem 11 (3):267–271

ChemSafetyPRO (2018.) http://www.chemsafetypro.com/Topics/CRA/What_is_Point_of_Departure_(POD)_in_Toxicology_and_How_to_Use_It_to_Calculate_Reference_Dose_RfD.html. Accessed Jun 2018

Chen L, Mulder PPJ, Louisse J et al (2017) Risk assessment for pyrrolizidine alkaloids detected in (herbal) teas and plant food supplements. Regul Toxicol Pharmacol 86:292–302

Das L, Bhaumik E, Raychaudhuri U et al (2012) Role of nutraceuticals in human health. J Food Sci Technol 49(2):173–183

Desbrow B, McCormack J, Burke LM (2014) Sports dietitians Australia position statement: sports nutrition for the adolescent athlete. Int J Sport Nutr Exerc Metab 24(5):570–584

Directive 89/398/EEC (1989.) https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex%3A31989L0398. Accessed Jun 2018

Doke SK, Dhawale SC (2015) Alternatives to animal testing: a review. Saudi Pharm J 23(3):223–229

Eggel M, Grimm H (2018) Necessary, but not sufficient. The benefit concept in the project evaluation of animal research in the context of directive 2010/63/EU. Animals (Basel) 8(3):E34

Elgawish RAR, Rahman HGA, Abdelrazek HMA (2015) Green tea extract attenuates CCl4-induced hepatic injury in male hamsters via inhibition of lipid peroxidation and p53-mediated apoptosis. Toxicol Rep 2:1149–1156

Espín JC, García-Conesa MT, Tomás-Barberán FA (2007) Nutraceuticals: facts and fiction. Phytochemistry 68(22–24):2986–3008

European Commission (2010) Sixth report on the statistics on the number of animals used for experimental and other scientific purposes in the member states of the European Union, Brussels

Ferdowsian HR, Beck N (2011) Ethical and scientific considerations regarding animal testing and research. PLoS One 6(9):e24059

Filippich LJ, Zhu J, Oelrichs P et al (1991) Hepatotoxic and nephrotoxic principles in Terminalia oblongata. Res Vet Sci 50(2):170–177

Fischer K, Kettunen J, Würtz P et al (2014) Biomarker profiling by nuclear magnetic resonance spectroscopy for the prediction of all-cause mortality: an observational study of 17,345 persons. PLoS Med 11(2):e1001606

Franco NH (2013) Animal experiments in biomedical research: a historical perspective. Animals (Basel) 3(1):238–273

Gerhauser C (2018) Impact of dietary gut microbial metabolites on the epigenome. Philos Trans R Soc Lond Ser B Biol Sci 373 (1748):20170359

Ghezzi P, Davies K, Delaney A et al (2018) Theory of signs and statistical approach to big data in assessing the relevance of clinical biomarkers of inflammation and oxidative stress. Proc Natl Acad Sci USA 115(10):2473–2477

Goncharov NV, Ukolov AI, Orlova TI et al (2015) Metabolomics: on the way to an integration of biochemistry, analytical chemistry, and informatics. Biol Bull Rev 5(4):296–307

Goncharov N, Maevsky E, Voitenko N et al (2016a) Nutraceuticals in sports activities and fatigue. In: Gupta RC (ed) Nutraceuticals: efficacy, safety and toxicity. Academic Press/Elsevier, Amsterdam, pp 177–188

Goncharov N, Orekhov A, Voitenko N et al (2016b) Organosulfur compounds as nutraceuticals. In: Gupta RC (ed) Nutraceuticals: efficacy, safety and toxicity. Academic Press/Elsevier, Amsterdam, pp 555–568

Goncharov NV, Belinskaia DA, Shmurak VI et al (2017a) Serum albumin binding and esterase activity: mechanistic interactions with organophosphates. Molecules 22(7):E1201

Goncharov NV, Nadeev AD, Jenkins RO, Avdonin PV (2017b) Markers and biomarkers of endothelium: when something is rotten in the state. Oxidative Med Cell Longev 2017:9759735, 27 pp

Goncharov NV, Terpilovskii MA, Shmurak VI et al (2017c) Comparative analysis of esterase and paraoxonase activities of different serum albumin species. J Evol Biochem Physiol 53(4):271–281

Goncharov NV, Terpilowski MA, Nadeev AD et al (2018) Cytotoxic power of hydrogen peroxide effect on endothelial cells *in vitro*. Biochemistry (Moscow), Supplement Series A: Membr Cell Biol 12(2):180–188

Greek R, Menache A (2013) Systematic reviews of animal models: methodology versus epistemology. Int J Med Sci 10 (3):206–221

Grimm H, Eggel M, Deplazes-Zemp A et al (2017) The road to hell is paved with good intentions: why harm-benefit analysis and its emphasis on practical benefit jeopardizes the credibility of research. Animals (Basel) 7(9):E34

Gupta RC (2016) Nutraceuticals: efficacy, safety and toxicity. Academic Press/Elsevier, Amsterdam, 1040 pp

Habs M, Binder K, Krauss S et al (2017) A balanced risk-benefit analysis to determine human risks associated with pyrrolizidine alkaloids (PA)—the case of tea and herbal infusions. Nutrients 9 (7):E717

Hassanin LA, Salama AM, Essa EA et al (2017) Potential role of some nutraceuticals in neurotoxicity induced by aluminum oxide in experimental animal model. Int J Adv Res Biol Sci 4(11):72–89

Hui L, Qigui L, Sashuang R et al (2014) Nonspecific changes in clinical laboratory indicators in unselected terminally ill patients and a model to predict survival time based on a prospective observational study. J Transl Med 12:78

Ismail T, Calcabrini C, Diaz AR et al (2016) Ellagitannins in cancer chemoprevention and therapy. Toxins (Basel) 8(5):E151

James KD, Kennett MJ, Lambert JD (2018) Potential role of the mitochondria as a target for the hepatotoxic effects of (-)-epigallocatechin-3-gallate in mice. Food Chem Toxicol 111:302–309

Jurgens TM, Whelan AM, Killian L et al (2012) Green tea for weight loss and weight maintenance in overweight or obese adults. Cochrane Database Syst Rev 12:CD008650

Kilkenny C, Browne WJ, Cuthill IC et al (2010) Improving bioscience research reporting: the ARRIVE guidelines for reporting animal research. PLoS Biol 8:e1000412

Kim KB, Nam YA, Kim HS et al (2014) α-Linolenic acid: nutraceutical, pharmacological and toxicological evaluation. Food Chem Toxicol 70:163–178

Knight A (2008) Systematic reviews of animal experiments demonstrate poor contributions toward human healthcare. Rev Recent Clin Trials 3(2):89–96

Konar D, Devarasetty M, Yildiz DV et al (2016) Lung-on-a-chip technologies for disease modeling and drug development. Biomed Eng Comput Biol 7(Suppl 1):17–27

Korf EA, Kubasov IV, Vonsky MS et al (2017) Green tea extract increases the expression of genes responsible for regulation of calcium balance in rat slow-twitch muscles under conditions of exhausting exercise. Bull Exp Biol Med 164(1):6–9

Kruger CL, Mann SW (2003) Safety evaluation of functional ingredients. Food Chem Toxicol 41(6):793–805

Mahady G, Parrot J, Lee C et al (2003) Botanical dietary supplement use in peri- and postmenopausal women. Menopause 10(1):65–72

Manteiga R, Park DL, Ali SS (1997) Risks associated with consumption of herbal teas. Rev Environ Contam Toxicol 150:1–30

Mason BC, Lavallee ME (2012) Emerging supplements in sports. Sports Health 4(2):142–146

Mazzanti G, Menniti-Ippolito F, Moro PA et al (2009) Hepatotoxicity from green tea: a review of the literature and two unpublished cases. Eur J Clin Pharmacol 65(4):331–341

Mazzanti G, Di Sotto A, Vitalone A (2015) Hepatotoxicity of green tea: an update. Arch Toxicol 89(8):1175–1191

Mazzanti G, Moro PA, Raschi E et al (2017) Adverse reactions to dietary supplements containing red yeast rice: assessment of cases from the Italian surveillance system. Br J Clin Pharmacol 83:894–908

Merz KH, Schrenk D (2016) Interim relative potency factors for the toxicological risk assessment of pyrrolizidine alkaloids in food and herbal medicines. Toxicol Lett 263:44–57

Mindukshev I, Kudryavtsev I, Serebriakova M et al (2016) Flow cytometry and light scattering technique in evaluation of

nutraceuticals. In: Gupta RC (ed) Nutraceuticals: efficacy, safety and toxicity. Academic Press/Elsevier, Amsterdam, pp 319–332
Mueller C (1999) The regulatory status of medical foods and dietary supplements in the United States. Nutrition 15:249–251
Munday R (2012) Harmful and beneficial effects of organic monosulfides, disulfides, and polysulfides in animals and humans. Chem Res Toxicol 25(1):47–60
Navarro SL, Li F, Lampe JW (2011) Mechanisms of action of isothiocyanates in cancer chemoprevention: an update. Food Funct 2(10):579–587
Ning J, Cui X, Kong X et al (2018) Risk assessment of genotoxic and carcinogenic alkenylbenzenes in botanical containing products present on the Chinese market. Food Chem Toxicol 115:344–357
Novozhilov AV, Tavrovskaya TV, Voitenko NG et al (2015) Efficacy of green tea extract in two exercise models. Bull Exp Biol Med 158 (3):342–345
Oelrichs PB, Pearce CM, Zhu J et al (1994) Isolation and structure determination of terminalin A toxic condensed tannin from Terminalia oblongata. Nat Toxins 2(3):144–150
Olson H, Betton G, Robinson D et al (2000) Concordance of the toxicity of pharmaceuticals in humans and in animals. Regul Toxicol Pharmacol 32:56–67
Päivärinta E, Pajari AM, Törrönen R et al (2006) Ellagic acid and natural sources of ellagitannins as possible chemopreventive agents against intestinal tumorigenesis in the Min mouse. Nutr Cancer 54(1):79–83
Patel S (2016) Functional food red yeast rice (RYR) for metabolic syndrome amelioration: a review on pros and cons. World J Microbiol Biotechnol 32:32–87
Pound P, Nicol CJ (2018) Retrospective harm benefit analysis of pre-clinical animal research for six treatment interventions. PLoS One 13(3):e0193758
Pound P, Ebrahim S, Sandercock P et al (2004) Where is the evidence that animal research benefits humans? BMJ 328:514–517
Prakash M, Shetty JK, Rao L et al (2008) Serum paraoxonase activity and protein thiols in chronic renal failure patients. Indian J Nephrol 18(1):13–16
Prokofieva DS, Goncharov NV (2014) Effects of biogenic and abiogenic disulphides upon endothelial cells in culture: comparison of three methods of viability assessment. Tsitologiya 56(6):410–418
Ried K, Fakler P (2014) Potential of garlic (Allium sativum) in lowering high blood pressure: mechanisms of action and clinical relevance. Integr Blood Press Control 7:71–82
Ronis M, Hennings L, Gomez-Acevedo H et al (2014) Different responses to soy and estradiol in the reproductive system of prepubertal male rats and neonatal male pigs. FASEB J 28:373.5
Ronis MJ, Gomez-Acevedo H, Blackburn ML et al (2016) Uterine responses to feeding soy protein isolate and treatment with 17-β-estradiol differ in ovariectomized female rats. Toxicol Appl Pharmacol 297:68–80
Ronis MJJ, Pedersen KB, Watt J (2018) Adverse effects of nutraceuticals and dietary supplements. Annu Rev Pharmacol Toxicol 58:583–601
Santini A, Cammarata SM, Capone G et al (2018) Nutraceuticals: opening the debate for a regulatory framework. Br J Clin Pharmacol 84(4):659–672
Sauer S, Luge T (2015) Nutriproteomics: facts, concepts, and perspectives. Proteomics 15(5–6):997–1013
Sauer S, Plauth A (2017) Health-beneficial nutraceuticals-myth or reality? Appl Microbiol Biotechnol 101(3):951–961
Shimshoni JA, Duebecke A, Mulder PP et al (2015) Pyrrolizidine and tropane alkaloids in teas and the herbal teas peppermint, rooibos and chamomile in the Israeli market. Food Addit Contam Part A Chem Anal Control Expo Risk Assess 32(12):2058–2067
Stahl BU, Kettrup A, Rozman K (1992) Comparative toxicity of four chlorinated dibenzo-*p*-dioxins (CDDs) and their mixture. Part I: acute toxicity and toxic equivalency factors (TEFs). Arch Toxicol 66(7):471–477
Stoner GD, Chen T, Kresty LA et al (2006) Protection against esophageal cancer in rodents with lyophilized berries: potential mechanisms. Nutr Cancer 54(1):33–46
Sugihara G, May R, Ye H et al (2012) Detecting causality in complex ecosystems. Science 338(6106):496–500
Tan KAL, Walker M, Morris K et al (2006) Infant feeding with soy formula milk: effects on puberty progression, reproductive function and testicular cell numbers in marmoset monkeys in adulthood. Hum Reprod 21:896–904
Terpilowski MA, Korf EA, Jenkins RO, Goncharov NV (2018) An algorithm for deriving combinatorial biomarkers based on ridge regression. J Bioinform Genom 1(6). https://doi.org/10.18454/jbg.2018.1.6.2
Terry C, Rasoulpour RJ, Knowles S et al (2015) Utilizing relative potency factors (RPF) and threshold of toxicological concern (TTC) concepts to assess hazard and human risk assessment profiles of environmental metabolites: a case study. Regul Toxicol Pharmacol 71(2):301–317
Toyokuni S (2014) Iron and thiols as two major players in carcinogenesis: friends or foes? Front Pharmacol 5:200
Ukolov AI, Kessenikh ED, Radilov AS, Goncharov NV (2017) Toxicometabolomics: identification of markers of chronic exposure to low doses of aliphatic hydrocarbons. J Evol Biochem Physiol 53 (1):25–36
Vanacloig-Pedros E, Proft M, Pascual-Ahuir A (2016) Different toxicity mechanisms for citrinin and ochratoxin A revealed by transcriptomic analysis in yeast. Toxins 8:273
Vaughan RA, Conn CA, Mermier CM (2014) Effects of commercially available dietary supplements on resting energy expenditure: a brief report. ISRN Nutr 2014:650264
Weinberg R (2010) Point: hypotheses first. Nature 464(7289):678
Wiedenfeld H, Edgar J (2011) Toxicity of pyrrolizidine alkaloids to humans and ruminants. Phytochem Rev 10:137–151
Zhang CL, Zeng T, Zhao XL, Xie KQ (2013) Garlic oil attenuated nitrosodiethylamine-induced hepatocarcinogenesis by modulating the metabolic activation and detoxification enzymes. Int J Biol Sci 9(3):237–245

Evaluation of Safety and Efficacy of Nutraceuticals Using *Drosophila* as an *in vivo* Tool

Anurag Sharma, Clinton D'Souza, Vipin Rai, and Subash Chandra Gupta

Abstract

The word nutraceutical refers to the natural bioactive compounds including functional food, medicinal food, and phytochemicals. The sources of nutraceuticals such as fruits, legumes, nuts, spices, and vegetables have been consumed since ancient time. The safety and efficacy of nutraceuticals has been examined using experimental models such as cell lines and rodents. During recent years, an emphasis has been given on the use of alternative experimental models from animal right organization. In this regard, the fruit fly, *Drosophila*, has emerged as an ideal alternative animal model to study the disease pathogenesis. More specifically, the high conservation of genome sequences, reduced genetic redundancy, short life cycle, and ease in the genetic manipulations are some of the advantages associated with *Drosophila*. The efficacy of nutraceuticals for human use against cancer, aging, and neurodegenerative diseases has also been examined using *Drosophila*. *Drosophila* has also been used to examine the safety of nutraceuticals. The metabolism, dose-response, and the pharmacological and toxicological profile of nutraceuticals have been examined. The common nutraceuticals evaluated using *Drosophila* include curcumin, celastrol, and genistein. The safety and efficacy of combination therapy has also been examined using *Drosophila*. In this chapter, we discuss the utility of *Drosophila* in examining the safety and efficacy of nutraceuticals against chronic diseases. The advantages and disadvantages associated with this experimental model are discussed.

Keywords

Alternative animal model · *Drosophila melanogaster* · Efficacy · Nutraceutical · Toxicity testing · Safety evaluation

1 Introduction

The modern medicine is highly advanced in the human society today. However, the concept of using our local food and natural products as medicine for the disease cure and improving quality of life was first recognized way back in the fifth century by Hippocrates. We now know that Mother Nature is a rich source of medicine and that as much as 70% of the current drugs have their origin from the Nature. The term nutraceutical (nutrition + pharmaceutical) that refers to a food or part of a food with nutritional values was first coined in 1989. Nutraceuticals are the natural bioactive compounds, which include dietary supplementary functional food, medicinal food, and phytochemicals. The use of nutraceuticals for chronic diseases has received considerable attention during the last two decades. Nutraceuticals have been consumed as dietary supplements since ancient time. It is estimated that on an average, 70% of Americans and 20% of Europeans are using nutraceuticals in some form every day (Ronis et al. 2018; The Lancet Gastroenterology Hepatology 2018). Growing scientific evidence indicates the beneficial effects of nutraceuticals for overall health or managing some health conditions (Cencic and Chingwaru 2010; Gupta et al. 2010a; Das et al. 2012; Abdelhamid et al. 2018). Although nutraceuticals are generally believed to be safe, some investigators have reported that these molecules

The original version of this chapter was revised. A correction to this chapter is available at https://doi.org/10.1007/978-3-030-04624-8_65.

A. Sharma (✉) · C. D'Souza
Division of Environmental Health and Toxicology, Nitte University Centre for Science Education and Research (NUCSER), Mangaluru, KA, India
e-mail: anuragsharma@nitte.edu.in

V. Rai · S. C. Gupta (✉)
Department of Biochemistry, Laboratory for Translational Cancer Research, Institute of Science, Banaras Hindu University, Varanasi, UP, India
e-mail: sgupta@bhu.ac.in

R. C. Gupta et al. (eds.), *Nutraceuticals in Veterinary Medicine*, https://doi.org/10.1007/978-3-030-04624-8_49

produce unexpected side effects and toxicity (Hudson et al. 2018; Ronis et al. 2018). Therefore, it is critical to evaluate the safety and efficacy of the nutraceuticals before they can be prescribed by the physicians.

Nutraceuticals exert their beneficial and harmful effects by interaction with multiple cell signaling molecules. However, the molecular basis for the efficacy of nutraceuticals continues to grow. In this context, the development of inexpensive, rapid, reliable, and high-throughput in vitro and in vivo assays will help in examining the metabolism, dose-response, and pharmacological and toxicological profile of these molecules. *Drosophila melanogaster*, an alternative to mammalian system, has been used as a versatile model in basic and applied biomedical research. This chapter provides a brief overview of the utility of *Drosophila* as an alternative model system to examine the efficacy and safety of nutraceuticals.

2 *Drosophila melanogaster* as a Model Organism

The choice of a model organism has always been at the core of biological research for many decades. Prokaryotes, protists, fungi, plants, and animals have been used as model organisms for a long time. Mice, rats, hamsters, rabbits, chicken, guinea pigs, *Xenopus*, primates, dogs, and cats are being used as surrogates to human. However, due to regulatory, scientific, and ethical concern, reduction or ban in the use of higher mammalian organism in research and testing has been recommended. In this context, Russell and Burch (1959) proposed "if animals were to be used in experiments, every effort should be made to replace them with non-sentient alternatives." Further a "3R" strategy has been developed which refers to *R*efinement, refining the experimental protocols to minimize unnecessary suffering to the animals; *R*eduction, reducing the animal numbers per experiment; and *R*eplacement, developing alternative methods which avoid or replace the use of animals (Tannenbaum and Bennett 2015). *D. melanogaster*, commonly known as "fruit fly," fulfills the criteria of "3R" and has been enormously used in biomedical research. In 1901, William Castle (1867–1962) laid the foundation of fly genetics. However, the power of fly genetics was truly explored by T. H. Morgan (1866–1945) and H. J. Muller by two great discoveries: (1) the concept of sex-linked inheritance (1926) and (2) mutation in genes by ionizing radiation (1945), respectively. At present, the versatility of this model has been proven in various disciplines of biomedical and basic research such as disease modeling, pharmacology, toxicology, genetics, development, and behavior (Pandey and Nichols 2011; Jansen et al. 2014; Rand et al. 2014). *Drosophila* embryos, larvae, and adults have been used to assess the efficacy and safety of various nutraceuticals. The following features make *Drosophila* an alternative model in nutraceutical research:

1. Closest invertebrate model organism to human; flies and human share functional homology with two-thirds of the human disease genes.
2. Fewer genes in flies reduce redundancy as compared to vertebrates.
3. Short life cycle (~10–12 days) and life span (60–70 days); high reproductive potentiality offers to study the effect of nutraceuticals on several generations.
4. *Drosophila* model has produced substantial insights in understanding the pathogenesis of human diseases.
5. *Drosophila* can be easily fed with nutraceuticals through food and the impact of the agents can be assessed at cellular and organismal levels. Likewise, the efficacy of the nutraceuticals can be examined by using fly disease model.
6. The conservation of signaling pathways and their regulators between flies and mammals may be pertinent to decipher the mechanistic insights into efficacy and safety of nutraceuticals.
7. The maximum information associated with *Drosophila* such as gene sequence, availability of transgenic lines, mutants, and RNAi stocks is available in the online database "FlyBase" (http://flybase.org/).
8. Use of *Drosophila* as an alternate animal model conforms to the regulations of animal rights organization and is recommended by European Centre for the Validation of Alternative Methods (ECVAM).

3 Utility of *Drosophila* in Examining the Anticancer Potential of Nutraceuticals

In recent years, nutraceuticals have emerged as potent agents for the prevention and treatment of cancer (Gupta et al. 2010a; Salami et al. 2013). Monotargeted drugs once called smart drugs or magic bullets have added only little to the overall survival of cancer patients. Moreover, these drugs produce numerous side effects and cannot be afforded by more than 80% of the human population. Accumulating evidence from preclinical and clinical studies suggests that nutraceuticals can target multiple cancer-related pathways. In addition, these agents have been consumed since ancient time and thus have well tested safety. *Drosophila* has been used as an alternate animal model to examine the efficacy of nutraceuticals. Numerous lines of evidence support the vital contribution of *Drosophila* not only in deciphering the fundamental molecular mechanism but also facilitating the drug development for cancer (Rudrapatna et al. 2012; Yadav et al. 2016). Also, *Drosophila* has been well established to deliver valuable information about the mode of action of various nutraceuticals in cancer models.

The health benefits of cruciferous vegetables such as broccoli and cabbage have been well documented (Armah et al. 2015). Lozano-Baena et al. (2015) reported antimutagenic property of Ethiopian mustard *Brassica carinata* and its glucosinolate sinigrin using somatic mutation and recombination test (SMART) assay in *Drosophila*. The anticancer effect of artemisinin and curcumin was studied by Das et al. (2014). *Lethal(2)giant* is a tumor suppressor gene and its deletion leads to a brain tumor in fly (Froldi et al. 2008). The administration of artemisinin and curcumin to *lethal(2)giant* organism enhanced ROS production (beyond the threshold) and was beneficial in several aspects of cancer pathogenesis. *UBIAD1/heix* (tumor suppressor gene) mutation in *Drosophila* exhibits strong lymphoma phenotypes including hypotrophy in the brain, hyperplasia of lymph gland (*Drosophila* hematopoietic organ), and hemocyte over proliferation. Vitamin K2 is a fat-soluble vitamin that has various health benefits including anticancer activities. Recently, Dragh et al. (2017) demonstrated that vitamin K2 exposure to *Drosophila* larvae prevents lymphoma progression by restoring mitochondria function. Many studies established that chemical and physical stressors can produce DNA instability, which may lead to malignant transformation (Wakeford et al. 2010; Rodgers et al. 2018). Nagpal and Abraham (2017) evaluated the antigenotoxic effect of β-carotene and tea polyphenols in γ-radiation-exposed *Drosophila*. The group demonstrated that co-exposure of β-carotene and tea polyphenols was able to decrease the γ-radiation-induced sex-linked recessive lethal (SLRL) mutation. Likewise, Kaya (2003) suggested the antigenotoxic effect of ascorbic acid against ethyl methanesulfonate (EMS), methyl methanesulfonate (MMS), and *N*-nitroso-*N*-ethylurea (ENU) exposure to *Drosophila*.

4 Utility of *Drosophila* in Examining the Efficacy of Nutraceuticals Against Aging

Aging is a multifactorial biological process that leads to a progressive decline in physiological and cognitive function (Khan et al. 2017). Although aging itself is a complex biological process, genetic and environmental factors make it even more complex. Therefore, the development of effective interventions for promoting healthy aging is one of the major and challenging biomedical concerns. Among different theories of aging, the free radical theory of aging is directly connected to nutraceuticals due to their free radical scavenging activities. Compared to other conventional biological models in aging research, *Drosophila* is a valuable organism to assess the efficacy and to identify the mode of action of nutraceuticals due to its short life span and well-defined genetics. Superoxide dismutase 1 (SOD1) and Forkhead box O (dfoxo), the critical players of the antioxidant defense system, are associated with aging. Laslo et al. (2013) and Wang et al. (2014a) demonstrated that supplementation of freeze-dried acai (*Euterpe oleracea*) pulp and cranberry promotes healthy aging in *SOD1* knockdown and *dfoxo* mutant flies. Similarly, riboflavin administration to *Drosophila* promoted the survival with increased reproductive capacity through modulation in antioxidant stress pathway (Zou et al. 2017). In addition, Chandrashekara et al. (2014) showed that curcumin supplementation leads to increased survival of *Drosophila* through modulation in energy metabolism such as increased levels of glycogen levels and pAkt and aconitase activity. Recently, transcriptome data of palm fruit juice (PFJ)-exposed *Drosophila* larvae and larval fat bodies revealed the modulation of stress-responsive genes (*heat shock proteins 70 and 26, SOD2, TOR*) that might be associated with increased longevity phenotype after PFJ supplementation to *Drosophila* (Leow et al. 2018). Nectarine (*Prunus persica* var. nectarine; subspecies of peach) contains dietary fibers, ample amount of vitamin C, β-carotenoids, and polyphenols that can promote a healthy life. Study on *Drosophila* showed that nectarine supplementation (4%) modulates glucose metabolism and controls oxidative damage leading to increased life span of the organism (Boyd et al. 2011). In another study, pharmacological inhibition of kynurenine from tryptophan by supplementation of berberine (bioactive component of natural Chinese medicine *Rhizoma coptidis*) leads to increase the life span of *Drosophila* (Navrotskaya et al. 2012). High-fat diet is associated with increased body weight and a decrease in life span (Muller et al. 2013; Lamont et al. 2016). Likewise, *Drosophila* fed with high-fat diet showed increased body weight and shortened life span. The effects of rosemary (perennial herb) extract on *Drosophila* life span under high-fat diet condition were evaluated by Wang et al. (2017). The group observed that supplementation of rosemary rescued the fat diet-induced body weight and enhanced the life span of *Drosophila* through increased antioxidant enzyme (SOD and CAT) activity and decreased *methuselah* expression. Similarly, supplementation of Royal Jelly proteins to *Drosophila* prolonged the life span via increased activity of the antioxidant enzyme and EGFR-mediated signaling pathway (Xin et al. 2016).

5 Utility of *Drosophila* in Examining the Efficacy of Nutraceuticals Against Neurodegenerative Diseases

The progressive and complex nature of neurodegenerative diseases poses a serious threat to human being worldwide. According to World Alzheimer's Report in 2015, an estimated 46.8 million people are affected with Alzheimer's disease (AD). Furthermore, this number is expected to touch

75 million in 2030. Likewise, more than ten million people are suffering with Parkinson's disease (PD) worldwide. Since the last decade, *Drosophila* has been extensively used to study various neurodegenerative diseases (Lu and Vogel 2009). Moreover, the use of this model to examine the therapeutic values of various agents has increased significantly. Epicatechin gallate (EC), a well-known antioxidant extracted from the green tea, has demonstrated multiple therapeutic potential (Chu et al. 2017). Siddique et al. (2014b) evaluated the efficacy of EC on transgenic *Drosophila* PD model (expression of human alpha synuclein in the fly brain using the UAS-Gal4 system) in the neurons. The results showed improved climbing ability and reduction in oxidative stress and apoptosis in the brain of diseased *Drosophila* after EC supplementation. The same group examined the therapeutic application of an Indian herb *Ocimum sanctum* L. (known as tulsi) against *Drosophila* PD model. Supplementation of *Ocimum sanctum* was found to enhance the locomotory ability in *Drosophila* PD model. This could be due to a reduction in the oxidative stress in the fly brain (Siddique et al. 2014a). Faust et al. (2009) used *Drosophila* DJ-1A model of PD to evaluate the therapeutic potential of Chinese herb celastrol and the antibiotic minocycline. The results revealed that administration of celastrol and minocycline inhibits the loss of dopaminergic neuron and restores the brain dopamine levels in the brain of *Drosophila* PD model. Likewise, spirulina (*Arthrospira platensis*), a biomass of cyanobacteria, is usually considered as superfood (Karkos et al. 2011). Administration of this spirulina to paraquat-sensitive PD *Drosophila* (DJ-1$\beta^{\Delta 93}$) increases life span and locomotor activity and decreases cellular stress in the organism (Kumar et al. 2017). The *Drosophila* strain mutant for PTEN-induced putative kinase 1 (PINK1B9) gene is another prevailing tool to study PD (Song et al. 2013).

Poddighe et al. (2014) examined the neuroprotective effects of *Mucuna pruriens* (contain genistein and polyunsaturated fatty acids) in *Drosophila* PINK1B9 mutant. The authors observed that supplementation of 0.1% *Mucuna pruriens* in the food of PINK1B9 mutant improved survival, rescued impaired climbing behavior, and restored brain bruchpilot and tyrosine hydroxylase expression. In another study, the neuroprotective effect of α-lipoic acid was studied against amyotrophic lateral sclerosis (ALS) using transgenic *Drosophila* (carrying G85R and G93A hSOD1 mutations) (Wang et al. 2018). The author observed that supplementation of α-lipoic acid exhibits a neuroprotection against ALS by upregulating antioxidant activity through the activation of the ERK/Akt pathway. Moreover, loss of *parkin* in *Drosophila* brain is associated with PD through evaluated oxidative stress and mitochondria dysfunction (Julienne et al. 2017). Administration of folic acid to *parkin* knockdown in *Drosophila* brain was associated with amelioration of locomotor ability, reduced mortality, oxidative stress, and restored energy metabolism in the organism (Srivastav et al. 2015). Mohandas et al. (2017) showed that manganese exposure to *Drosophila* induces locomotory dysfunction which can be ameliorated by supplementation of whey protein isolate. Likewise, paraquat exposure to *Drosophila* leads to severe neurodegeneration through altered mitochondria content, increased ROS production, and accelerated aging. The authors showed that administration of eicosapentaenoic and docosahexaenoic acid (omega-3 fatty acid) in combination with PQ protects the organism against neurodegeneration by inhibiting H_2O_2 production and reducing the levels of glutathione and oxidized proteins (de Oliveira Souza et al. 2017). Formation of amyloid plaques due to the aggregation of amyloid-β peptides is one of the hallmarks of AD (Murphy and LeVine 2010). The effect of flavonoid derivative against *Drosophila* AD model (expression of $A\beta_{42}$ in *Drosophila* brain tissue using brain driver; elav-Gal4) was evaluated by Singh et al. (2014). The authors observed that oral administration of flavonoid to AD flies exhibited anti-Aβ activity by improving motor function and increasing longevity. The authors also observed 70% rescue in eye degeneration ($A\beta_{42}$ expression using eye-specific driver; ey-gal4) phenotype by oral administration of flavonoid at 100 μM. In another study, a neuroprotective effect of *Curcuma longa* was observed in another transgenic *Drosophila* model (BACE-1 expression using brain- and eye-specific driver) (Wang et al. 2014b).

6 Utility of *Drosophila* in the Toxicity Evaluation of Nutraceuticals

During the last two decades, several simple and reliable cellular and organismal assays have been developed in *Drosophila* model for toxicity evaluation (Sharma et al. 2012; Chandra et al. 2015; Pandey et al. 2015). These assays can be used to evaluate the safety of nutraceuticals (Fig. 1). Genistein is a plant-derived estrogen with therapeutic potential against human diseases such as cancer, osteoporosis, and cardiovascular diseases (Miao et al. 2012; Spagnuolo et al. 2015; De Gregorio et al. 2017). Altun et al. (2011) observed a 45% reduction in mean life span of *Drosophila* population after genistein exposure. However, Siddique et al. (2018) demonstrated the reduction of PD symptoms in genistein-exposed transgenic *Drosophila*. The excessive consumption of green tea polyphenol (GTP) produces negative effects in *Drosophila* (Lopez et al. 2016). The exposure of *Drosophila* larvae to GTP (10 mg/mL) was found to delay the development of an organism. Furthermore, reduction in reproductive output, morphological defects in reproductive organs, and reduction in the survival of female organisms were observed. The exposure of *Drosophila* to *Croton campestris* and *Duguetia furfuracea* (medicinal plants originating in Brazil;

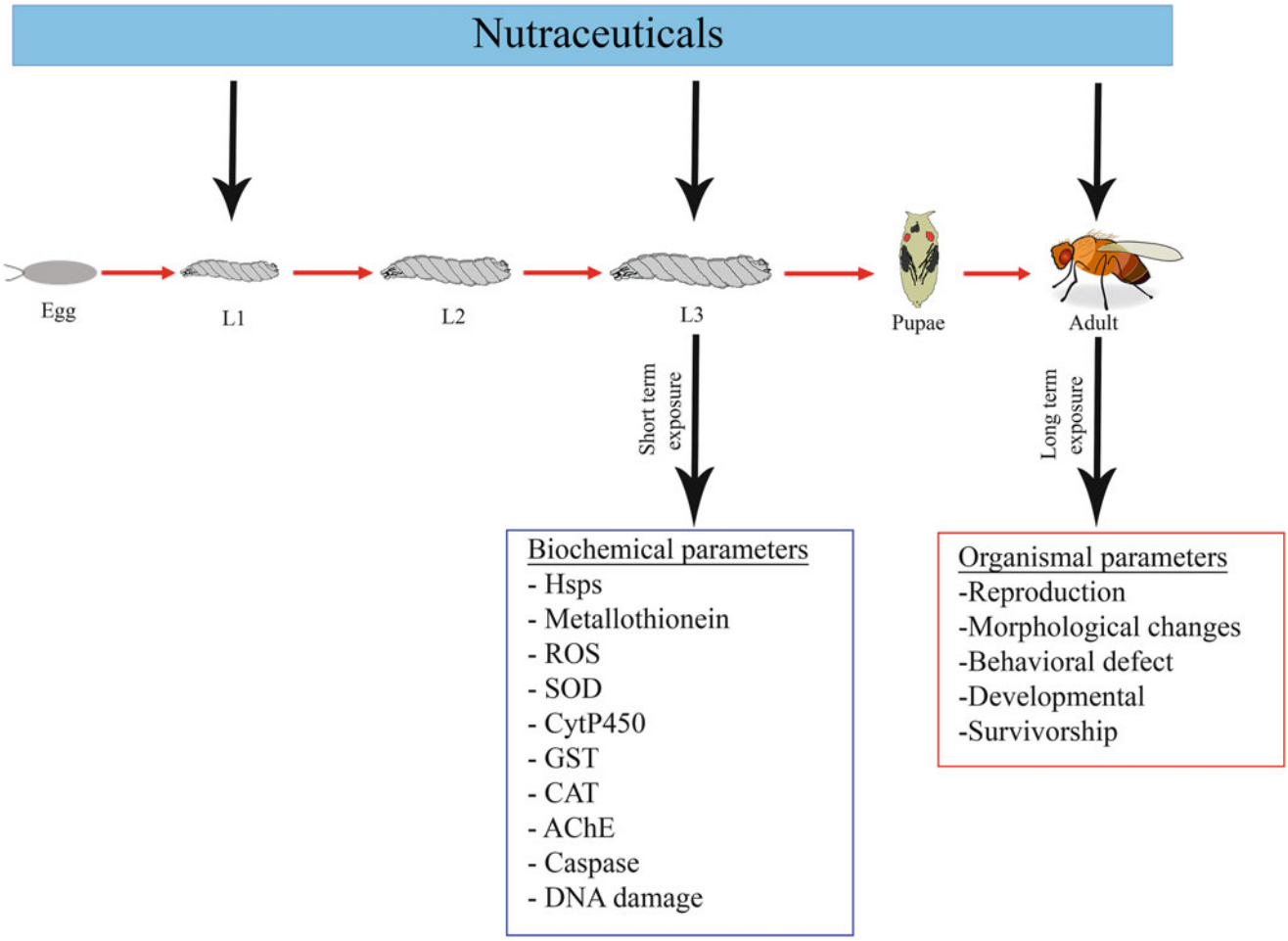


Fig. 1 Schematic diagram showing the utility of *Drosophila* in the assessment of efficacy and toxicity of nutraceuticals at the molecular and organismal level

used to treat numerous health problems) was found to increase mortality and impaired locomotor behavior due to oxidative stress (Valeria Soares de Araujo Pinho et al. 2014; Junior et al. 2016). Boulahbel (2015) examined the developmental and reproductive effect of neem oil on *Drosophila*. Neem oil exposure to *Drosophila* resulted in reduced pupal weight and fecundity and fertility.

Transgenic *Drosophila* has also been used for the assessment of nutraceutical toxicity. Using transgenic technology, the safety- and toxicity-associated genes are easily assessed. For example, expression of heat shock proteins (HSPs) is considered as first-tier bioindicator of cellular stress. Ample evidence has shown that the activity of *hsps* (*hsp70*, *hsp83*, *hsp26*) promoter can be evaluated by tagging it with a reporter gene such as *β-galactosidase* or *GFP* for the assessment of toxicity (Gupta et al. 2010b). Equally, the activity of glutathione S-transferases (GST) is directly proportional to increased ROS levels. Transgenic *Drosophila* carrying GST promoter tagged with β-galactosidase or GFP (*GST-lacZ or GST-GFP*) can be used for the easy assessment of ROS levels after nutraceutical administration (Veal et al. 2002; Sykiotis and Bohmann 2008; Louradour et al. 2017). Expression of the *reaper* and *hid* is considered as the initiators of cell death (Xu et al. 2009). Assessment of β-galactosidase in *reaper-lacZ* or *hid-lacZ* transgenic *Drosophila* can be used as cell death evaluation after nutraceutical administration (Fan et al. 2010; Wu et al. 2015). The Gal4/UAS system is one of the most powerful tools for in vivo target gene expression. The yeast transcriptional activator Gal4 binds to the upstream activating sequence (UAS) and activates transcription. Gal4 activity stimulates the transcription of a reporter gene under the control of UAS in *Drosophila* (Duffy 2002). This system can be used to decipher the molecular insights of the nutraceuticals.

7 Concluding Remarks and Future Directions

Due to the increasing popularity of nutraceutical use in our daily life, the safety and efficacy assessment of these agents are crucial. This chapter describes the *Drosophila* as an excellent in vivo model system for efficacy and safety

Table 1 Assessment of safety and efficacy of nutraceuticals and plant extracts using *Drosophila* model system

S. no.	Name of the agent	Source of the agent	Effect of the agent	References
1.	Glucosinolate sinigrin	*Brassica carinata*	Anticancer	Lozano-Baena et al. (2015)
2.	Artemisinin	*Artemisia annua*	Anticancer	Das et al. (2014)
3.	Curcumin	*Curcuma longa*	Anticancer	Das et al. (2014)
4.	Vitamin K2	Fermented foods	Anticancer	Dragh et al. (2017)
5.	β-carotene	Yellow and orange fruits	Anticancer	Nagpal and Abraham (2017)
6.	Tea polyphenols	*Camellia sinensis*	Anticancer	Nagpal and Abraham (2017)
7.	Ascorbic acid	Fruits and vegetables	Anticancer	Kaya (2003)
8.	Freeze-dried acai	*Euterpe oleracea*	Antiaging	Laslo et al. (2013)
9.	Cranberry	*Vaccinium macrocarpon*	Antiaging	Wang et al. (2014a)
10.	Riboflavin	Leafy vegetables	Stress reliever	Zou et al. (2017)
11.	Curcumin	*Curcuma longa*	Antiaging	Chandrashekara et al. (2014)
12.	Palm fruit juice	*Borassus flabellifer*	Increased longevity	Leow et al. (2018)
13.	Nectarine	*Prunus persica*	Increased longevity	Boyd et al. (2011)
14.	Berberine	*Berberis aristata*	Increased longevity	Navrotskaya et al. (2012)
15.	Rosemary extract	*Rosmarinus officinalis*	Increased longevity	Wang et al. (2017)
16.	Royal jelly proteins	Honey	Increased longevity	Xin et al. (2016)
17.	Epicatechin gallate	*Camellia sinensis*	Neuroprotective and antioxidant	Siddique et al. (2014b)
18.	Tulsi extract	*Ocimum sanctum* L.	Neuroprotective	Siddique et al. (2014a)
19.	Celastrol	*Celastrus regelii*	Neuroprotective	Faust et al. (2009)
20.	Minocycline	Natural tetracycline antibiotics	Neuroprotective	Faust et al. (2009)
21.	Spirulina	*Arthrospira platensis*	Neuroprotective	Kumar et al. (2017)
22.	Genistein extract	*Mucuna pruriens*	Neuroprotective	Poddighe et al. (2014)
23.	α-Lipoic acid	Dietary supplements	Neuroprotective	Wang et al. (2018)
24.	Whey protein isolate	Dietary supplements	Neuroprotective	Mohandas et al. (2017)
25.	Omega-3 fatty acid	Dietary supplements	Neuroprotective	de Oliveira Souza et al. (2017)
26.	Turmeric extract	*Curcuma longa*	Neuroprotective	Wang et al. (2014b)
27.	Genistein	Phytoestrogen	Reduction in life span	Altun et al. (2011)
28.	Green tea polyphenol	*Camellia sinensis*	Developmental delay	Lopez et al. (2016)
29.	Extract	*Croton campestris*	Oxidative damage and reduction in life span	Junior et al. (2016)
30.	Extract	*Duguetia furfuracea*	Oxidative damage and reduction in life span	Valeria Soares de Araujo Pinho et al. (2014)
31.	Neem oil	*Azadirachta indica*	Developmental defects	Boulahbel (2015)

assessments of nutraceuticals (Table 1). Because of significant similarity with the human genome, short life span, and high reproductive capacity, *Drosophila* can be used for the assessment of safety and efficacy of nutraceuticals. Several assays at the cellular and organismal level have been developed in this model to give a comprehensive view of safety/efficacy of nutraceutical compounds. In addition, reporter gene assay and the UAS-Gal4 system can also be utilized for the rapid assessment and to decode the mechanistic understanding of these agents, respectively.

Acknowledgment Gupta's and Sharma's laboratories are supported by Science and Engineering Research Board (ECR/2016/000034 to SCG and ECR/2016/001863 to AS). Additionally, Gupta's laboratory is supported by University Grants Commission [No. F.30-112/2015 (BSR)]. CD wishes to thank the Directorate of Minorities (DOM/FELLOWSH I P/CR-33/2018-19) for the financial support.

References

Abdelhamid AS, Brown TJ, Brainard JS et al (2018) Omega-3 fatty acids for the primary and secondary prevention of cardiovascular disease. Cochrane Database Syst Rev 7:CD003177

Altun D, Uysal H, Askin H et al (2011) Determination of the effects of genistein on the longevity of *Drosophila melanogaster* meigen (Diptera; Drosophilidae). Bull Environ Contam Toxicol 86:120–123

Armah CN, Derdemezis C, Traka MH et al (2015) Diet rich in high glucoraphanin broccoli reduces plasma LDL cholesterol: evidence from randomised controlled trials. Mol Nutr Food Res 59:918–926

Boulahbel B (2015) Activity of neem oil in *Drosophila melanogaster*: toxicity and delayed effect on the progeny. J Entomol Zool Stud 3:306–310

Boyd O, Weng P, Sun X et al (2011) Nectarine promotes longevity in *Drosophila melanogaster*. Free Radic Biol Med 50:1669–1678

Cencic A, Chingwaru W (2010) The role of functional foods, nutraceuticals, and food supplements in intestinal health. Nutrients 2:611–625

Chandra S, Pandey A, Chowdhuri DK (2015) MiRNA profiling provides insights on adverse effects of Cr(VI) in the midgut tissues of *Drosophila melanogaster*. J Hazard Mater 283:558–567

Chandrashekara KT, Popli S, Shakarad MN (2014) Curcumin enhances parental reproductive lifespan and progeny viability in *Drosophila melanogaster*. Age 36:9702

Chu C, Deng J, Man Y et al (2017) Green tea extracts epigallocatechin-3-gallate for different treatments. Biomed Res Int 2017:5615647

Das L, Bhaumik E, Raychaudhuri U et al (2012) Role of nutraceuticals in human health. J Food Sci Technol 49:173–183

Das SS, Nanda GG, Alone DP (2014) Artemisinin and curcumin inhibit Drosophila brain tumor, prolong life span, and restore locomotor activity. IUBMB Life 66:496–506

De Gregorio C, Marini H, Alibrandi A et al (2017) Genistein supplementation and cardiac function in postmenopausal women with metabolic syndrome: results from a pilot strain-echo study. Nutrients 9:584

de Oliveira Souza A, Couto-Lima CA, Rosa Machado MC et al (2017) Protective action of Omega-3 on paraquat intoxication in *Drosophila melanogaster*. J Toxicol Environ Health A 80:1050–1063

Dragh MA, Xu Z, Al-Allak ZS et al (2017) Vitamin K2 prevents lymphoma in *Drosophila*. Sci Rep 7:17047

Duffy JB (2002) GAL4 system in *Drosophila*: a fly geneticist's Swiss army knife. Genesis 34:1–15

Fan Y, Lee TV, Xu D et al (2010) Dual roles of Drosophila p53 in cell death and cell differentiation. Cell Death Differ 17:912–921

Faust K, Gehrke S, Yang Y et al (2009) Neuroprotective effects of compounds with antioxidant and anti-inflammatory properties in a Drosophila model of Parkinson's disease. BMC Neurosci 10:109

Froldi F, Ziosi M, Tomba G et al (2008) Drosophila lethal giant larvae neoplastic mutant as a genetic tool for cancer modeling. Curr Genomics 9:147–154

Gupta SC, Kim JH, Prasad S et al (2010a) Regulation of survival, proliferation, invasion, angiogenesis, and metastasis of tumor cells through modulation of inflammatory pathways by nutraceuticals. Cancer Metastatis Rev 29:405–434

Gupta SC, Sharma A, Mishra M et al (2010b) Heat shock proteins in toxicology: how close and how far? Life Sci 86:377–384

Hudson A, Lopez E, Almalki AJ et al (2018) A review of the toxicity of compounds found in herbal dietary supplements. Planta Med 84:613–626

Jansen RL, Brogan B, Whitworth AJ et al (2014) Effects of five Ayurvedic herbs on locomotor behaviour in a *Drosophila melanogaster* Parkinson's disease model. Phytother Res 28:1789–1795

Julienne H, Buhl E, Leslie DS et al (2017) Drosophila PINK1 and parkin loss-of-function mutants display a range of non-motor Parkinson's disease phenotypes. Neurobiol Dis 104:15–23

Junior FE, Macedo GE, Zemolin AP et al (2016) Oxidant effects and toxicity of Croton campestris in *Drosophila melanogaster*. Pharm Biol 54:3068–3077

Karkos PD, Leong SC, Karkos CD et al (2011) Spirulina in clinical practice: evidence-based human applications. Evid-Based Complement Alternat Med 2011:531053

Kaya B (2003) Anti-genotoxic effect of ascorbic acid on mutagenic dose of three alkylating agents. Turk J Biol 27:241–246

Khan SS, Singer BD, Vaughan DE (2017) Molecular and physiological manifestations and measurement of aging in humans. Aging Cell 16:624–633

Kumar A, Christian PK, Panchal K et al (2017) Supplementation of spirulina (Arthrospira platensis) improves lifespan and locomotor activity in paraquat-sensitive DJ-1beta(Delta93) flies, a Parkinson's disease model in drosophila melanogaster. J Diet Suppl 14:573–588

Lamont BJ, Waters MF, Andrikopoulos S (2016) A low-carbohydrate high-fat diet increases weight gain and does not improve glucose tolerance, insulin secretion or beta-cell mass in NZO mice. Nutr Diabetes 6:e194

Laslo M, Sun X, Hsiao CT et al (2013) A botanical containing freeze dried acai pulp promotes healthy aging and reduces oxidative damage in sod1 knockdown flies. Age 35:1117–1132

Leow SS, Luu A, Shrestha S et al (2018) *Drosophila* larvae fed palm fruit juice (PFJ) delay pupation via expression regulation of hormetic stress response genes linked to ageing and longevity. Exp Gerontol 106:198–221

Lopez TE, Pham HM, Barbour J et al (2016) The impact of green tea polyphenols on development and reproduction in *Drosophila melanogaster*. J Funct Foods 20:556–566

Louradour I, Sharma A, Morin-Poulard I et al (2017) Reactive oxygen species-dependent Toll/NF-kappaB activation in the *Drosophila* hematopoietic niche confers resistance to wasp parasitism. elife 6: e25496

Lozano-Baena MD, Tasset I, Obregon-Cano S et al (2015) Antigenotoxicity and tumor growing inhibition by leafy *Brassica carinata* and Sinigrin. Molecules 20:15748–15765

Lu B, Vogel H (2009) *Drosophila* models of neurodegenerative diseases. Annu Rev Pathol 4:315–342

Miao Q, Li JG, Miao S et al (2012) The bone-protective effect of genistein in the animal model of bilateral ovariectomy: roles of phytoestrogens and PTH/PTHR1 against post-menopausal osteoporosis. Int J Mol Sci 13:56–70

Mohandas G, Rao SV, Muralidhara R (2017) Whey protein isolate enrichment attenuates manganese-induced oxidative stress and neurotoxicity in *Drosophila melanogaster*: relevance to Parkinson's disease. Biomed Pharmacother 95:1596–1606

Muller AP, Dietrich Mde O, Martimbianco de Assis A et al (2013) High saturated fat and low carbohydrate diet decreases lifespan independent of body weight in mice. Longev Healthspan 2:10

Murphy MP, LeVine H 3rd (2010) Alzheimer's disease and the amyloid-beta peptide. J Alzheimers Dis 19:311–323

Nagpal I, Abraham SK (2017) Protective effects of tea polyphenols and beta-carotene against gamma-radiation induced mutation and oxidative stress in *Drosophila melanogaster*. Genes Environ 39:24

Navrotskaya VV, Oxenkrug G, Vorobyova LI et al (2012) Berberine prolongs life span and stimulates locomotor activity of *Drosophila melanogaster*. Am J Plant Sci 3:1037–1040

Pandey UB, Nichols CD (2011) Human disease models in *Drosophila melanogaster* and the role of the fly in therapeutic drug discovery. Pharmacol Rev 63:411–436

Pandey A, Khatoon R, Saini S et al (2015) Efficacy of methuselah gene mutation toward tolerance of dichlorvos exposure in Drosophila melanogaster. Free Radic Biol Med 83:54–65

Poddighe S, De Rose F, Marotta R et al (2014) *Mucuna pruriens* (Velvet bean) rescues motor, olfactory, mitochondrial and synaptic impairment in PINK1B9 *Drosophila melanogaster* genetic model of Parkinson's disease. PLoS One 9:e110802

Rand MD, Montgomery SL, Prince L et al (2014) Developmental toxicity assays using the *Drosophila* model. Curr Protoc Toxicol 59(1):12 11–12 20

Rodgers KM, Udesky JO, Rudel RA et al (2018) Environmental chemicals and breast cancer: an updated review of epidemiological literature informed by biological mechanisms. Environ Res 160:152–182

Ronis MJJ, Pedersen KB, Watt J (2018) Adverse effects of nutraceuticals and dietary supplements. Annu Rev Pharmacol Toxicol 58:583–601

Rudrapatna VA, Cagan RL, Das TK (2012) *Drosophila* cancer models. Dev Dyn 241:107–118

Russell WMS, Burch RL (1959) The principles of humane experimental technique. Methuen, London

Salami A, Seydi E, Pourahmad J (2013) Use of nutraceuticals for prevention and treatment of cancer. Iran J Pharm Res 12:219–220

Sharma A, Mishra M, Shukla AK et al (2012) Organochlorine pesticide, endosulfan induced cellular and organismal response in Drosophila melanogaster. J Hazard Mater 221–222:275–287

Siddique YH, Faisal M, Naz F et al (2014a) Role of Ocimum sanctum leaf extract on dietary supplementation in the transgenic *Drosophila* model of Parkinson's disease. Chin J Nat Med 12:777–781

Siddique YH, Jyoti S, Naz F (2014b) Effect of epicatechin gallate dietary supplementation on transgenic *Drosophila* model of Parkinson's disease. J Diet Suppl 11:121–130

Siddique YH, Naz F, Jyoti S et al (2018) Effect of genistein on the transgenic *Drosophila* model of Parkinson's disease. J Diet Suppl:1–14

Singh SK, Gaur R, Kumar A et al (2014) The flavonoid derivative 2-(4′ Benzyloxyphenyl)-3-hydroxy-chromen-4-one protects against Abeta42-induced neurodegeneration in transgenic *Drosophila*: insights from *in silico* and *in vivo* studies. Neurotox Res 26:331–350

Song S, Jang S, Park J et al (2013) Characterization of PINK1 (PTEN-induced putative kinase 1) mutations associated with Parkinson disease in mammalian cells and *Drosophila*. J Biol Chem 288:5660–5672

Spagnuolo C, Russo GL, Orhan IE et al (2015) Genistein and cancer: current status, challenges, and future directions. Adv Nutr 6:408–419

Srivastav S, Singh SK, Yadav AK et al (2015) Folic acid supplementation rescues anomalies associated with knockdown of parkin in dopaminergic and serotonergic neurons in Drosophila model of Parkinson's disease. Biochem Biophys Res Commun 460:780–785

Sykiotis GP, Bohmann D (2008) Keap1/Nrf2 signaling regulates oxidative stress tolerance and lifespan in *Drosophila*. Dev Cell 14:76–85

Tannenbaum J, Bennett BT (2015) Russell and Burch's 3Rs then and now: the need for clarity in definition and purpose. J Am Assoc Lab Anim Sci 54:120–132

The Lancet Gastroenterology Hapatology (2018) Herbal assault: liver toxicity of herbal and dietary supplements. Lancet Gastroenterol Hapatol 3:141

Valeria Soares de Araujo Pinho F, Felipe da Silva G, Echeverria Macedo G et al (2014) Phytochemical constituents and toxicity of *Duguetia furfuracea* hydroalcoholic extract in *Drosophila melanogaster*. Evid Based Complement Alternat Med 2014:838101

Veal EA, Toone WM, Jones N et al (2002) Distinct roles for glutathione S-transferases in the oxidative stress response in Schizosaccharomyces pombe. J Biol Chem 277:35523–35531

Wakeford R, Little MP, Kendall GM (2010) Risk of childhood leukemia after low-level exposure to ionizing radiation. Expert Rev Hematol 3:251–254

Wang C, Yolitz J, Alberico T et al (2014a) Cranberry interacts with dietary macronutrients to promote healthy aging in Drosophila. J Gerontol A Biol Sci Med Sci 69:945–954

Wang X, Kim JR, Lee SB et al (2014b) Effects of curcuminoids identified in rhizomes of Curcuma longa on BACE-1 inhibitory and behavioral activity and lifespan of Alzheimer's disease *Drosophila* models. BMC Complement Altern Med 14:88

Wang HL, Sun ZO, Rehman RU et al (2017) Rosemary extract-mediated lifespan extension and attenuated oxidative damage in *Drosophila melanogaster* fed on high-fat diet. J Food Sci 82:1006–1011

Wang T, Cheng J, Wang S et al (2018) Alpha-lipoic acid attenuates oxidative stress and neurotoxicity via the ERK/Akt-dependent pathway in the mutant hSOD1 related *Drosophila* model and the NSC34 cell line of amyotrophic lateral sclerosis. Brain Res Bull 140:299–310

Wu C, Chen Y, Wang F et al (2015) Pelle modulates dFoxO-mediated cell death in *Drosophila*. PLoS Genet 11:e1005589

Xin XX, Chen Y, Chen D et al (2016) Supplementation with major Royal-Jelly Proteins increases lifespan, feeding, and fecundity in *Drosophila*. J Agric Food Chem 64:5803–5812

Xu D, Woodfield SE, Lee TV et al (2009) Genetic control of programmed cell death (apoptosis) in *Drosophila*. Fly 3:78–90

Yadav AK, Srikrishna S, Gupta SC (2016) Cancer drug development using *Drosophila* as an *in vivo* tool: from bedside to bench and back. Trends Pharmacol Sci 37:789–806

Zou YX, Ruan MH, Luan J et al (2017) Anti-aging effect of riboflavin via endogenous antioxidant in fruit fly *Drosophila Melanogaster*. J Nutr Health Aging 21:314–319

Biomarkers of Foods and Nutraceuticals: Applications in Efficacy, Safety, and Toxicity

Ramesh C. Gupta, Ajay Srivastava, Anita Sinha, and Rajiv Lall

Abstract

Currently, nutraceuticals, primarily plant extracts or their constituents, are used for prevention and treatment of diseases in both humans and animals. The use of nutraceuticals (commonly referred to as herbal supplements), compared to modern medicines, has become more popular because they (1) offer health benefits, (2) are effective in prevention and treatment of diseases, (3) have a wide margin of safety, (4) are more compatible with other nutraceuticals and/or modern medicines in polypharmacy, (5) are inexpensive, and (6) are easily available and do not require physician's prescription. Countless plant extracts have been investigated for their detailed phytochemistry, phytopharmacology, and phytotherapy/phytomedicine. A single plant can have more than one hundred phytoconstituents with many of them having biological and pharmacological potential. This makes evaluation of phytoconstituents efficacy and safety very complicated and challenging. Another challenging issue with nutraceuticals is contamination with metals, pesticides, and mycotoxins and adulteration with illegal drugs. Compared to modern medicines, nutraceuticals lack information on pharmacokinetics/toxicokinetics and interaction with other nutraceuticals and therapeutic drugs and biomarkers. The presence of food compounds, phytochemicals, and/or their metabolites in body fluids and tissues serves as biomarkers. This chapter describes the biomarkers of common foods of nutritional value and plant extracts of nutraceutical potential.

Keywords

Biomarkers · Nutraceuticals · Veterinary nutraceuticals · Phytochemicals · Polyphenols · Functional foods · Phytotherapy

1 Introduction

The necessity and importance of biomarkers in the field of dietary/functional foods and nutraceuticals have been well recognized around the world (Davis and Milner 2007; Caligiani et al. 2010; Gupta 2016; Savolainen et al. 2017). Biomarkers are often used to identify exposure of animals and humans to a type and composition of food, plant extracts, or their ingredients and for the evaluation for efficacy and safety. Dietary ingredients not only provide required nutrients but also aid in managing certain chronic diseases, such as diabetes, cardiovascular, gastrointestinal, cognition, cancer, and other health conditions. Phytochemistry of plant extracts has proven to be very complex because a single plant can have more than one hundred chemical constituents, for example, *Perilla frutescens* L. (Yu et al. 2017a). As a result, a plant extract can exert multiple biological and pharmacological effects. Molecular targets and mechanisms have been identified for many bioactive components present in foods and nutraceuticals (Gupta 2016). Different plant extracts can be used in combination with an objective to be more effective than a single plant extract against a disease, and biomarkers can aid in explaining the dosage-toxicity-efficacy relationships (Shen et al. 2017). A large number of plant extracts exert antioxidant and anti-inflammatory effects, and therefore they are used in prevention and treatment of many diseases in animals and humans (Gessner et al. 2016; Gupta 2016). By now, the consequences of oxidative stress and inflammation in animal health and their impact on the animal industry are well recognized. Many phytochemicals have anti-inflammatory and analgesic effects and therefore can be

R. C. Gupta (✉)
Toxicology Department, Breathitt Veterinary Center, Murray State University, Hopkinsville, KY, USA
e-mail: rgupta@murraystate.edu

A. Srivastava · A. Sinha · R. Lall
Vets Plus Inc., Menomonie, WI, USA

R. C. Gupta et al. (eds.), *Nutraceuticals in Veterinary Medicine*, https://doi.org/10.1007/978-3-030-04624-8_50

indicated in pain management (Alonso-Castro et al. 2017; Tamrat et al. 2017). Those nutraceuticals that exert immunomodulatory and apoptotic activity against tumor or neoplastic cells can be employed as antitumor and/or anticancer agents (Ayeka et al. 2017; Davis and Milner 2007; Yu et al. 2017a, b). In production animals, nutraceuticals are used to enhance their growth and performance, in addition to preventing and treating diseases. Today, the use of prebiotics, probiotics, and synbiotics is greater than ever before in both monogastrics and ruminants. Some nutraceuticals, such as resveratrol, are used for life extension, in addition to their antioxidative and cardioprotective activities (Gessner et al. 2016). Many nutraceuticals are used to ameliorate poisonings caused by metals, pesticides, mycotoxins, and venoms. This chapter is prepared to address some of the biomarkers of exposure, effects, and susceptibility to foods, nutraceuticals, and the plant extracts that have potential for nutraceutical use.

2 Complexities in Food and Nutraceutical Biomarkers

In the food and nutraceutical industries, biomarkers play pivotal roles in identification and quantification of chemical ingredients, pharmacokinetics and toxicokinetics, food-herb-drug interactions, safety and toxicity evaluations, and decision-making policies. Biomarkers are also important for identification and quantitation of illegal drugs that are involved in adulterating nutraceuticals (Gupta 2016; Cheng et al. 2017). However, it needs to be mentioned that biomarker research is very expensive, as it requires highly qualified chemists, biochemists, pharmacologists, and toxicologists, as well as state-of-the-art equipment.

Matrices of both food and phytoextracts are complex and require highly sophisticated and sensitive equipment, such as HPLC-MS, HPLC-DAD-MS, UPLC-PDA, UHPLC-MS-MS, UHPLC-Q-TOF-MS, GC-MS, GC-MS-MS, MALDI-TOF-MS, NMR/MRI, micellar electrokinetic chromatography, and many others. Currently, an *omics* (nutrigenomics, proteomics, metabolomics, and nutriphenomics) approach has also become very useful in identifying biomarkers in the field of food and nutraceutical research. In food science, separation, isolation, identification, confirmation, and quantification of bioactive compounds are needed before they can be qualified and validated as biomarkers.

Complexity comes from multiple compounds present in a single food ingredient. For example, anthocyanins in the Chilean maqui berry are characterized by using HPLC-DAD-ESI/MS^n and NMR spectroscopy (Brauch et al. 2017). Soybean extracts contain genistein, daidzein, genistin, daidzin, glycitein, malonylgenistin, acetyl genistin, malonyldaidzin, and many other bioactive compounds; and their identification, confirmation, and quantification are conducted by 1H NMR spectrometry (Caligiani et al. 2010). Scientists have extensively explored the use of NMR and MRI to develop a wide range of applications for food research and manufacturing. MRI offers not only information about the chemical composition and internal structure of certain foods but also permits monitoring of internal compositional and structural modification of foods when they experience different agricultural practices and industrial processes (Marcone et al. 2013). Recently, Rossi et al. (2017) identified 32 proteins (including novel storage proteins: phaseolins, legumins, and lectins) in the Brazilian common bean (*Phaseolus vulgaris* L.) using 2-D gel electrophoresis and tandem mass spectrometry (MS/MS). The fermentation process further complicates identification and quantification of compounds as it modifies the chemical structures of their metabolites.

The matrix of a plant extract is even more complex than the food matrix. For example, bee pollen from nectar plants (*Brassica campestris* L., *Camellia sinensis* L., *Nelumbo nucifera* Gaertn.) is characterized for identification and quantitation of bioactive compounds (phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylserine, lysophosphatidylcholine, ceramide, diglyceride, triglyceride, fatty acids, phenols, flavonoids, carbohydrates, vitamins, and minerals) using UPLC-Q-exactive orbitrap/MS-based lipidomics (Li et al. 2017a). Very recently, Sima et al. (2018) used micellar electrokinetic chromatography (chemometric assessment) and HPLC to fingerprint and authenticate herbal medicines. Measurement of an individual phytochemical and/or its metabolite(s) needs to be quantitated in the body fluid and/or tissues to serve as biomarkers of exposure. Biomarkers of effects are also measured in the body fluids. For example, ABTS for antioxidative state and cytokines and prostaglandins for anti-inflammatory activity serve as biomarkers. Similarly, for anticancer, immunomodulatory, antidiabetic, and other health effects, biomarkers have been identified that are measured in body fluids.

3 Analysis of Antioxidant Activity

Oxidative stress plays a major role in etiologies of several diseases in humans and animals, and protein thiolation index (PTI) is commonly used as a biomarker (Giustarini et al. 2012). To counteract oxidative stress, antioxidants are used. Antioxidants are an extremely heterogeneous family of compounds, and a distinction between direct antioxidants (such as vitamin E, curcumin, ascorbic acid, phenols, polyphenols) and indirect antioxidants (catalase, superoxide dismutase, glutathione peroxidase) has been made (Amorati and Valgimigli 2015a, b). The antioxidant activity of phytochemicals and plant extracts can be measured using

ABTS (2, 2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid), DPPH (2, 2-diphenyl-1-picrylhydrazyl), FRAP (ferric reducing antioxidant power), ORAC (oxygen radical absorbance capacity) assays, and others (Ou et al. 2002; Cheng et al. 2006; Thaipong et al. 2006; Amorati and Valgimigli 2015a, b). Results in these assays are expressed in μmol Trolox [(±)-6 hydroxy-2,5,7,8 tetra-methylchromane-2-carboxylic acid] equivalents (TE)/100 g or 100 mL sample. Among common solid and liquid foods, the total antioxidant capacity (TAC) was found to be maximum in red wine (dry) (247,800) and least in the red pepper (40) (Kusano and Ferrari 2008).

In humans, animals, birds, and fish, the separate measurement of different antioxidant molecules is not practical, and their antioxidant effects are additive, so measurement of TAC is advised (Erel 2004). The other names for TAC are total antioxidant activity (TAA), total antioxidant power (TAOP), total antioxidant status (TAS), and total antioxidant response (TAR). Various methods have been developed to measure TAC, but the ABTS-based method is the most widely used, and the results of TAC are expressed in mmol TE/L. TAC, measured by ABTS or any other test, is currently used as a biomarker of antioxidative status. In some studies, plasma and urinary levels of the antioxidant flavonols (quercetin and kaempferol) are used as biomarkers for dietary intake (De Vries et al. 1998).

4 Analysis of Anti-inflammatory Activity

A large number of nutraceuticals, including phytochemicals, exert anti-inflammatory activity. In experimental animal models (mostly rats and mice), inflammation is induced by injecting carrageenan or formalin into the paws, and the volume of raw paw edema or the volume of the exudates in air pouch is measured (Omote et al. 1998; Ajayi et al. 2017; Fahmy et al. 2017; Tamrat et al. 2017). The anti-inflammatory effect of a nutraceutical can be determined by measuring the reduction in the volume of paw edema and/or air pouch exudate and reduction in inflammatory mediator (histamine, bradykinin, substance P, nitric oxide, IL-1β, IL-6, TNF-α, PGE_2, glutamate, aspartate, myeloperoxidase, and others) release in rat, mouse, or any other lab animal. These inflammatory mediators can be measured in the body fluids (primarily in serum) and be used as biomarkers of inflammatory and anti-inflammatory activities.

5 Analgesic Activity

The radiant heat tail-flick test, as originally described by D'Amour and Smith (1941) with slight modifications (Milind and Monu 2013), is performed to measure analgesic activity.

6 Biomarkers of Foods and Nutraceuticals

In the case of foods and functional foods, the biomarkers are bioactive compounds that are present. These compounds can be identified and quantitated in the food ingredient and in body fluids (mainly plasma and urine) and tissues, and they often serve as biomarkers of food intake. Some of the biomarkers of common foods are listed in Table 1. Plasma levels of alkylresorcinol C17 and alkylresorcinol C19 serve as good biomarkers of whole grain wheat and rye intake; β-alanine for beef; eicosapentaenoic acid (EPA) and 3-carboxy-4-methyl-5-propyl-2-furanpropanoic acid (CMPF) for fish; linoleic acid for seeds, nuts, and vegetable oil; etc. (Table 1).

During food processing, foods can be contaminated with a number of chemicals, such as acrylamide, 3-monochloropropane-1, 2-diol (3-MCPD) esters, glycidyl esters, furan, acrolein, etc. Recently, Rietjens et al. (2018) reported that detecting residues of these contaminants or their metabolites and adducts in urine, blood, or tissue can serve as biomarkers of exposure and aid in physiologically based pharmacokinetics, biomonitoring, and risk assessment.

The presence of chemical compounds and/or their metabolites in body fluids/tissues of animals and humans serves as biomarkers of phytochemicals/marine organisms and other nutraceutical consumption. The nutraceuticals and their biomarkers are listed in Table 2. Some biomarkers are specific, while others are neither specific nor validated. These biomarkers not only indicate exposure to a particular nutraceutical but also as a further guide for pharmacological and therapeutic interventions. A majority of phytoconstituents exert antioxidative and anti-inflammatory activities, while others exert immunomodulatory, anti-apoptotic, antiplatelet aggregating, anticancer, anti-osteoarthritic, anti-acetylcholinesterase, anti-butyrylcholinesterase, and many other properties. Furthermore, some biomarkers are useful in the practice of polypharmacy where herb-herb or herb-nutraceutical may not be compatible.

These biomarkers can aid in identifying the food or nutraceutical consumed and also in pharmacokinetics and toxicokinetics of phytochemicals, interaction in polypharmacy, and phytotherapy.

7 Biomarkers of Polyphenols and Related Compounds: A Pharmacokinetic Perspective

7.1 Rationale

Plants produce a variety of secondary metabolites, including phenolic compounds, commonly referred to as polyphenols. There are more than 500 dietary polyphenols belonging to the classes of phenolic acids, flavonoids, lignans, and stilbenes,

Table 1 Biomarkers of some common foods/functional foods and their contaminants

Diet	Biomarker(s)	References
Chicken	Anserine in urine	Cheung et al. (2017)
Fish	Trimethylamine *N*-oxide in urine, species-specific protein (masses) pattern, DNA barcoding, eicosapentaenoic acid (EPA), 3-carboxy-4-methyl-5-propyl-2-furanpropanoic acid (CMPF), and n-3 fatty acids	Cheung et al. (2017), Stahl and Schröder (2017), and Savolainen et al. (2017)
Meat (beef)	β-Alanine and heterocyclic amines	Savolainen et al. (2017) and Hsiao et al. (2017)
Fish and meat	Carnosine, acylcarnitines (acetylcarnitine, propionylcarnitine, 2-methylbutyrylcarnitine), polyunsaturated fatty acids, amino acids	Marcone et al. (2013) and Cheung et al. (2017)
Shrimp	Arginine kinase peptide	Ortea et al. (2009)
Dairy foods	Amino acids, peptides and proteins, food colloids, essential fatty acids	Mariette (2009)
Eggs	Alpha-tocopherol, cholesterol, and gamma linolenic acid	Mariette (2009) and Savolainen et al. (2017)
Common bean (*Phaseolus vulgaris* L.)	Phaseolins, legumins, lectins	Rossi et al. (2017)
Soybean (*Glycine max.*)	Isoflavones (genistein, genistin, daidzein, biochanin, malonylgenistin, malonyldaidzin, acetyl genistin), saponins, amino acids, organic acids, etc.	Caligiani et al. (2010)
Cereals, wheat, rye, and other grains	β-Glucan, alkylresorcinols C17 and C19, and octacosanol	Ross et al. (2003), Woo et al. (2011), and Savolainen et al. (2017)
Avocados	Oleic acid	Savolainen et al. (2017)
Mushroom (*Russula senecis*)	Monosaccharides, polysaccharides, and β-glucan	Khatua and Acharya (2017)
Brassica vegetables (Brussels sprouts, broccoli, cauliflower, cabbage, mustard)	Glucosinolates	Bischoff (2016)
Liquid foods	Amino acids, organic acids, anthocyanins, phenolic compounds	van Gorsel et al. (1992) and Belton et al. (1996)
Margarine and vegetable oil	Oleic acid, linoleic acid, and α-tocopherol	Savolainen et al. (2017)
Food process-related contaminants	Acrylamide, 3-MCPD esters, glycidyl esters, furan, and acrolein, their metabolites and adducts in urine, blood, and tissues	Rietjens et al. (2018)

and as a result, their chemistry is very complex. Some of the groups of polyphenols are mentioned here in brief with suitable example(s) in parentheses. Hydroxybenzoic acids (a monomer gallic acid and a dimer ellagic acid, collectively referred to as ellagitannins) are common ingredients used in nutraceuticals. Flavonoids that are commonly found in plant extracts include flavonols (quercetin), flavones (apigenin), flavanols [(−)-epicatechin and proanthocyanidin B2)], flavanones (naringenin), anthocyanins (anthocyanidins: cyanidin, delphinidin, malvidin, pelargonidin, peonidin, and petunidin), and isoflavones (genistein) (Pinto and Santos 2017). It needs to be mentioned that there are about 700 anthocyanins reported to be isolated from plants (Faria et al. 2014). In humans, the major polyphenol classes consumed are biflavones (45%), anthocyanins (17%), and flavonols, flavones, and flavanones (16%). There are variations, of course, during different seasons and in different countries.

Polyphenols can act as antioxidants by quenching reactive oxygen species (ROS) or indirectly by altering gene expression via intracellular signaling cascades by reducing NFκB or enhancing nuclear factor-like 2 (Nrf2), thus stimulating the body's own antioxidant and detoxification mechanism (Bohn 2014). Polyphenols exert multiple phytopharmacological mechanisms and effects and thereby prevent chronic diseases, such as diabetes, cardiovascular, neurodegenerative, cancer, and many other illnesses.

7.2 Analysis of Total Phenolic Content

The total phenolic content (TPC) can be measured spectrophotometrically on a microplate reader (EPOCH 2 BioTek, BioTek Instruments Inc., Highland Park, Winooski, USA) according to Folin-Ciocalteu's method (Bailon-Moscoso et al. 2017). The amount of TPC is expressed in mg gallic acid equivalent (GAE)/g sample. The polyphenol content has been reported to be maximum in the cloves (15,188.2 mg/100 g) and least in the whole grain pasta and cauliflower (21.9 mg/100 g and 22.3 mg/100 g, respectively) (Pinto and Santos 2017).

Table 2 Potential biomarkers of selected plant extracts, marine organisms, and nutraceuticals

Plant extract/nutraceutical	Potential biomarker(s)	Biological/pharmacological effect(s)	References
Açai (*Euterpe oleracea*)	Lignans (dihydrobenzofuran, tetrahydrofuran, etc.), (+)-lariciresinol, (+)-pinoresinol, (+)-syringaresinol	Antioxidative, cytoprotective	Chin et al. (2008a)
Acacia nilotica (L.) (Babul)	Tannins, flavonoids, alkaloids, fatty acids, and polysaccharides	Antioxidative, anti-inflammatory, antidiarrheal, antihypertensive, antispasmodic, antibacterial, anthelmintic, antiplatelet aggregatory, anticancer, and acetylcholinesterase inhibitory	Rather et al. (2015)
Alpinia galanga	1,8-cineole, β-bisabolene, β-selinene, α-selinene, farnesene, 1, 2-benzenedicarboxylic acid, germacrene B, 1′S′-1′-acetoxychavicol acetate, and α-fenchyl acetate	Anti-inflammatory, antioxidant, analgesic, psychostimulant, hypoglycemic, anti-allergic, antimicrobial, gastroprotective, antiplatelet, anticancer, immunostimulatory, cholesterol reducing	Chudiwal et al. (2010) and Srivastava et al. (2017)
Amaranth (*Amaranthus hypochondriacus* L.)	Amaranth Alb, amaranth Glob, 11S globulin tetrapeptides, and polyphenols	ACE inhibitor, antihypertensive, antioxidative, hypocholesterolemia, immunomodulatory, antitumor, hypoglycemic, anti-anemia	Rastogi and Shukla (2013), Soriano-Santos and Escalona-Buendia (2015), and Quiroga et al. (2017)
Ashwagandha (*Withania somnifera*)	Anaferine, anahygrine, withanine, somniferine, withaferin-A, withanolides, withanol, diosgenin, sitoindosides, etc.	Antidepressant, antifungal, antimicrobial, antimalarial, chondroprotective, cholinesterase inhibiting and promoting learning and memory in Alzheimer's, antioxidative, anti-inflammatory, antiaging, anxiolytic, and antitumor	Choudhary et al. (2004) and Bharti et al. (2016)
Astaxanthin (*Haematococcus pluvialis*)	Astaxanthin	Antioxidative	Satoh (2016)
Astragali radix	Astragaloside IV, calycosin, and formononetin	Pain relieving, antioxidative, anti-inflammatory, and anti-arthritic	Maresca et al. (2017)
Baccharis dracunculifolia DC	Caffeic acid, *trans*-cinnamic acid, *p*-coumaric acid, and ferulic acid	Antioxidative, anti-inflammatory, and immunomodulatory	Figueiredo-Rinchel et al. (2017)
Barleria lupulina Lindl.	Alkaloids, saponins, tannins, flavonoids, and proteins	Antioxidative, antibacterial, and immunomodulating	Kumari et al. (2017)
Bee pollen from nectar plants (*Brassica campestris* L., *Camellia sinensis* L., *Nelumbo nucifera* Gaertn.)	Phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylserine, ceramide, phenols, flavonoids, fatty acids, carbohydrates, vitamins, and minerals	Antioxidative, anti-inflammatory, anticarcinogenic, antibacterial, fungicidal, hepatoprotective, anti-atherosclerotic, and immune-regulating properties	Denisow and Denisow-Pietrzyk (2016) and Li et al. (2017a)
Berberine (*Berberis vulgaris*, *Berberis aristata*, *Berberis aquifolium*, *Berberis canadensis*, *Coptis chinensis*, *Argemone mexicana*)	Berberine, thalifendine, berberrubine, and jatrorrhizine	Antidiabetic, cardiovascular and metabolic disorders, gastroenteritis, and neurodegenerative diseases	Kumar et al. (2015)
Bidens pilosa L. leaves extract	Catechin, kaempferol, ferulic acid, gallic acid, and paclitaxel	Antioxidative, anti-inflammatory, analgesic, antimicrobial, immunosuppressive, antiproliferative, antitumor, and anticancer	Fotso et al. (2014) and Singh et al. (2017)
Blueberry (*Vaccinium corymbosum*)	Anthocyanins/anthocyanidins (delphinidin, cyanidin, petunidin, peonidin, and malvidin)	Anticancer	Aqil et al. (2014)

(continued)

Table 2 (continued)

Plant extract/nutraceutical	Potential biomarker(s)	Biological/pharmacological effect(s)	References
Blue-green algae	C-phycocyanin	Antioxidative, anti-inflammatory, antihyperalgesic, selective COX-2 inhibitor, and cartilage repair in osteoarthritis	Shih et al. (2009), Pieloch (2006), and Martinez et al. (2015)
Brassica juncea	Sinapic acid	Peroxynitrite scavenging activity	Zou et al. (2002)
Butea monosperma Lam.	Aurones, butin, butein, butrin, isobutrin, chalcones, palasitrin, coreopsin, and others	Anti-diarrhea, antidiabetic, astringent, and diuretic	Khan et al. (2017)
Calafate (*Berberis microphylla*)	Polyphenols	Antioxidative	Ruiz et al. (2010)
Cassia absus	Chaksine, iso-chaksine, chrysophanol, and aloe emodin	Antioxidative, anti-inflammatory, antihypertensive, antifertility, antibacterial, antifungal, antihyperglycemic, anti-glycation, alpha-amylase, and inhibitory	Ahmad et al. (2018)
Calophyllum brasiliense	Jacareubin	Antibacterial, antitumor, anticancer, and gastroprotective	Garcia-Nino et al. (2017)
Cashew nut shell liquid (*Anacardium occidentale*)	Anacardic acids	Anti-Alzheimer's disease, anti-apoptotic, antioxidative, and antibacterial	Filho et al. (2018)
Cat's claw (*Uncaria guianensis* and *U. tomentosa*)	Indole alkaloid, tannins, rutin, ellagic acid, gallic acid, flavonoids, sterol, proanthocyanidin, etc.	Antioxidative, anti-inflammatory, and wound healing	Clark (2007)
Celastrus regelii and *Tripterygium wilfordii* (thunder god vine)	Celastrol	Antioxidative, anti-inflammatory, anticancer, anti-osteoarthritic, and insecticidal	Wang et al. (2018)
Chilean maqui berry/blackberry (*Aristotelia chilensis*)	Gallic acid, luteolin, myricetin, and quercetin	Free radical scavenging and antioxidative, anti-inflammatory, hypoglycemic, and anti-hemolytic	Cespedes et al. (2017)
Chromolaena odorata leaf extract	Stigmasterol and scutellarein tetramethyl ether	Antioxidative, lipid peroxide inhibitory, hydrogen peroxide inhibitory, and wound healing	Vijayaraghavan et al. (2017)
Cinnamon oil and cinnamon extract (*Cinnamomum zeylanicum*)	Cinnamic acid, cinnamyl acetate, cinnamaldehyde, eugenol, benzoic acid, and benzaldehyde	Bactericidal, bacteriostatic, fungicidal, fungistatic, antidiabetic, antiparasitic, antioxidative, anti-hyperlipidemia, antihypertensive, and cardioprotective	Gupta et al. (2008) and Ranasinghe et al. (2017)
Copaiba oil (*Copaifera multijuga* Hayne; *Copaifera duckei* Dwyer; and *Copaifera langsdorffii* Desf.)	Oleoresin, β-caryophyllene, β-bisabolene, α-bergamotene, copalic acid methyl ester	Anti-inflammatory, antibacterial, and lavicidal	Rodrigues et al. (2014), Lucca et al. (2015), and Xavier et al. (2017)
Corymbia citriodora	α-Citronellal, citronellol acetate, isopulegol, and eucalyptol	Antifungal and antioxidant	Salem et al. (2018)
Cupressus macrocarpa Hartw.	Terpinen-4-ol, α-phellandrene, α-citronellol, and α-citronellal	Antibacterial and antifungal	Salem et al. (2018)
Curcumin/turmeric (*Curcuma longa*)	Curcumin, mitocurcumin	Neuroprotective, antioxidative, anti-inflammatory, anti-osteoarthritis, anti-Parkinson's, anti-Alzheimer's, antidiabetic, anti-cardiovascular diseases, anticonvulsant, anti-obesity, anticancer, and wound healing	Henroitin et al. (2010), Aggarwal et al. (2013), Comblain et al. (2015), Javeri and Chand (2016), Risuleo (2016), Levine et al. (2017), Jayakumar et al. (2017), and Mohanty and Sahoo (2017)
Date palm pollen (*Phoenix dactylifera* L.)	Phenols and flavonoids	Cardiopreventive, ACE inhibiting, and antioxidative	Daoud et al. (2017)
Daylily (*Hemerocallis fulva*)	Phlomuroside, lariciresinol, adenosine, quercetin, isorhamnetins, and pinnatanine, roseoside	Lipid peroxidation inhibitory, antioxidative, anti-inflammatory, and anti-jaundice	Zhang et al. (2004)

(continued)

Table 2 (continued)

Plant extract/nutraceutical	Potential biomarker(s)	Biological/pharmacological effect(s)	References
Drosera rotundifolia, *D. anglica*, *D. intermedia*, *D. madagascariensis*	2′-*o*-galloylhyperoside, myricetin-3-*o*-β-glucopyranoside, kaempferol, and others	Anti-respiratory diseases	Zehl et al. (2011)
Essential oils from *Piper hispidimervium*, *P. sarmentosum*, *P. hancei*	Asaricin	Anticholinesterase	Xiang et al. (2017)
Eucalyptus bark (*Eucalyptus globulus* and *Eucalyptus macrocarpa*)	Pinoresinol, vomifoliol, methyl gallate, rhamnazin, rhamnetin, eucalyptone, eriodictyol, quercetin, taxifolin, engelitin, catechin, eupatriol, macrocarpals, and several terpenoids	Lipid peroxidation inhibitory, antibacterial	Yamakoshi et al. (1992) and Yun et al. (2000)
Fenugreek (*Trigonella foenum-graecum*)	Diosgenin, trigonelline, coumarin, galactomannan, 4-hydroxyisoleucine	Anti-hyperglycemic, hypolipidemic, antimetabolic syndrome, anti-inflammatory, anticancer, galactogogue, hepatoprotective, and enhancing male libido	Garg (2016)
Ficus racemosa	Lupeol and β-sitosterol	Antibacterial, antifungal, and wound healing	Bopage et al. (2018)
Ficus sycomorus	Flavonoids, saponins, tannins, triterpines, phenols, and cardiac glycosides	Antioxidative, antidiabetic, antidepressant, and spatial memory enhancing, antiepileptic, anticonvulsant	Foyet et al. (2017)
Flaxseed	Lignans (secoisolariciresinol, enterodiol, enterolactone, enterolignan), α-linolenic acid	Anti-inflammatory, antidiabetic, antihyperlipidemic, antihypertensive, antimetabolic syndrome, anti-obesity	Machado et al. (2015), Di et al. (2017), and Yu et al. (2017a, b)
Forskolin (Forsk) (*Coleus forskohlii*)	Forskolin	Anti-inflammatory, anticancer, anti-obesity, anticancer, increasing cAMP, and enhancing protein biosynthesis	Risuleo (2016)
Garlic (*Allium sativum*)	Alliin, allicin, *S*-alkyl-L-cysteine sulfoxides, diallyl thiosulfinate, sodium-2-propenyl thiosulfate, diallyl sulfide, plasma C-peptide, and many others	Antimicrobial, antioxidative, anti-inflammatory, antimutagenic, anticancer, anti-asthmatic, immunomodulatory, prebiotic, and antidiabetic	Goncharov et al. (2016) and Wang et al. (2017)
Glycyrrhizae radix	18 β-Glycyrrhetinic acid	Anti-inflammatory, anti-asthmatic	Kim et al. (2017)
Glycyrrhiza uralensis Fisch.	Licorice compounds (glycyrrhizin, glabridin, liquiritin, isoliquiritin, isoliquiritigenin)	Immunomodulatory, anti-inflammatory, anticancer, and antitumor	Ayeka et al. (2017)
Guava (*Psidium guajava*)	Phytofluene, β-carotene, β-cryptoxanthin, lycopene, cryptoflavin, lutein, neochrome	Antioxidative	Thaipong et al. (2006)
Grape seed (*Vitis vinifera*)	Proanthocyanidin	Antioxidative, anti-osteoarthritic	Jayaprakasha et al. (2001) and Woo et al. (2011)
Green tea (*Camellia sinensis*)	Epigallocatechin-3-gallate, epicatechin, epigallocatechin, catechin, gallocatechin, theaflavin, apigenin, luteolin, quercetin, myricetin, and cyclodextrin	Antioxidant, anti-inflammatory, anti-apoptotic, genital warts, immunomodulatory, anti-obesity, antidiabetic, antimicrobial, anticancer, wound healing, and anti-cardiovascular diseases	Tarachiwin et al. (2007), Clarke et al. (2014), Svoboda et al. (2015), Coppock and Dziwenka (2016), Li et al. (2016), Bacanli et al. (2017), and Oda and Murakami (2017)
Gymnosperma glutinosum	Dihydrotucumanoic acid	Antinociceptive, anti-inflammatory, antimicrobial, antidiarrheal	Alonso-Castro et al. (2017)
Hempseed oil	Polyunsaturated fatty acids	Atopic dermatitis	Callaway et al. (2005)
Honeybees	Propolis	Antiseptic	Bacanli et al. (2017)
Hymenaea martiana	Engeletin	Antidiabetic	Ye et al. (2017)
Indian blackberry/jamun (*Syzygium cumini* L.)	Anthocyanins, ellagic acid/ellagitannins, and polyphenolics	Antioxidative, antiproliferative	Aqil et al. (2012)

(continued)

Table 2 (continued)

Plant extract/nutraceutical	Potential biomarker(s)	Biological/pharmacological effect(s)	References
Indian frankincense (*Boswellia serrata*)	Boswellic acids: β-boswellic acid, acetyl-β-boswellic acid, 11-keto-β-boswellic acid, and acetyl-11-keto-β-boswellic acid	5-Lipoxygenase inhibitor, anti-inflammatory, anti-arthritic, prostate tumor inhibitor, neuroprotective in Parkinson's disease	Pang et al. (2009), Poeckel and Werz (2006), Siddiqui (2011) and Ameen et al. (2017)
Indian gooseberry/Amla (*Phyllanthus emblica*)	Hydrolysable tannoids (emblicanin A, emblicanin B, punigluconin, pedunculagin), punicafolin, phyllanemblinin, and other polyphenols	Antioxidative, anti-inflammatory, anti-arthritis	Fleck et al. (2013) and May et al. (2015)
Juniper (*Juniperus communis* L.)	α-Pinene, chlorogenic acid, rutin, apigenin, and quercetin	Antioxidative, anti-inflammatory, genoprotective, immunostimulatory, and antifungal	Fierascu et al. (2018)
Knotweed (*Reynoutria* spp.)	Resveratrol, polydatin, rutin, reynoutrin, emodin, physcion, citrorosein, lapathoside A, quercetin, and kaempferol	Antioxidative, anti-inflammatory, neuroprotective, antibacterial, antifungal, and antitumor	Patocka et al. (2017)
Limnophila aromatica	Phenols and flavonoids	Antioxidative	Do et al. (2014)
Limonene (oil from citrus plants, orange, lemon, grapefruit)	Limonene (*p*-Mentha-1,8-diene)	Anti-gastric diseases and antiproliferative effect in cancer cells	Bacanli et al. (2017)
Lycopene	Lycopene	Free radical scavenging and antioxidative, anti-inflammatory, neuroprotective, anti-dementia, anticancer	Bacanli et al. (2017) and Zhao et al. (2017)
Lotus (*Nelumbo nucifera* Gaertn)	Polyphenols (gallic acid, catechin, peltatoside, rutin, isoquercitrin, miquelianin, and astragalin)	Antioxidative, hepatoprotective, and anticancer	Horng et al. (2017)
Mangosteen (*Garcinia mangostana*)	Xanthones, mangostanin, and α-mangostin	Antioxidative, anti-inflammatory, antiproliferative, neuroprotective, hypoglycemic, anti-obesity, and quinone reductase induction	Chin et al. (2008b) and Ovalle-Magallanes et al. (2017)
Melastoma malabathricum and *Muntingia calabura*	Gallic acid, ellagic acid, quercetin, vitexin, isovitexin, myricetin, kaempferol, naringenin, luteolin, diosmetin, apigenin, and many other polyphenols	Gastroprotective, antiulcer, gastric secretion inhibitory, antinociceptive, cytoprotective, antioxidative, free radical scavenging, anti-lipid peroxidation, anti-inflammatory, and antipyretic	Zakaria et al. (2007) and Halim et al. (2007)
Mulberry bark powder (*Ramulus mori*)	Flavonoids, polysaccharides, resveratrol, phenolic acids	Antioxidative, anti-inflammatory, anti-apoptotic to pancreas cells, and antidiabetic	Yin et al. (2017)
Maqui berry (*Aristotelia chilensis*)	Anthocyanins, polyphenols	Antioxidative, antibacterial, antidiabetic	Rojo et al. (2012), Genskowsky et al. (2016), Brauch et al. (2017), and Li et al. (2017c)
Marine organisms (*Cladiella krempfi*, *Klyxum molle*, and others)	Diterpenes: krempfielins, hirsutalins, klymollins, klysimplexin, klysimplexin sulfoxide, simplexin, cladieunicellin, and others	Anti-inflammatory, anti-arthritic	González et al. (2015)
Naringin (from grapefruit and related citrus species)	Naringin	Antioxidative, anti-inflammatory, antimicrobial, and anticancer	Bacanli et al. (2017)
Neem extract (*Azadirachta indica*)	Azadirachtins, nimbin, nimbidin, nimbolide, margolone, gedunin, catechin, epicatechin, gallocatechin, epigallocatechin, and several others	Nutritive, anxiolytic, antimicrobial, antiparasitic, immunomodulatory, antioxidative, antidiabetic, antitumor, chemopreventive, pest control, wound healing	Tandan et al. (1995), Tepsuwan et al. (2002), Aruwayo and Maigandi (2013), and Kumar et al. (2016)
Nigella sativa	Thymoquinone	Antioxidant, anti-inflammatory, anticancer, pro-apoptotic effect, anti-autoimmune diseases, anti-asthma, antidiabetic	Gupta et al. (2016)

(continued)

Table 2 (continued)

Plant extract/nutraceutical	Potential biomarker(s)	Biological/pharmacological effect(s)	References
Nonea micrantha	Crodacid, neophytadiene, vanicol, chrysarobin, clionasterol, and many others	Cholinesterase inhibiting and promoting learning and memory and antioxidative	Choudhary et al. (2017)
Ocimum gratissimum (Ocimum oil)	Rutin, rosmarinic acid, chicoric acid, kaempferol, caffeic acid, *trans*-ferulic acid, quercetin, vicetin, salvigenin, nevadensin, and cirsimaritin	Anti-inflammatory and antioxidative, antirheumatism, anti-bronchitis, antimicrobial	Ajayi et al. (2017)
Olax nana	Gallic acid derivatives, flavonoids, rutin, tannins, sterols, saponins, and terpenoids	Antioxidative, free radical scavenging, anticholinesterase, and alpha-glucosidase inhibiting	Ovais et al. (2018)
Olive oil	Tyrosol, hydroxytyrosol, (phenyl-ethyl alcohols, lignans, and secoiridoids)	Antioxidative	Servili et al. (1999), Carrasco-Pancorbo et al. (2005), and Marcone et al. (2013)
Panax notoginseng Ledeb saponins	Ginsenoside Rgl, ginsenoside Rbl, and ginsenoside Rd	Antioxidative, anti-pancreatitis, anti-apoptotic, anti-autophagy, and anticancer	Liu et al. (2018)
Phyllostachys heterocycla var. *pubescens*	2,6-Dimethoxy-*p*-benzoquinone	Antibacterial	Nishina et al. (1991)
Pinus sylvestris L.	Phenolic compounds	Anti-inflammatory	Karonen et al. (2004)
Pueraria lobata	Puerarin	Antihypertensive, anti-genotoxic, and against fever, diarrhea, and eye disorder	Bacanli et al. (2017)
Rabdosia rubescens (Donglingcao)	Oridonin	Antioxidative, anti-inflammatory, immunomodulatory, and anticancer	Wu et al. (2018)
Ramaria largentii Marr & D. E. Stuntz (wild edible mushroom)	Protocatechuic acid, vanillic acid, sterols, phenolic compounds, and triterpenes	Antioxidative and anti-genotoxic	Aprotosoaie et al. (2017)
Resveratrol	*Trans*-resveratrol, *cis*-resveratrol, *trans*-resveratrol-glucuronoside cinnamic acid, *p*-coumaric acid	Antioxidative, antitumor, antiviral, anti-atheroma	Risuleo (2016)
Rhodiola rosea L.	Salidroside, rosarin, rosavin, and rosin	Antioxidative, anti-inflammatory, antidiabetic, anticancer, and antiviral	Marchev et al. (2017)
Rhizoma smilacis Glabrae	Astilbin	Antioxidative, anti-inflammatory, immunomodulatory and anti-hepatic, anti-arthritic, and anti-renal injury	Gao et al. (2017)
Rosmarinic acid (*Rosmarinus officinalis*, *Perilla frutescens*)	Rosmarinic acid, apigenin, caffeic acid, catechin, ferulic acid, luteolin, *m*-coumaric acid, and carnosic acid	Chemopreventive, antiangiogenesis, antiproliferative, antitumor, growth promoting, antioxidative, anti-inflammatory, antidepressive, immunomodulatory, antimicrobial, antidiabetic, anti-allergic, hepato- and renal-protective, and anti-Alzheimer's disease	Baba et al. (2004), Krajčovičová and Melus (2013), Alagawany et al. (2017), Levine et al. (2017), Yu et al. (2017a, b), and Rong et al. (2018)
Royal fern (*Osmunda regalis* root)	Tannins, glycosides, steroids, saponins, and ferulic acid	Anticancer	Schmidt et al. (2017)
Royal jelly proteins	Dipeptide YY	Renin inhibiting, ACE inhibiting, antihypertensive, wound healing	Fujii et al. (1990) and Sultana et al. (2008)
Safflower seed oil cake (*Carthamus tinctorius* L.)	Carthatins	Acetylcholinesterase-inhibiting activity	Peng et al. (2017)
Saffron (*Crocus sativus* L.)	Crocin-1, crocetin, *trans*-crocetin, safranal	Anti-apoptotic, antioxidative, anti-inflammatory, anticancer, antitumor, neuroprotective, cardioprotective, hepatoprotective	Lautenschläger et al. (2014) and Hoshyar and Mollaei (2017)

(continued)

Table 2 (continued)

Plant extract/nutraceutical	Potential biomarker(s)	Biological/pharmacological effect(s)	References
Sarcandra glabra	Sesquiterpenes, phenolic acid, flavonoids, chalcones, polysaccharides, coumarins, triterpenoids	Antioxidative, anti-inflammatory, immune-modulatory, anticancer, antidiarrheal, and antirheumatism	Tsai et al. (2017)
Saururus chinensis	Sauchinone	Antioxidative, anti-inflammatory, chondroprotective, and anti-osteoarthritic	Gao et al. (2017)
Sclerocarya birrea (A. Rich.) Hochst (Marula extract/oil)	Quinic acid, catechin, epigallocatechin gallate, and epicatechin gallate	Antiaging, anti-elastase, and anti-collagenase	Shoko et al. (2018)
Scutellaria baicalensis Georgi	Baicalin and baicalein	Antioxidative, anti-inflammatory, anticancer, anti-dementia, antimicrobial, antipyretic, and anti-jaundice	Jeong et al. (2011)
Shilajit	Dibenzo-α-pyrones, dibenzo-α-pyrone-chromoproteins, fulvic acids	Antioxidative, immunomodulatory, anti-inflammatory, analgesic, learning augmentation, antidiabetic, antiulcerogenic, anti-osteoarthritic, cardioprotective, and antimicrobial	Aggarwal et al. (2007), Wilson et al. (2011), Lawley et al. (2013), and Bhavsar et al. (2016)
Spirulina platensis/*Arthrospira platensis*	C-phycocyanin	Nutritive, anti-anemia, antioxidative, antimicrobial, anti-inflammatory, anti-allergy, antidiabetic, chemopreventive, anticancer, and neuroprotective	Sarada et al. (2011) and Wan et al. (2016)
Stenoloma chusanum (L.) ching	Filicinic acid, chlorogenic acid, pyracrimycin, theophylline, chaplacin, bisabolenone, and scadenone	Antioxidative, anti-tyrosinase, and antitumor	Wu et al. (2017)
Tamarind seed and pericarp (*Tamarindus indica* L.)	2-Hydroxy-3,4-dihydroxyacetophenone, methyl 3,4-dihydroxybenzoate, 3,4-dihydroxyphenyl acetate, (+)-catechin, (−)-epicatechin, proanthocyanidins, and procyanidins	Free radical scavenging, antioxidative, and antidiabetic	Tsuda et al. (1994), Maiti et al. (2004), Sudjaroen et al. (2005), and Sandesh et al. (2014)
Tea tree oil (*Melaleuca alternifolia*)	Terpinen-4-ol, terpinolene, and 1,8-cineole	Antifungal, antibacterial, anti-pneumonic, anti-lice, anti-inflammatory	Carson et al. (2006) and Li et al. (2017b)
Terminalia chebula	Chebulagic acid, chebulinic acid, chebulic acid, gallic acid, ellagic acid, tannic acid, corilagin, triterpenoids, polyphenolic compounds, and ascorbate	Antioxidative, anti-inflammatory, analgesic, anti-arthritic, antiproliferative, adaptogenic, immunomodulatory, and cytoprotective	Upadhyay et al. (2014) and Murdock et al. (2016)
Terminalia muelleri Benth. (Australian almond)	Isoorientin, vitexin, ellagic acid, ellagitannin	Anti-inflammatory and analgesic	Fahmy et al. (2017)
Thyme and oregano oil	Carvacrol and γ-terpinene	Antioxidative, antibacterial, antimycotic, and antiplatelet	Bacanli et al. (2017)
Tibouchina granulosa	Proanthocyanidins, flavonoid glycosides	Anti-inflammatory	Sobrinho et al. (2017)
Tiger milk mushroom (*Lignosus rhinocerotis*)	A- and β-glucan, phenolic compounds, polysaccharide	Anti-inflammatory, antioxidant, anticoagulant, fibrinolytic, antimicrobial, anti-obesity, anticancer, hepatoprotective, antitumor, antiviral, neuroprotective, and immunomodulatory	Nallathamby et al. (2018)
Turbinaria ornata	Sulfated polysaccharides	Anti-inflammatory, anti-rheumatoid arthritis	Ananthi et al. (2017)

(continued)

Table 2 (continued)

Plant extract/nutraceutical	Potential biomarker(s)	Biological/pharmacological effect(s)	References
Ursolic acid (*Malus pumila*, *Ocimum basilicum*, and many others)	Ursolic acid	Anti-arthritis, antiulcer, anticancer, antidiabetes	Bacanli et al. (2017)
Wheat bran oil	Sterols, oryzanol-like compounds, tocopherols, and carotenoids	Free radical scavenging, antioxidative, and hypolipidemic	Talawar et al. (2017)
Wild blueberries (*Vaccinium angustifolium* Aiton and *V. myrtilloides* Michaux)	Anthocyanins	Antioxidative	Chorfa et al. (2016)
Wild garlic (*Allium ursinum*)	Methyl isorinate, alliins (methylalliin, methiin, and allylalliin), organosulfur compounds (allyl methyl disulfide and allicin), and phenolic compounds	Antioxidative, antimicrobial, calcium channel inhibition, spasmolytic, antiplatelet, and other GI disturbances	Hiyasat et al. (2009) and Pavlovic et al. (2017)

7.3 Nutraceutical-Gut Microbiota Interaction and Biomarkers of Bioavailability

Upon ingestion, food/nutraceuticals first encounter the gut microbiota, which is considered a metabolic organ. Bacteria appear to participate in the metabolism of polyphenols of phase I and phase II reactions. Common reactions include deglycosylation, dehydroxylation, demethylation, or conjugation. Some examples of metabolites formed from isoflavones by the microbiota are (1) dihydrodaidzein, equol, and *O*-desmethylangolensin (*O*-DMA) from daidzein, (2) dihydrogenistein from genistein, (3) urolithin B from ellagitannins, (4) enterodiol and enterolactone from secoisolariciresinol di-*O*-glucoside, and (5) flavanone 8-prenylnaringenin from prenylchalcone isoxanthohumol. Biomarkers often explain how the nutraceuticals affect the level and composition of gut microbiota and how the gut microbiota affects the nutraceutical levels, their bioavailability, and metabolism.

From the interaction between nutraceuticals and microbiota, one or more of the following outcomes can be expected (Faria et al. 2014; Most et al. 2017; Williams and Clifford 2017; Tomas-Barberan et al. 2018):

1. Level and composition of microbiota can be altered by nutraceuticals (e.g., polyphenols and resveratrol).
2. Nutraceuticals can be metabolized, and their bioactivity can be altered.
3. Microbiota can alter the bioavailability and other aspects of pharmacokinetics of nutraceuticals.
4. The interaction of nutraceuticals with microbiota can influence the metabolomic profile.
5. Polyphenols may offer prebiotic activity by modulating the microbiota composition.
6. Gender and inter-individual variabilities play a significant role.

In some cases, phytopharmacological and phytotherapeutic outcome from these factors proves to be conflicting. For example, some reports indicate that dietary polyphenols promote growth of the gut microbiota (Roopchand et al. 2015), while others suggest that they are antimicrobial (Marin et al. 2015). Based on these interactions, qualitative and quantitative biomarkers can be identified and validated. For example, enterolactone and hydroxytyrosol are considered good biomarkers of lignan and olive phenolic alcohol intake, respectively.

In general, microbiological fermentation decreases the bioavailability of the native polyphenols, but in some cases, it can give rise to metabolites that are more bioactive than the native polyphenols, for example, equol from daidzein and dihydroresveratrol from resveratrol.

Interestingly, in a very recent study, foregut microbiota of camel and cattle was shown to degrade (99%) a non-polyphenolic compound indospicine (a well-known hepatotoxin) from *Indigofera spicata* to 2-aminopimelamic acid and 2-aminopimelic acid (Tan et al. 2017).

In addition to indigenous factors, such as microbiota and digestive enzymes, the food matrix (dietary fiber, lipids, proteins, and digestible carbohydrates) can significantly affect bioavailability and bioaccessibility, uptake, and further metabolism of polyphenols (Bohn 2014).

In general, polyphenols may have greater bioavailability when they are ingested in combination with other polyphenols than when taken alone, such as in supplement. About 48% of dietary polyphenols are bioaccessible in the small intestine, while 42% become bioaccessible in the large intestine (Saura-Calixto et al. 2007). Faria et al. (2014) noted that the majority of dietary anthocyanins are not absorbed at the upper GI level, hence reaching the intestinal microbiota where they are biotransformed into their metabolites, which are then absorbed.

7.4 Absorption

Polyphenols may be transported in the blood in free form, bound to proteins, or bound to lipoproteins (Bohn 2014). Quercetin was found to be bound to albumin at over 99%. Absorption of monomers, such as epicatechin, is usually high (45%) compared to dimers of procyanidins (<1%) (Bohn 2014). Polyphenols can be absorbed by passive diffusion (e.g., chlorogenic acid) or the facilitated transport system (e.g., caffeic acid). Polyphenols, such as caffeic acid and ferulic acid, are absorbed by facilitated transport by monocarboxylic acid transporters (MCTs). Other transporters can also be involved, such as P-glycoprotein, multidrug resistance proteins (MRPs), and ATP-binding cassette transporters.

7.5 Blood/Urinary Metabolites as Biomarkers of Polyphenol Intake

The presence of polyphenols and other nutraceuticals and/or their metabolites in body fluids (mainly in serum and urine) serve as biomarkers of exposure. The relationship between polyphenol intake and specific biomarkers measured in the blood and urine is highly complex (Pinto and Santos 2017). This may be due to multiple factors: (1) poor release of polyphenols from the food matrix; (2) interaction with other food compounds and with microbiota; (3) individual polyphenols that are not digested in the digestive tract, resulting in poor absorption from the intestine; (4) extensive biotransformation by phase I and phase II reactions; and (5) rapid excretion. The method of analysis, sensitivity of instrumentation, concentration of the polyphenol metabolite in the blood/urine, and availability of standards are other factors that may influence the accuracy of metabolite analysis and identification.

Human studies suggest that a biomarker in pooled 24-h urine collection (total urinary polyphenol) and a surrogate biomarker in plasma (carotenoids) may provide a suitable estimate to assess polyphenol intake (Burkholder-Cooley et al. 2017; Pinto and Santos 2017). Of course, estimation based on urinary polyphenol excretion is limited by genetic variance, metabolism, and bioavailability.

Pérez-Jiménez et al. (2010) investigated urinary metabolites as biomarkers of polyphenol intake in humans and found that polyphenols such as daidzein, genistein, glycitein, enterolactone, and hydroxytyrosol are good biomarkers of polyphenol intake, while hesperidin, naringenin, (−)-epicatechin, (−)-epigallocatechin, quercetin, and three microbial metabolites of isoflavones (dihydrodaidzein, equol, and *O*-desmethylangolensin) are less suitable biomarkers of intake. Additional urinary metabolites are in the form of conjugates (sulfate and glucuronide). By now, about 160 polyphenol metabolites have been identified in urine, and only some of them could be ascribed with certainty to known precursors (Pérez-Jiménez et al. 2010). Likewise, quantitation of parent and/or metabolites in urine can be used to determine the intake of other nutraceuticals. It needs to be pointed out that the value of urinary metabolites of polyphenols or other classes of phytochemicals as biomarkers of intake depends on their specificity, sensitivity, and variability.

7.6 Biomarkers of Polyphenol Tissue Exposure

Following absorption, polyphenols can be distributed in most tissues, and they cross the blood-brain barrier. Tissue levels of polyphenols and their metabolites are better biomarkers than plasma concentrations, and they serve as better biomarkers of health effects than their intake because of a large individual variability in absorption and metabolism.

7.7 Excretion

Polar compounds such as gallic acid and isoflavones excrete via the kidney, while apolar compounds such as curcumin excrete via bile.

8 Biomarkers of Toxicity and Safety Evaluation of Foods and Nutraceuticals

For the toxicity and safety assessment of foods and nutraceuticals, serum samples are analyzed for biomarkers of liver (alanine aminotransferase, ALT; aspartate aminotransferase, AST; gamma-glutamyl transferase, GGT; alkaline phosphatase, ALP; and bilirubin), kidney (blood urea nitrogen (BUN) and creatinine), and heart (creatine kinase (CK) and troponin) functions. Other serum biomarkers specific for liver injury include microRNA-122 (miR-122), glutamate dehydrogenase (GLDH), caspase-cleaved cytokeratin-18 (cc-K18), and full-length keratin-18 (FL-K18) (Longo et al. 2017). FL-K18 is passively released from necrotic cells, while cc-K18 is released from apoptotic cells after loss of membrane integrity, and the ratio of cc-K18 to FL-K18 is regarded as the "apoptotic index." In hepatotoxicity, macrophage colony-stimulating factor (CSF-1) is also used as a biomarker. The "apoptotic index" has been proposed to estimate the relative contributions of necrosis and apoptosis to cell death. Some of these biomarkers may not typically satisfy Hy's law criteria. Kinetics of all these hepatotoxicity biomarkers can be interpreted using DILIsym® software, which is a mechanistic mathematical

model for drug-induced liver injury (Shoda et al. 2014). In case of acute kidney injury, biomarkers, such as neutrophil gelatinase-associated lipocalin (NGAL), kidney injury molecule-1 (KIM-1), and high-mobility group box protein 1 (HMGB1), can also be taken into consideration while assessing effects of herbal medicines (Oh et al. 2017). Currently, intense research is underway for more tissue-specific and tissue-sensitive biomarkers, such as microRNAs, which are still considered mechanistic biomarkers for mechanistic modeling (Penman et al. 2014; Gupta 2014).

9 Biomarkers of Nutraceuticals with Toxic Potential

During the last few decades, the global use of nutraceuticals has increased tremendously for humans and animals and so has health risks emerging from active components as well as from toxic contaminants of supplements (Gupta 2016; Gupta et al. 2018). Some plant extracts are inherently toxic by virtue of having toxic phytoconstituents, while others are likely to be contaminated with other plant alkaloids (e.g., pyrrolizidine alkaloids), metals (arsenic, cadmium, lead, and mercury), pesticides (insecticides, herbicides, fungicides, PCBs, PAHs, etc.), and mycotoxins (aflatoxins, ochratoxin A, citrinin, T-2, and HT2) (Gupta et al. 2018). Sometimes, nutraceuticals are also adulterated with drugs of abuse. Some common plant extracts that are used as nutraceuticals are mentioned here with their toxic principle in parentheses: St. John's wort (hypericin), goldenseal (hydrastine), ginkgo biloba (ginkgotoxins), kava (flavokawain B), ephedra (ephedrine), pennyroyal oil (ketone pulegone), *Aristolochia* (aristolochic acid), bitter melon (momordins and vicine), lychee (hypoglycin A), comfrey (pyrrolizidine alkaloid), green tea (epigallocatechin-3-gallate), and many others (Gupta et al. 2018). Additionally, some plants, such as *Indigofera spicata*, that are edible, high in protein, and easily digestible by livestock are toxic by having a phytotoxin indospicine (Tan et al. 2017).

Detection of a phytotoxic principle and/or its metabolite (s) in the blood, urine, or tissue often serves as a biomarker of exposure, and the level of exposure can serve as a biomarker of effect. Adverse effects associated with toxic plant extracts may include from minor nausea, vomiting, or GI upset to as severe as liver, kidney, or cardiac failure, seizures, or death (Gupta et al. 2018). Most nutraceuticals are multi-targeted small molecules, and they affect multiple signaling pathways to exert their effects. There are even some nutraceuticals that exert tissue-specific adverse effects. For example, the target organs for toxicity of *Aristolochia*, comfrey, and ephedra are the renal, hepatic, and cardiac systems, respectively. In addition to residue detection, nutraceutical/contaminant-induced tissue damage can be evaluated using conventional biomarkers, as described in the previous section (Gupta et al. 2018).

Detection of residue of a metal, pesticide, mycotoxin, or drug of abuse in body fluids confirms the exposure to contaminants. Many times, toxicity outcome arises from toxic herb-drug interaction in polypharmacy. For further details, readers are referred to Gupta (2016) and Gupta et al. (2018).

10 Concluding Remarks and Future Directions

Biomarkers are of utmost importance, as they offer a plethora of information on various aspects of foods and nutraceuticals, including identification of food ingredients and phytochemicals, pharmacokinetics and toxicokinetics of phytochemicals, interaction with other nutraceuticals and modern medicines in the case of polypharmacy, toxicity and safety evaluation of nutraceuticals, clinical trials, and their regulations. Choice of the most promising biomarkers that are validated plays a vital role in the field of nutrition and nutraceuticals and in the assessment of associations with health and disease outcome. With a rapid advancement in equipment and biotechnologies, novel biomarkers are continuously emerged with a greater sensitivity and reproducibility, and the quest will continue. Thus, with the aid of validated biomarkers, the food and nutraceutical industries will have a greater confidence in high quality foods and nutraceuticals.

Acknowledgment The authors would like to thank Ms. Robin B. Doss for her technical assistance in preparation of this chapter.

References

Aggarwal SP, Khanna R, Karmarkar R et al (2007) Shilajit: a review. Phytother Res 21:401–405

Aggarwal BB, Gupta SC, Sung B (2013) Curcumin: an orally bioavailable blocker of TNF and other pro-inflammatory biomarkers. Br J Pharmacol 169:1672–1692

Ahmad S, Hassan A, Abbasi WM et al (2018) Phytochemistry and pharmacological potential of *Cassia absus*—a review. J Pharm Pharmacol 70:27–41

Ajayi AM, Umukoro S, Ben-Azu B et al (2017) Toxicity and protective effect of phenolic- enriched ethylacetate fraction of *Ocimum gratissimum* (Linn.) leaf against acute inflammation and oxidative stress in rats. Drug Dev Res 78:135–145

Alagawany M, Abd El-Hack ME, Farag MR et al (2017) Rosmarinic acid: modes of action, medicinal values and health benefits. Anim Health Res Rev 7:1–10

Alonso-Castro AJ, González-Chávez MM, Zapata-Morales JR et al (2017) Antinociceptive activity of ent-dihydrotucumanoic acid isolated from *Gymnosperma glutinosum* Spreng Less. Drug Dev Res 78:340–348

Ameen AM, Elkazaz AY, Mohammad HMF (2017) Anti-inflammatory and neuroprotective activity of boswellic acids in rotenone parkinsonian rats. Can J Physiol Pharmacol 95(7):819–829

Amorati R, Valgimigli L (2015a) Advantages and limitations of common testing methods for antioxidants. Free Radic Res 49:633–649

Amorati R, Valgimigli L (2015b) Methods to measure the antioxidant activity of phytochemicals and plant extracts. J Agric Food Chem 66:3324–3329

Ananthi S, Gayathri V, Malarvizhi R et al (2017) Anti-arthritic potential of marine macroalgae *Turbinaria ornata* in complete Freund's adjuvant induced rats. Exp Toxicol Pathol 69(8):672–680

Aprotosoaie AC, Zavastin DE, Mihai C-T et al (2017) Antioxidant and antigenotoxic potential of *Ramaria largentii* Marr and D. E. Stuntz, a wild edible mushroom collected from Northeast Romania. Food Chem Toxicol 108:429–437

Aqil F, Gupta A, Munagala R et al (2012) Antioxidant and antiproliferative activities of anthocyanin/ellagitannins-enriched extracts from *Syzygium cumini* L. (Jamun, the Indian blackberry). Nutr Cancer 64(3):428–438

Aqil F, Vadhanam MV, Jeyabalan J et al (2014) Detection of anthocyanins/anthocyanidins in animal tissues. J Agric Food Chem 62:3912–3918

Aruwayo A, Maigandi SA (2013) Neem (*Azadirachta indica*) seed cake/kernel as protein source in ruminants feed. Am J Exp Agric 3 (3):482–494

Ayeka PA, Bian YH, Githaiga PM et al (2017) The immunomodulatory activities of licorice polysaccharides (*Glycyrrhiza uralensis* Fisch.) in CT 26 tumor-bearing mice. BMC Complement Altern Med 17:536

Baba S, Osakabe M, Natsume J et al (2004) Orally administered rosmarinic acid is present as the conjugated and/or methylated forms in plasma, and is degraded and metabolized to conjugated forms of caffeic acid, ferulic acid and m-coumaric acid. Life Sci 75 (2):165–178

Bacanli M, Aydin S, Başaran AA et al (2017) Are all phytochemicals useful in the preventing of DNA damage? Food Chem Toxicol 109:210–217

Bailon-Moscoso N, Tinitana F, Martínez-Espinosa R et al (2017) Cytotoxic, antioxidative, genotoxic and antigenotoxic effects of *Horchata*, beverage of South Ecuador. BMC Complement Altern Med 17:539

Belton PS, Delgadillo I, Holmes E et al (1996) Use of high-field ^{1H} NMR spectroscopy for the analysis of liquid foods. J Agric Food Chem 44:1483–1487

Bharti VK, Malik JK, Gupta RC (2016) Ashwagandha: multiple health benefits. In: Gupta RC (ed) Nutraceuticals: efficacy, safety and toxicity. Academic Press/Elsevier, Amsterdam, pp 717–733

Bhavsar SK, Thaker AM, Malik JK (2016) Shilajit. In: Gupta RC (ed) Nutraceuticals: efficacy, safety and toxicity. Academic Press/Elsevier, Amsterdam, pp 707–716

Bischoff KL (2016) Glucosinolates. In: Gupta RC (ed) Nutraceuticals: efficacy, safety and toxicity. Academic Press/Elsevier, Amsterdam, pp 551–554

Bohn T (2014) Dietary factors affecting polyphenol bioavailability. Nutr Rev 72(7):429–452

Bopage NS, Gunaherath KB, Jayawardena KH et al (2018) Dual function of active constituents from bark of *Ficus racemosa* L in wound healing. BMC Complement Altern Med 18:29

Brauch JE, Reuter L, Conrad J et al (2017) Characterization of anthocyanins in novel Chilean maqui berry clones by HPLC-DAD-ESI/MS^n and NMR-spectroscopy. J Food Compos Anal 58:16–22

Burkholder-Cooley NM, Rajaram SS, Haddad EH et al (2017) Validating polyphenol intake estimates from a food-frequency questionnaire by using repeated 24-h dietary recalls and a unique method-of-triads approach with 2 biomarkers. Am J Clin Nutr 105 (3):685–694

Caligiani A, Palla G, Maietti A et al (2010) 1H NMR fingerprinting of soybean extracts, with emphasis on identification and quantification of isoflavones. Nutrients 2:280–289

Callaway J, Schwab U, Harvima I et al (2005) Efficacy of dietary hempseed oil in patients with atopic dermatitis. J Dermatol Treat 16(2):87–94

Carrasco-Pancorbo A, Cerretani L, Bendini A et al (2005) Evaluation of the antioxidant capacity of individual phenolic compounds in virgin olive oil. J Agric Food Chem 53:8918–8925

Carson CF, Hammer KA, Riley TV (2006) *Melaleuca alternifolia* (Tea tree) oil: a review of antimicrobial and other medicinal properties. Clin Microbiol Rev 19(1):50–62

Cespedes CL, Pavon N, Dominguez M et al (2017) The Chilean Maquiberry *Aristotelia chilensis* (Elaeocarpaceae), Maqui as mediator in inflammation-associated disorders. Food Chem Toxicol 108: 438–450

Cheng Z, Moore J, Yu L (2006) High-throughput relative DPPH radical scavenging capacity assay. J Agric Food Chem 54:7429–7436

Cheng Q, Shou L, Chen C et al (2017) Application of ultra-high-performance liquid chromatography coupled with LTQ-orbitrap mass spectrometry for identification, confirmation and quantitation of illegal adulterated weight-loss drugs in plant dietary supplements. J Chromatogr B 1064:92–99

Cheung W, Keski-Rakhonen P, Assi N et al (2017) A metabolomic study of biomarkers of meat and fish intake. Am J Clin Nutr 105 (3):600–608

Chin Y-W, Chai H-B, Keller WJ et al (2008a) Lignans and other constituents of the fruits of *Euterpe oleracea* (Açai) with antioxidant and cytoprotective activities. J Agric Food Chem 56:7759–7764

Chin Y-W, Jung H-A, Chai H et al (2008b) Xanthones with quinone reductase-inducing activity from the fruits of *Garcinia mangostana* (Mangosteen). Phytochemistry 69:754–758

Comblain F, Serisier S, Barthelemy N et al (2015) Review of dietary supplements for the management of osteoarthritis in dogs in studies from 2004–2014. J Vet Pharmacol Ther 39(1):1–15

Chorfa N, Savard S, Belkacemi K et al (2016) An efficient method for high-purity anthocyanin isomers isolation from wild blueberries and their radical scavenging activity. Food Chem 197:1226–1234

Choudhary MI, Yousuf S, Nawaz SA et al (2004) Cholinesterase inhibiting withanolides from *Withania somnifera*. Chem Pharm Bull (Tokyo) 52(11):1358–1361

Choudhary I, Ullah F, Ayaz M et al (2017) Anticholinesterase and antioxidant potentials of *Nonea micrantha* Bios. and Reut along with GC-MS analysis. BMC Complement Altern Med 17:499

Chudiwal AK, Jain DP, Somani RS (2010) *Alpinia galanga* Willd.—an overview on phyto-pharmacological properties. Indian J Nat Prod Resour 1(2):143–149

Clark KL (2007) Nutritional considerations in joint health. Clin Sports Med 26:101–118

Clarke KA, Dew TP, Watson REB et al (2014) High performance liquid chromatography tandem mass spectrometry dual extraction method for identification of green tea catechin metabolites excreted in human urine. J Chromatogr B 972:29–37

Coppock RW, Dziwenka M (2016) Green tea extract. In: Gupta RC (ed) Nutraceuticals: efficacy, safety and toxicity. Academic Press/Elsevier, Amsterdam, pp 633–652

D'Amour F, Smith DA (1941) A method for determining loss of pain sensation. J Pharmacol Exp Ther 72(1):74–79

Daoud A, Mefteh FB, Mnafgui K et al (2017) Cardiopreventive effect of ethanolic extract of Date Palm Pollen against isoproterenol induced myocardial infarction in rats through the inhibition of the angiotensin-converting enzyme. Exp Toxicol Pathol 69(8): 656–665

Davis CD, Milner JA (2007) Biomarkers for diet and cancer prevention research: potentials and challenges. Acta Pharmacol Sin 28(9): 1262–1273

De Vries JH, Hollman PC, Meyboom S et al (1998) Plasma concentrations and urinary excretion of the antioxidant flavonols quercetin and kaempferol as biomarkers for dietary intake. Am J Clin Nutr 68:60–65

Denisow B, Denisow-Pietrzyk M (2016) Biological and therapeutic properties of bee pollen: a review. J Sci Food Agric 96:4303–4309

Di Y, Jones J, Mansell K et al (2017) Influence of flaxseed lignan supplementation to older adults on biochemical and functional outcome measures of inflammation. J Am Coll Nutr 36(8):646–653

Do QD, Angkawijaya AE, Tran-Nguyen PL et al (2014) Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophila aromatica*. J Food Drug Anal 22:296–302

Erel O (2004) A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. Clin Biochem 37:277–285

Fahmy NM, El-Sayed E, Abdel-Daim MA et al (2017) Anti-inflammatory and analgesic activities of *Terminalia muelleri* Benth. (Combretaceae). Drug Dev Res 78:146–154

Faria A, Fernandes I, Norberto S et al (2014) Interplay between anthocyanins and gut microbiota. J Agric Food Chem 62:6898–6902

Fierascu I, Ungureanu C, Avramescu SM et al (2018) Genoprotective, antioxidant, antifungal, and anti-inflammatory evaluation of hydroalcoholic extract of wild-growing *Juniperus communis* L. (*Cupresseae*) native to Romanian southern sub-Carpathian hills. BMC Complement Altern Med 18:1–14

Figueiredo-Rinchel ASG, de Melo LL, Bortot LO et al (2017) *Baccharis dracunculifolia* DC (Asteraceae) selectively modulates the effector functions of human neutrophils. J Pharm Pharmacol 69:1829–1845

Filho FO, Alcântra DB, Rodrigues THS et al (2018) Development and validation of a reversed phase HPLC method for determination of anacardic acids in cashew (*Anacardium occidentale*) nut shell liquid. J Chromatogr Sci 56(4):300–306

Fleck A, Gupta RC, Goad JT et al (2013) Anti-arthritic efficacy and safety of Crominex®+ (trivalent chromium, *Pyllanthus emblica* extract, and Shilajit) in moderately arthritic dogs. J Vet Sci Anim Hus 1(4):1–8

Fotso AF, Longo F, Djomeni PD et al (2014) Analgesic and anti-inflammatory activities of the ethyl acetate fraction of *Bidens pilosa* (Asteraceae). Inflammopharmacology 22:105–114

Foyet HS, Deffo ST, Yewo PK et al (2017) *Ficus sycomorus* extract reversed behavioral impairment and brain oxidative stress induced by unpredictable chronic mild stress in rats. BMC Complement Altern Med 17:502

Fujii A, Kobayashi S, Kuboyama N et al (1990) Augmentation of wound healing by royal jelly (RJ) in streptozotocin-diabetic rats. Jpn J Pharmacol 53:331–337

Gao Y, Li C, Wang Y et al (2017) Nonclinical safety of astilbin: a 4-week oral toxicity study in rats with genotoxicity, chromosomal aberration, and mammalian micronucleus tests. Food Chem Toxicol 107:1–9

Garcia-Nino WR, Estrada-Muniz E, Valverde M et al (2017) Cytogenetic effects of Jacareubin from *Calophyllum brasiliense* on human peripheral blood mononucleated cells *in vitro* and on mouse polychromatic erythrocytes *in vivo*. Toxicol Appl Pharmacol 335:6–15

Garg RC (2016) Fenugreek: multiple health benefits. In: Gupta RC (ed) Nutraceuticals: efficacy, safety and toxicity. Academic Press/Elsevier, Amsterdam, pp 599–617

Genskowsky E, Puente LA, Pérez-Álvarez JA et al (2016) Determination of polyphenolic profile, antioxidant activity and antibacterial properties of maqui [*Aristotelia chilensis* (Molina) Stunts] a Chilean blackberry. J Sci Food Agric 96:4235–4242

Gessner DK, Ringseis R, Eder K (2016) Potential of plant polyphenols to combat oxidative stress and inflammatory processes in farm animals. J Anim Physiol Anim Nutr 101:605–628

Giustarini D, Dalle-Donne I, Lorenzini S et al (2012) Protein thiolation index (PTI) as a biomarker of oxidative stress. Free Radic Biol Med 53:907–915

Goncharov N, Orekhov AN, Voitenko N et al (2016) Organosulfur compounds as nutraceuticals. In: Gupta RC (ed) Nutraceuticals: efficacy, safety and toxicity. Academic Press/Elsevier, Amsterdam, pp 555–568

González Y, Torres-Mendoza D, Jones GE et al (2015) Marine diterpenoids as potential anti-inflammatory agents. Mediators Inflamm 2015:1–14

Gupta RC (2014) In: Gupta RC (ed) Biomarkers in toxicology. Academic Press/Elsevier, Amsterdam, pp 1–1128

Gupta RC (2016) In: Gupta RC (ed) Nutraceuticals: efficacy, safety and toxicity, Academic Press/Elsevier, Amsterdam. pp 1–1022

Gupta C, Garg AP, Uniyal RC et al (2008) Comparative analysis of the antimicrobial activity of cinnamon oil and cinnamon extract on some food-borne microbes. Afr J Microbiol Res 2(9):247–251

Gupta B, Ghosh KK, Gupta RC (2016) Thymoquinone. In: Gupta RC (ed) Nutraceuticals: efficacy, safety and toxicity. Academic Press/Elsevier, Amsterdam, pp 541–550

Gupta RC, Srivastava A, Lall R (2018) Toxicity potential of nutraceuticals. In: Nicolotti O (ed) Computational toxicology—methods and protocols. Springer Nature, New York

Halim SZ, Zakaria ZA, Omar MH et al (2007) Synergistic gastroprotective activity of methanolic extract of a mixture of *Melastoma malabathricum* and *Muntingia calabura* leaves in rats. BMC Complement Altern Med 17:488

Henroitin Y, Clutterbuck AL, Allaway D et al (2010) Biological actions of curcumin on articular chondrocytes. Osteoarthr Cart 18:141–149

Hiyasat B, Sabha D, Grötzinger K et al (2009) Antiplatelet activity of *Allium ursinum* and *Allium sativum*. Pharmacology 83:197–2004

Horng C-T, Huang C-W, Yang M-Y et al (2017) *Nelumbo nucifera* leaf extract treatment attenuated preneoplastic lesions and oxidative stress in the livers of diethylnitrosamine-treated rats. Environ Toxicol 32:2327–2340

Hoshyar R, Mollaei H (2017) A comprehensive review on anticancer mechanisms of the main carotenoid of saffron, crocin. J Pharm Pharmacol 69(11):1419–1427

Hsiao HY, Chen BH, Kao TH (2017) Analysis of heterocyclic amines in meat by the quick, easy, cheap, effective, rugged, and safe method couples with LC-DAD-MS-MS. J Agric Food Chem 65 (51):11329–11329

Javeri I, Chand N (2016) Curcumin. In: Gupta RC (ed) Nutraceuticals: efficacy, safety and toxicity. Academic Press/Elsevier, Amsterdam, pp 435–445

Jayakumar S, Patwardhan RS, Pal D et al (2017) Mitochondrial targeted curcumin exhibits anticancer effects through disruption of mitochondrial redox and modulation of TrxR2 activity. Free Radic Biol Med 113:530–538

Jayaprakasha GK, Singh RP, Sakariah KK (2001) Antioxidant activity of grape seed (*Vitis vinefera*) extracts on peroxidation models *in vitro*. Food Chem 73:285–290

Jeong K, Shin Y-C, Park S et al (2011) Ethanol extract of *Scutellaria baicalensis* Georgi prevents oxidative damage and neuroinflammation and memorial impairments in artificial senescense mice. J Biomed Sci 18:14

Karonen M, Hamalainen M, Nieminen R et al (2004) Phenolic extractives from the bark of *Pinus sylvestris* L. and their effects on inflammatory mediators nitric oxide and prostaglandin E_2. J Agric Food Chem 52:7532–7540

Khan W, Gupta S, Ahmad S (2017) Toxicology of the aqueous extract from the flowers of *Butea monosperma* Lam. and it's metabolomics in yeast cells. Food Chem Toxicol 108:486–497

Khatua S, Acharya K (2017) Alkaline extractive crude polysaccharide from *Russula senecis* possesses antioxidant potential and stimulates innate immunity response. J Pharm Pharmacol 69:1817–1828

Kim SH, Hong JH, Lee JE et al (2017) 18 beta-Glycyrrhetinic acid, the major bioactive component of *Glycyrrhizae radix*, attenuates airway inflammation by modulating Th2 cytokines, GATA-3, STAT6, and Foxp3 transcription factors in an asthmatic mouse model. Environ Toxicol Pharmacol 52:99–113

Krajčovičová Z, Melus V (2013) Bioactivity and potential health benefits of Rosmarinic acid. Univ Rev 7(2):8–14

Kumar A, Chopra EK, Mukherjee M et al (2015) Current knowledge and pharmacological profile of berberine: an update. Eur J Pharmacol 761:288–297

Kumar D, Rahal A, Malik JK (2016) Neem extract. In: Gupta RC (ed) Nutraceuticals: efficacy, safety and toxicity. Academic Press/ Elsevier, Amsterdam, pp 585–597

Kumari R, Kumar S, Kumar A et al (2017) Antibacterial, antioxidant and immunomodulatory properties in extracts of *Barleria lupulina* Lindl. BMC Complement Altern Med 17:484

Kusano C, Ferrari B (2008) Total antioxidant capacity: a biomarker in biomedical and nutritional studies. J Cell Mol Biol 7(1):1–15

Lautenschläger M, Lechtenberg M, Sendker K et al (2014) Effective isolation protocol for secondary metabolites from saffron: semi-preparative scale preparation of crocetin-1 and *trans*-crocetin. Fitoterapia 92:290–295

Lawley S, Gupta RC, Goad JT et al (2013) Anti-inflammatory and anti-arthritic efficacy and safety of purified shilajit in moderately arthritic dogs. J Vet Sci Anim Hus 1(3):302

Levine CB, Bayle J, Biourge V et al (2017) Cellular effects of a turmeric root and rosemary leaf extract on canine neoplastic cell lines. BMC Vet Res 13:388

Li M, Xu J, Shi T et al (2016) Epigallocatechin-3-gallate augments therapeutic effects of mesenchymal stem cells in skin wound healing. Clin Exp Pharmacol 43:1115–1124

Li Q, Liang X, Zhao L et al (2017a) UPLC-Q-exactive orbitrap/MS-based lipidomics approach to characterize lipid extracts from bee pollen and their *in vitro* anti-inflammatory properties. J Agric Food Chem 65:6848–6860

Li M, Zhu LF, Zhang TT et al (2017b) Pulmonary delivery of tea tree oil-beta-cyclodextrin inclusion complexes for the treatment of fungal and bacterial pneumonia. J Pharm Pharmacol 69(11):1458–1467

Li J, Yuan C, Pan P et al (2017c) Bioassay-guided isolation of antioxidant and cytoprotective constituents from a Maqui berry (*Aristotelia chilensis*) dietary supplement ingredient as markers for qualitative and quantitative analysis. J Agric Food Chem 65:8634–8642

Liu M-W, Wei R, Su M-X et al (2018) Effects of *Panax notoginseng* saponins on severe acute pancreatitis through the regulation of mTOR/Akt and caspase-3 signaling pathway by upregulating miR-181b expression in rats. BMC Complement Altern Med 18:51

Longo DM, Generaux GT, Howell BA et al (2017) Refining liver safety risk assessment: application of mechanistic modeling and serum biomarkers to cimaglermin alpha (GGF2) clinical trials. Clin Pharmacol Ther 102(6):961–969

Lucca LG, de Matos SP, Borille BT et al (2015) Determination of β-caryophyllene skin permeation/retention from crude copaiba oil (*Copaifera multijuga* Hayne) and respective oil-based nanoemulsion using a novel HS-GC/MS method. J Pharm Biomed Anal 104:144–148

Machado AM, de Paula H, Cardoso LD et al (2015) Effects of brown and golden flaxseed on the lipid profile, glycemia, inflammatory biomarkers, blood pressure and body composition in overweight adolescents. Nutrition 31:90–96

Maiti R, Jane D, Das UK et al (2004) Antidiabetic effect of aqueous extract of seed of *Tamarindus indica* L. in streptozotocin-induced diabetic rats. J Ethnopharmacol 92(1):85–91

Marchev AS, Dimitrova P, Koycheva IK et al (2017) Altered expression of TRAIL on mouse T cells via ERK phosphorylation by *Rhodiola rosea* L. and its marker compounds. Food Chem Toxicol 108: 419–428

Marcone MF, Wang S, Albabish W et al (2013) Diverse food-based applications of nuclear magnetic resonance (NMR) technology. Food Res Int 51:729–747

Maresca M, Micheli L, Cinci L et al (2017) Pain relieving and protective effects of Astragalus hydroalcoholic extract in rat arthritis models. J Pharm Pharmacol 69:1858–1870

Mariette F (2009) Investigations of food colloids by NMR and MRI. Curr Opin Colloid Interface Sci 14(3):203–211

Marin L, Miguelez EM, Villar CJ et al (2015) Bioavailability of dietary polyphenols and gut microbiota metabolism: antimicrobial properties. Biomed Res Int 2015:905215

Martinez SE, Chen Y, Ho EA et al (2015) Pharmacological effects of a c-phycocyanin-based multicomponent nutraceutical in an *in-vitro* canine chondrocyte model of osteoarthritis. Can J Vet Res 79: 241–249

May K, Gupta RC, Miller J et al (2015) Therapeutic efficacy and safety evaluation of a novel chromium supplement (Crominex®+3-) in moderately arthritic horses. J J Vet Sci Res 2(1):014

Milind P, Monu Y (2013) Laboratory models for screening analgesics. Int Res J Pharm 4:15–19

Mohanty C, Sahoo SK (2017) Curcumin and its topical formulations for wound healing applications. Drug Discov Today 22(10):1582–1592

Most J, Penders J, Lucchesi M et al (2017) Gut microbiota composition in relation to the metabolic response to 12-week combined polyphenol supplementation in overweight men and women. Eur J Clin Nutr 71:1040–1045

Murdock N, Gupta RC, Vega N et al (2016) Evaluation of *Terminalia chebula* extract for anti-arthritic efficacy and safety in osteoarthritic dogs. J Vet Sci Technol 7(1):1–8

Nallathamby N, Phan C-W, Seow SLS et al (2018) A status review of the bioactive activities of tiger milk mushroom *Lignosus rhinocerotis* (Cooke) Ryvarden. Front Pharmacol 8:998

Nishina A, Hasegawa K-I, Uchibori T et al (1991) 2, 6-Dimethoxy-*p*-benzoqiuinone as an antibacterial substance in the bark of *Phyllostachys heterocycla* var. *pubescens,* a species of thick stemmed bamboo. J Agric Food Chem 39:266–269

Oda K, Murakami T (2017) Pharmacokinetic interaction of green tea beverage containing cyclodextrins and high concentration catechins with p-glycoprotein substrates in LLC-GA5-COL150 cells *in vitro* and in the small intestine of rats *in vivo*. J Pharm Pharmacol 69:1736–1744

Oh S-M, Park G, Lee SH et al (2017) Assessing the recovery from prerenal and renal acute kidney injury after treatment with single herbal medicine via activity of the biomarkers HMGB1, NGAL, and KIM-1 in kidney proximal tubular cells treated by cisplatin with different doses and exposure. BMC Complement Altern Med 17:544

Omote K, Kawamata T, Kawamata M et al (1998) Formalin-induced release of excitatory amino acids in the skin of the hindpaw. Brain Res 787(1):161–164

Ortea I, Canas B, Calo-Mata P et al (2009) Arginine kinase peptide mass fingerprinting as a proteomic approach for species identification and taxonomic analysis of commercially relevant shrimp species. J Agric Food Chem 57:5665–5672

Ou B, Huang D, Hampsch-Woodill M et al (2002) Analysis of antioxidant activities of common vegetables employing oxygen radical absorbance capacity (ORAC) and ferric reducing antioxidant power (FRAP) assays: a comparative study. J Agric Food Chem 50:3122–3128

Ovais M, Ayaz M, Khalil AT et al (2018) HPLC-DAD finger printing, antioxidant, cholinesterase, and α-glucosidase inhibitory potentials of a novel plant *Olax nana*. BMC Complement Altern Med 18:1–13

Ovalle-Magallanes B, Eugenio-Perez D, PedrazaCharerri J (2017) Medicinal properties of Mangosteen (*Garcinia mangostana* L.): a comprehensive update. Food Chem Toxicol 109:102–122

Pang X, Yi Z, Zhang X et al (2009) Acetyl-11-keto-β-boswellic acid inhibits prostate tumor growth by suppressing vascular endothelial growth factor receptor 2-mediated angiogenesis. Cancer Res 69: 5893–9000

Patocka J, Navratilova Z, Ovando M (2017) Biologically active compounds of Knotweed (*Reynoutria* Spp.). Mil Med Sci Lett 86 (1):17–31

Pavlovic DR, Veljkovic M, Stojanovic NM et al (2017) Influence of different wild-garlic (*Allium ursinum*) extracts on the gastrointestinal system: spasmolytic, antimicrobial and antioxidant properties. J Pharm Pharmacol 69:1208–1218

Peng XR, Wang X, Dong JR et al (2017) Rare hybrid dimers with anti-acetylcholinesterase activities from a Safflower (Carthamus tinctorius L.) seed oil cake. J Agric Food Chem 65(43):9453–9459

Penman AD, Kaufman GE, Daniels KK (2014) MicroRNA expression as an indicator of tissue toxicity. In: Gupta RC (ed) Biomarkers in toxicology. Academic Press/Elsevier, Amsterdam, pp 1003–1018

Pérez-Jiménez J, Hubert J, Hooper L et al (2010) Urinary metabolites as biomarkers of polyphenol intake in humans: a systematic review. Am J Clin Nutr 92(4):801–809. https://doi.org/10.3945/ajcn.2010.29924

Pieloch MJ (2006) Method of use and dosage composition of bluegreen algae extract for inflammation in animals. United States Patent; US 7, 025, 965 B1, pp 1–10

Pinto P, Santos CN (2017) Worldwide (poly)phenol intake: assessment methods and identified gaps. Eur J Nutr 56:1393–1408

Poeckel D, Werz O (2006) Biological actions and molecular targets. Curr Med Chem 13:3359–3369

Quiroga AV, Aphalo P, Nardo AE et al (2017) *In vitro* modulation of renin-angiotensin system enzymes by Amaranth (*Amaranthus hypochondriacus*) protein-derived peptides: alternative mechanisms different from ACE inhibition. J Agric Food Chem 65:7415–7423

Ranasinghe P, Jayawardena R, Pigera S et al (2017) Evaluation of pharmacodynamic properties and safety of *Cinnamomum zeylanicum* (Ceylon cinnamon) in healthy adults: a phase I clinical trial. BMC Complement Altern Med 17:550

Rastogi A, Shukla S (2013) Amaranth: a new millennium crop of nutraceutical values. Crit Rev Food Sci Nutr 53(2):109–125

Rather LJ, Islam S-U, Mohammad F (2015) *Acacia nilotica* (L): a review of its traditional uses, phytochemistry, and pharmacology. Sustain Chem Pharm 2:12–30

Rietjens IMCM, Dussort P, Günther H et al (2018) Exposure assessment of process-related contaminants in food by biomarker monitoring. Arch Toxicol 92:15–40

Risuleo G (2016) Resveratrol: multiple activities on the biological functionality of the cell. In: Gupta RC (ed) Nutraceuticals: efficacy, safety and toxicity. Academic Press/Elsevier, Amsterdam, pp 453–464

Rodrigues EDCR, Ferreira AM, Vilhena JCE et al (2014) Development of a larvicidal nanoemulsion with Copaiba (*Copaifera duckei*) oleoresin. Rev Bras Farm 24:699–707

Rojo LE, Ribnicky D, Logendra S et al (2012) *In vitro* and *in vivo* antidiabetic effects of anthocyanins from Maqui berry (*Aristotelia chilensis*). Food Chem 131:387–396

Rong H, Liang Y, Niu Y (2018) Rosmarinic acid attenuates β-amyloid-induced oxidative stress via Akt/GSK-3β/Fyn-mediated Nrf2 activation in PC2 cells. Free Radic Biol Med 120:114–123

Roopchand DE, Carmody RN, Kuhn P et al (2015) Dietary polyphenols promote growth of the gut bacterium *Akkermansia muciniphila* and attenuate high fat diet-induced metabolic syndrome. Diabetes 64:2847–2858

Ross AB, Shepherd MJ, Schüpphaus M et al (2003) Alkylresorcinols in cereals and cereal products. J Agric Food Chem 51:4111–4118

Rossi GB, Valentim-Neto PA, Blank M et al (2017) Comparison of grain proteome profiles of four Brazilian common bean (*Phaseolus vulgaris* L.) cultivars. J Agric Food Chem 65:7588–7597

Ruiz A, Hermosín-Gutiérrez I, Mardones C et al (2010) Polyphenols and antioxidant activity of calafate (*Berberis microphylla*) fruits and other native berries from Southern Chile. J Agric Food Chem 58:6081–6089

Salem MZM, Elansary HO, Ali HM et al (2018) Bioactivity of essential oils extracted from *Cupressus macrocarpa* branchlets and *Corymbia citriodora* leaves grown in Egypt. BMC Complement Altern Med 18:23

Sandesh P, Velu V, Singh RP (2014) Antioxidant activities of tamarind (*Tamarindus indica*) seed coat extracts using *in vitro* and *in vivo* models. J Food Sci Technol 51(9):1965–1973

Sarada DVL, Kumar CS, Rengasamy R (2011) Purified c-phycocyanin from *Spirulina platensis* (Nordstedt) Geitler: a novel and potent agent against drug resistant bacteria. World J Microbol Biotechnol 27:779–783

Satoh T (2016) Astaxanthin: health benefits and toxicity. In: Gupta RC (ed) Nutraceuticals: efficacy, safety and toxicity. Academic Press/Elsevier, Amsterdam, pp 531–539

Saura-Calixto F, Serrano J, Goñi I (2007) Intake and bioaccessibility of total polyphenols in a whole diet. Food Chem 101(2):492–501

Savolainen O, Lind MV, Bergstrom G et al (2017) Biomarkers of food intake and nutrient status are associated with glucose tolerance status and development of type 2 diabetes in older Swedish women. Am J Clin Nutr 106(5):1302–1310

Schmidt M, Skaf J, Gavril G et al (2017) The influence of *Osmunda regalis* root extract on head and neck cancer cell proliferation, invasion and gene expression. BMC Complement Altern Med 17:518

Servili M, Baldioli M, Selvaggini R et al (1999) High-performance liquid chromatography evaluation of phenols in olive fruit, virgin olive oil, vegetation waters, and pomace and 1D- and 2D-nuclear magnetic resonance characterization. J Am Oil Chem Soc 76: 873–882

Shen J, Pu ZJ, Kai J et al (2017) Comparative metabolomics analysis for the compatibility and incompatibility of kansui and kansui and licorice with different ratios by UHPLC-QTOF/MS and multivariate data analysis. J Chromatogr B 1057:40–45

Shih CM, Cheng SN, Wong CS et al (2009) Antiinflammatory and antihyperalgesic activity of c-phycocyanin. Anesth Analg 108:1302–1310

Shoda LKM, Woodhead JL, Siler SQ et al (2014) Linking physiology to toxicity using DILIsym®, a mechanistic mathematical model of drug-induced liver injury. Biopharm Drug Dispos 35:33–49

Shoko T, Maharaj VJ, Naidoo D et al (2018) Anti-aging potential of extracts from *Sclerocarya birrea* (A. Rich.) Hochst and its chemical profiling by UPLC-Q-TOF-MS. BMC Complement Altern Med 18:54

Siddiqui MZ (2011) *Boswellia serrata*, a potential antiinflammatory agent: an overview. Indian J Pharm Sci 73(3):255–261

Sima IA, Andrasi M, Sarbu C (2018) Chemometric assessment of chromatographic methods for herbal medicines authentication and fingerprinting. J Chromatogr Sci 56(1):49–55

Singh G, Passari AK, Singh P et al (2017) Pharmacological potential of *Bidens pilosa* L. and determination of bioactive compounds using UHPLC-QqQ_{LIT}-MS/MS and GC/MS. BMC Complement Altern Med 17:492

Sobrinho AP, Minho AS, Ferreira LC et al (2017) Characterization of anti-inflammatory effect and possible mechanism of action of *Tibouchina granulosa*. J Pharm Pharmacol 69:706–713

Soriano-Santos J, Escalona-Buendía H (2015) Angiotensin I-converting enzyme inhibitory and antioxidant activities and surfactant properties of protein hydrolysates as obtained of *Amaranthus hypochondriacus* L. grain. J Agric Food Chem 52(4):2073–2082

Srivastava S, Mennemeier M, Pimple S (2017) Effect of *Alpinia galanga* on mental-alertness and sustained attention with or without

caffeine: a randomized placebo-controlled study. J Am Coll Nutr 36 (8):631–639

Stahl A, Schröder U (2017) Development of a MALDI-TOF MS-based protein fingerprint database of common food fish allowing fast and reliable identification of fraud and substitution. J Agric Food Chem 65:7519–7527

Sudjaroen Y, Haubner R, Würtele G et al (2005) Isolation and structure elucidation of phenolic antioxidants from tamarind (*Tamarindus indica* L.) seeds and pericarp. Food Chem Toxicol 43(11): 1673–1682

Sultana A, Nurun Nabi AHM, Nasir UM et al (2008) A dipeptide YY derived from royal jelly proteins inhibits renin activity. Int J Mol Med 21:677–681

Svoboda P, Vičková H, Nováková L (2015) Development and validation of UHPLC-MS/MS method for determination of eight naturally occurring catechin derivatives in various tea samples and the role of matrix effects. J Pharm Biomed Anal 114:62–70

Talawar ST, Harohally NV, Ramakrishna C et al (2017) Development of wheat bran oil concentrates rich in bioactives with antioxidant and hypolipidemic properties. J Agric Food Chem 65(45):9838–9848

Tamrat Y, Nedi T, Assefa S et al (2017) Anti-inflammatory and analgesic activities of solvent fractions of the leaves of *Moringa stenopetala* Bak. (Moringaceae) in mice models. BMC Complement Altern Med 17:473

Tan ETT, Jassim RA, D'Arcy BR et al (2017) *In vitro* biodegradation of hepatotoxic indospicine in *Indigofera spicata* and its degradation derivatives by camel foregut and cattle rumen fluids. J Agric Food Chem 65:7528–7534

Tandan SK, Gupta S, Chandra S et al (1995) Safety evaluation of *Azadirachta indica* seed oil, a herbal wound dressing agent. Fitoterapia 66:69–72

Tarachiwin L, Ute K, Kobayashi E et al (2007) ^{1}H NMR based metabolic profiling in the evaluation of Japanese green teat quality. J Agric Food Chem 55(23):9330–9336

Tepsuwan A, Kupradinum P, Kusamran WR (2002) Chemopreventive potential of neem flowers on carcinogen-induced rat mammary and liver carcinogenesis. Asia Pac J Cancer Prev 3(3):231–238

Thaipong K, Boonprakob U, Crosby K et al (2006) Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. J Food Compos Anal 19:669–675

Tomas-Barberan FA, Selma MV, Espín JC (2018) Polyphenols' gut microbiota metabolites: bioactives or biomarkers? J Agric Food Chem 66:3593–3594

Tsai Y-C, Chen S-H, Lin L-C et al (2017) Anti-inflammatory principles from *Sarcandra glabra*. J Agric Food Chem 65:6497–6505

Tsuda T, Watanabe M, Ohshima K et al (1994) Antioxidative components isolated from the seed of tamarind (*Tamarindus indica* L.). J Agric Food Chem 42:2671–2674

Upadhyay A, Agrahari P, Singh DK (2014) A review on the pharmacological aspects of *Terminalia chebula*. Int J Pharmacol 10:289–298

van Gorsel H, Li C, Kerbel EL et al (1992) Compositional characterization of prune juice. J Agric Food Chem 40:784–789

Vijayaraghavan K, Rajkumar J, Seyed MA (2017) Efficacy of *Chromolaena odorata* leaf extracts for the healing of rat excision wounds. Vet Med 62(10):565–578

Wan D, Wu Q, Kuča K (2016) Spirulina. In: Gupta RC (ed) Nutraceuticals: efficacy, safety and toxicity. Academic Press/ Elsevier, Amsterdam, pp 569–583

Wang J, Zhang X, Lan H et al (2017) Effect of garlic supplement in the management of type 2 diabetes mellitus (T2DM): a meta-analysis of randomized controlled trials. Food Nutr Res 61:1377571

Wang WF, Ha CZ, Lin T et al (2018) Celastrol attenuates pain and cartilage damage via SDF-1/CXCR4 signaling pathway in osteoarthritis rats. J Pharm Pharmacol 70(1):81–88

Williams G, Clifford MN (2017) Role of the small intestine, colon and microbiota in determining the metabolic fate of polyphenols. Biochem Pharmacol 139:24–39

Wilson E, Rajamanickam GV, Dubey GP et al (2011) Review on shilajit used in traditional Indian medicine. J Ethnopharmacol 136:1–9

Woo YJ, Joo YB, Jung YO et al (2011) Grape seed proanthocyanidin extract ameliorates monosodium iodoacetate-induced osteoarthritis. Exp Mol Med 43:561–570

Wu S, Li J, Wang Q et al (2017) Seasonal dynamics of the phytochemical constituents and bioactivities of extracts from *Stenoloma chusanum* (L.) ching. Food Chem Toxicol 108:458–466

Wu QJ, Zheng XC, Wang T et al (2018) Effects of dietary supplementation with oridonin on the growth performance, relative organ weight, lymphocyte proliferation, and cytokine concentration in broiler chickens. BMC Vet Res 14:34

Xavier FH, Maciuk A, Morais ARD et al (2017) Development of a gas chromatography method for the analysis of copaiba oil. J Chromatogr Sci 55(10):969–978

Xiang C-P, Han J-X, Li X-C et al (2017) Chemical composition and acetylcholinesterase inhibitory activity of essential oils from *Piper* species. J Agric Food Chem 65:3702–3710

Yamakoshi Y, Murata M, Shimizu A et al (1992) Isolation and characterization of macrocarpals B-G antibacterial compounds from *Eucalyptus macrocarpa*. Biosci Biotechnol Biochem 56: 1570–1576

Ye W, Chen R, Sun W et al (2017) Determination and pharmacokinetics of engeletin in rat plasma by ultra-high performance liquid chromatography with tandem mass spectrometry. J Chromatogr B 1060: 144–149

Yin X-L, Liu H-Y, Zhang Y-Q (2017) Mulberry branch bark powder significantly improves hyperglycemia and regulates insulin secretion in type II diabetic mice. Food Nutr Res 61:1368847

Yu H, Qiu J-F, Ma L-J et al (2017a) Phytochemical and phytopharmacological review of *Perilla frutescens* L. (Labiatae), a traditional edible-medicinal herb in China. Food Chem Toxicol 108:375–391

Yu X, Tang YH, Liu PY (2017b) Flaxseed oil alleviates chronic HFD-induced insulin resistance through remodeling lipid homeostasis in obese adipose tissue. J Agric Food Chem 65(44):9635–9645

Yun B-S, Lee I-K, Kim J-P et al (2000) Lipid peroxidation inhibitory activity of some constituents isolated from the stem bark of *Eucalyptus globulus*. Arch Pharm Res 23:147–150

Zakaria ZA, Hazalin NM, Zaid SM et al (2007) Antinociceptive, anti-inflammatory and antipyretic effects of *Muntingia calabura* aqueous extract in animal models. J Nat Med 61(4):443–448

Zehl M, Braunberger C, Conrad J et al (2011) Identification and quantification of flavonoids and ellagic acid derivatives in therapeutically important *Drosera* species by LC-DAD, LC-NMR, NMR, and LC-MS. Anal Bioanal Chem 400(8):2565–2576

Zhang Y, Cicchewicz RH, Nair MG (2004) Lipid peroxidation inhibitory compounds from daylily (*Hemerocallis fulva*) leaves. Life Sci 75:753–763

Zhao B, Ren B, Guo R et al (2017) Supplementation of lycopene attenuates oxidative stress induced neuroinflammation and cognitive impairment via Nrf2/NF-κB transcriptional pathway. Food Chem Toxicol 109(1):505–516

Zou Y, Kim AR, Kim JE et al (2002) Peroxynitrite scavenging activity of sinapic acid (3, 5-dimethoxy-4-hydroxycinnamic acid) isolated from *Brassica juncea*. J Agric Food Chem 50:5884–5890

Toxicology and Drug Interactions of Nutraceuticals

Rhian B. Cope

Abstract

This chapter attempts to summarise and tabulate many of the better known adverse toxicological and drug–drug interactional effects of nutraceuticals and botanical supplements. Because of the large scope of these subjects, an extensive literature list is provided in the "References" section of the chapter. The safety properties of nutraceuticals are, in general, poorly studied, and there is absolutely no reason to assume that these products are "safe" for use in humans or animals despite claims of longstanding cultural or dietary use. Given this context it is possible that many problems currently go unrecognised and the burden of adverse effects may be substantially underestimated. This situation will likely continue until a shift towards a regime premarket regulatory approval with mandatory post-market pharmacovigilance is applied in higher-risk and/or data-poor substances.

Keywords

Veterinary nutraceuticals · Toxic interactions

1 Introduction

The best-known adverse effects of nutraceuticals and herbal supplements involve hepatotoxicity and drug-drug interactions. Due to the monumental scope of these issues, this chapter has taken the approach of summarizing the well-known adverse effects of nutraceutical and botanical supplements. Since most regulatory jurisdictions often require minimal to no information or studies on the adverse effects of nutraceuticals and herbal supplements, the available data is often of lower quality and is exploratory in nature. Often the critical safety properties of nutraceuticals are poorly studied, and much of the information is based on in vitro studies, small-scale exploratory studies in laboratory species, limited case series and single case reports.

Currently there is no reason to suspect that the classical high-risk organs and systems associated with well-characterised pharmaceuticals (CNS, liver, kidney and the cardiac conduction system) are not also at risk from poorly characterised nutraceutical and herbal extract products. In many jurisdictions, post-market safety evaluation can be at best described as minimal and, at worst, haphazard or absent. While the relatively lax regulatory approach that is currently applied to nutraceutical products remains, often an overwhelming lack of systematically evaluated safety data on many of these products will also remain. Furthermore, much of the current database is simply observational, and often there is a substantial lack of mechanistic and mode of action data.

Perhaps the saving grace in this situation is that, based largely on Poisons Information Centre data, most nutraceutical-/supplement-associated adverse events are minor although apparent adverse effects are relatively common. However serious adverse events and fatalities have certainly been detected. Given the known incidence of adverse events amongst study populations that is now available from the human pharmaceutical clinical trial literature, it is likely that the adverse effects of nutraceuticals are often seriously underestimated.

2 Hepatotoxicity

As noted in the introduction, unexpected hepatotoxicity is the best studied major cause of adverse events associated with nutraceutical and supplement use. Two major types of hepatotoxicity have been identified:

R. B. Cope (✉)
Australian Pesticides and Veterinary Medicines Authority, Armidale, NSW, Australia
e-mail: rhian.Cope@apvma.gov.au

R. C. Gupta et al. (eds.), *Nutraceuticals in Veterinary Medicine*, https://doi.org/10.1007/978-3-030-04624-8_51

- Classical Type A chemical-driven hepatotoxicity due to the inherent hepatocellular damage induced by the active ingredient and/or its metabolite(s) and/or its impurities. This type of hepatotoxicity has a predictable individual and population-dose responses and temporality that often has a well-characterised mode of action, and the majority of an appropriately exposed population will develop liver damage. Classical examples include many human pharmaceuticals, various mycotoxins such as the aflatoxins and plant toxins such as the pyrrolizidine alkaloids.
- Classical Type B idiosyncratic hepatotoxicity that occurs without warning is often non-predictable using classical toxicological techniques, usually does not have a clear or predictable dose response, often does not have predictable temporality postexposure, may occur rarely or sporadically across populations and may be associated with a particular hypersusceptible subpopulation and/or may have an immune-mediated component. Classical examples are the drugs troglitazone and trovafloxacin.

Important and well-described Type A hepatotoxicity due to sources of nutraceuticals is summarised in Table 1. Critically, often only limited information is available for many nutraceuticals; thus this summary cannot be regarded as anything like comprehensive. Furthermore, since Type B hepatotoxicity can be difficult to identify in large populations, this type of hepatotoxicity may be seriously underestimated with nutraceutical products (especially since these products are usually not subjected to systematic post-market safety evaluation).

Greater detail on specific agents listed in Table 1 can be found by referring to the papers in the "References" section of this chapter.

Classical Type B hepatotoxicity in humans has been recorded with black cohosh (*Actaea racemosa*, Franco et al. 2017). Classical immune-mediated hepatitis secondary to the formation of protein adducts has been identified. Autoimmune hepatitis has also been associated with the use of *Echinacea purpurea*-containing products (Enbom et al. 2014; Franco et al. 2017; Kocaman et al. 2008; Lim et al. 2013; Lynch et al. 2006; Teschke 2010a, b; Whiting et al. 2002).

3 Known Drug–Drug Interactions of Nutraceuticals

A comprehensive detailed description of the large number of drug–drug and xenosensor interactions of nutraceuticals would be the subject for an entire textbook on its own! Because of the length restrictions of this chapter, this information has been summarised in Table 2. Further details can be found in the relevant references. It is important to remember that these effects are not necessarily adverse in the biological sense: many are classical examples of the adaptive mechanisms that come into play when the body encounters a xenobiotic and enacts a series of stereotypical biochemical responses in an attempt to detoxify and/or enhance the excretion of the unwanted substance. Unfortunately, these responses can also be adverse and/or can affect the pharmacokinetic behaviour of many other substances. When referring to Table 1, it is important to remember that CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP3A4 and CYP3A5 members of cytochrome P450 enzyme family metabolise approximately 90% of current human pharmaceuticals.

4 Cardiovascular Effects

There is currently a serious lack of systematic evaluation of the possible cardiovascular effects of nutraceuticals and nutritional supplements. Sympathomimetic toxicity associated with caffeine and/or *Ephedra* sp.- (now banned in many countries) and/or *Yohimbe* sp.-containing products is now well-known (Gurley et al. 2015). Psychiatric adverse effects have also been associated with such products.

Citrus aurantium L. (bitter orange) use has been associated with adverse effects on the cardiovascular system including angina, hypertension, tachycardia, ventricular extrasystoles, and ischemic colitis (Gange et al. 2006; Stohs 2017; Sultan et al. 2006). The putative toxic principles are the sympathomimetic stimulant amines, synephrine and octopamine (Stohs 2017). The use in combination with other sympathomimetics may exacerbate these effects and has reputedly resulted in angina, acute myocardial infarction, ischaemic stroke and exercise-induced syncope. Co-morbidities are often present in the reported human adverse experience reports making definitive conclusions regarding causality less absolute (Stohs 2017). As with other sympathomimetic substances, bitter orange psychosis has also been described (Retamero et al. 2011).

Ginkgo biloba-based products have been ventricular arrhythmias (Cianfrocca et al. 2002). *Cimicifuga racemosa* (black cohosh) use has been associated with reversible heart block with bradycardia and cutaneous vasculitis in humans (McKenzie and Rahman 2010).

Hypertension and hypokalemic effects on the heart and skeletal muscle are classical well-known effects of glycyrrhetic acid derived from *Glycyrrhiza glabra* (liquorice). Chaste tree (*Vitex agnus castus*) products are known to cause arteriospasm (Daniele et al. 2005).

5 Allergic and Skin Effects Not Associated with Hepatotoxicity

Citrus aurantium L. (bitter orange) use has been associated with allergic bronchospasm (Felix et al. 2013). IgE-mediated and other types of hypersensitivity have reputedly associated

Table 1 Potential Type A hepatotoxicity due to nutraceuticals

Herbal supplement	Key compound or product form	Effects or circumstances	References
Ginseng *Panax ginseng*, *P. quinquefolius*	Extract	Possibly due to interaction with imatinib	Bilgi et al. (2010)
Greater celandine *Chelidonium majus*	Extract	Reports of hepatocellular injury and cholestasis in humans	Hardeman et al. (2008), Stickel et al. (2003), Teschke et al. (2011) and Teschke et al. (2012a, b)
Black pepper *Piper nigrum*	Piperidine	Experimental evidence of redox damage in rodents	De Souza Grinevicius et al. (2016) and Piyachaturawat et al. (1995)
St. John's wort *Hypericum perforatum*	Extract	Possibly due to interaction with copaiba and or due to a drug–drug or drug chemical interaction	Agollo et al. (2014), Domínguez Jiménez et al. (2007) and Valentao et al. (2004)
Green tea *Camellia sinensis*	Epigallocatechin-3-gallate	Hepatotoxic in rodents and humans. Cases in humans are reputedly due to *N*-nitroso-fenfluramine and/or epigallocatechin-3-gallate	Wang et al. (2015)
	Extract		Emoto et al. (2014), Federico et al. (2007), Galati et al. (2006), Gloro et al. (2005), Mazzanti et al. (2009), Mazzanti et al. (2015), Pillukat et al. (2014), Teschke et al. (2014) and Vial et al. (2003)
Germander *Teucrium* sp.	Extracts, teucrin A, teuchamaedryn A	Hepatoxic in rodents and humans	Goksu et al. (2012), Kouzi et al. (1994), Lekehal et al. (1996) and Sezer and Bozaykut (2012)
Camphor *Cinnamomum camphora*	Topical cream	Since case report of hepatotoxicity in a human child	Uc et al. (2000)
Kava kava *Piper methysticum*	Extract	Hepatotoxic in humans	Teschke (2010a, b)
Pennyroyal oil *Mentha pulegium*, *Hedeoma pulegioides*	Oil, (R)-(+)-pulegone, pulegone, menthol, menthone	Hepatotoxic in rodents and in humans	Anderson et al. (1996), Bakerink et al. (1996), Gordon et al. (1982), Gordon and Khojasteh (2015) and Sztajnkrycer et al. (2003)
Gardenia *Fructus gardenia*	Extract	Hepatotoxic in rodents	Yamano et al. (1988) and Yang et al. (2006)
	Geniposide		Wei et al. (2014)
Garlic *Allium sativum*	Homogenate	Hepatotoxic in rodents	Oboh (2006) and Rana et al. (2006)
Monocrotaline, dehydromonocrotaline, dehydrorectronecine	Found in many plant species, classically *Crotalaria* sp.	Classical hepatotoxic and pulmonary vasculotoxic pyrrolizidine alkaloids	Mingatto et al. (2007)
Garcinia cambogia	Extract	Hepatotoxic in humans	Corey et al. (2016)
Mistletoe *Viscum coloratum*	Extract	Hepatotoxic in humans	Kim et al. (2015)
Bitter orange *Citrus aurantium*	Not stated	Hepatotoxic in humans	Teschke and Andrade (2016)
Chaste tree *Vitex agnus-castus*	Not stated	Hepatotoxic in humans	Daniele et al.(2005)
Valerian (*Valeriana officinalis*)	Not stated	Hepatotoxic in humans	Cohen and Del Toro (2008)

with immunostimulation by products containing *Echinacea* sp. (Mullins and Heddle 2002). Allergic reactions to domestic bee royal jelly and propolis are well-known. Cutaneous pseudolymphoma has been associated with *Cimicifuga racemosa* (black cohosh) use (Meyer et al. 2007).

Cinnamon-containing products are well-known causes of adverse effects on the skin and oral cavity mucosa including contact sensitisation; stomatitis with swelling and burning of lips, tongue and cheeks; exacerbation of rosacea; and hyperkeratotic plaques covering most of the dorsal and lateral tongue and involving the buccal mucosa, allergic leukoplakia of oral mucosa and squamous cell carcinoma of the tongue (Campbell et al. 2008; Cohen and Bhattacharyya 2000; Mihail 1992; Pilapil 1989; Siqueira et al. 2009; Tremblay and Avon 2008; Westra et al. 1998).

Soybean (*Glycine max*) products are a well-known cause of serious allergic reactions in humans as are ginseng-containing products.

Table 2 Known potential drug–drug interactions of nutraceuticals

Herbal supplement	Key compound or product form	Effects	References
Inhibition of cytochrome P450 family enzymes			
Mangosteen (*Garcinia mangostana*)	Aqueous extract	Inhibition of CYPs 2C8, 2C9, 2C19	Foti et al. (2009)
Black cohosh (*Actaea racemosa* L. [*syn. Cimicifuga racemosa* L.])	Fukinolic acid and cimicifugic acids A and B	Inhibition of CYPs 1A2, 2D6, 2C9, 3A4	Gurley et al. (2008) and Huang et al. (2010)
Green tea (*Camellia sinensis*)	(−)-Epigallocatechin-3-gallate	Inhibition of liver and intestinal microsomal CYPs 2B6, 2C8, 2C19, 2D6, 3A	Misaka et al. (2013a, b, c)
	Extracts	Inhibition of CYP3A	Misaka et al. (2013a, b, c)
Menthol	Menthofuran	Inhibition of CYPs 2A6, 2A13	Kramlinger et al. (2012), Miyazawa et al. (2011) and Yamaguchi et al. (2013)
	(−)-menthol	Inhibition of CYPs 2A6, 2A13	
Garcinia jasminoides	Geniposide, extract	Inhibition of CYP 3A4	Kang et al. (1997)
	Genipin	Inhibition of CYPs 2C19 and 3A4	Gao et al. (2014) and Kang et al. (1997)
	Geniposide	Inhibition of CYP 3A4	
Garlic (*Allium sativum*)	Garlic oil	Inhibition of CYP 2E1	Gurley et al. (2005) and Zhang et al. (2006)
	Not defined	Inhibition of CYP 1A2	
Retrorsine	Various plant extracts	Inhibition of CYP3A4	Dai et al. (2010)
Grapefruit (*Citrus paradisi*)	Dihydroxybergamottin, bergamottin	Inhibition of CYP3A4	Cingi et al. (2013), Holmberg et al. (2014), Lee et al. (1999), Messer et al. (2012) and Tanaka et al. (2013)
	Juice	Inhibition of CYP3A4	
Echinacea purpurea	Root extract (pill)	Inhibition of CYPs 3A4, 1A2	Barrett (2003), Gorski et al. (2004) and Gurley et al. (2004)
	Maslinic acid, corosolic acid, ursolic acid	Inhibition of CYP3A4	
Cranberry (*Vaccinium macrocarpon*)	Extract	Inhibition of CYPs 1A2, 2D6	Kim et al. (2011)
Milk thistle (*Silybum marianum*)	Silybin, isosilybin	Inhibition of CYP 3A4, inhibition of PXR up-regulation	Mooiman et al. (2013)
Tomato (*Lycopersicon esculentum*)	Juice extract	Inhibition of CYP3A4	Sunaga et al. (2012)
Capsicum (*Capsicum annuum* L. var. grossum.)	Dried	Inhibition of CYP3A4	Sunaga et al. (2012)
Potato (*Solanum tuberosum* L.)	Dried	Inhibition of CYPs 1A2, 2D6, 3A4	Sunaga et al. (2012)
Eggplant (*Solanum melongena* L.)	Dried	Inhibition of CYPs 1A2, 2D6, 3A4	Sunaga et al. (2012)
Sweet pepper (*Capsicum annuum*)	Dried	Inhibition of CYPs 1A2, 2D6, 3A4	Sunaga et al. (2012)
Black elderberry (*Sambucus nigra*)	Extract	Inhibition of CYPs 1A2, 2D6, 3A4	Langhammer and Nilsen (2014)
Fennel (*Foeniculum vulgare*)	Tea	Inhibition of CYPs 1A2, 2D6, 3A4	Langhammer and Nilsen (2014)
Horsetail (*Equisetaceae family*)	Tea	Inhibition of CYPs 1A2, 2D6, 3A4	Langhammer and Nilsen (2014)
Raspberry leaf (*Rubus idaeus*)	Extract	Inhibition of CYPs 1A2, 2D6, 3A4	Langhammer and Nilsen (2014)
Cinnamon (*Cinnamomum verum*)	*O*-methoxy cinnamaldehyde	Inhibition of CYPs 1A2, 2E1	Hasegawa et al. (2002)
	Extract	Inhibition of CYPs 2C9, 3A4	
Ginger (*Zingiber officinale*)	Extract	Inhibition of CYPs 2C9, 2C19 and 3A4	Li et al. (2013a, b)
Mace (*Myristica fragrans*)	Extract	Inhibition of CYPs 2C9, 3A4	Kimura et al. (2010)
Nutmeg (*Myristica genus*)	Extract	Inhibition of CYPs 2C9, 3A4	Kimura et al. (2010)
Valerian (*Valeriana officinalis*)	Extract	Inhibition of CYP 2C8	Carrasco et al. (2009)

(continued)

Table 2 (continued)

Herbal supplement	Key compound or product form	Effects	References
Madagascar medicinal plant (*Catharanthus roseus*)	Ajmalicine	Inhibition of CYP 2D6	Usia et al. (2005)
	Vindolene	Inhibition of CYPs 2D6, 3A4	
	Serpentine	Inhibition of CYPs 2D6, 3A4	
Southern African medicinal plant (*Sutherlandia frutescens*)	Extract	Inhibition of CYP3A4	Bye and Dutton (1991) and Mills et al. (2005)
Southern African medicinal plant (*Moringa oleifera*)	Extract	Inhibition of CYP3A4	Monera et al. (2008)
West African medicinal plants *Aframomum cuspidatum* *Aframomum melegueta* *Harrisonia abyssinica* *Phyllanthus amarus* *Piper guineense* *Lonchocarpus sericeus* *Lippia multiflora*	Extracts	Inhibition of CYPs 3A4, 3A5, 3A7	Agbonon et al. (2010)
West African medicinal plants *Jatropha curcas* *Persea americana* *Oxytenanthera abyssinica* *Talinum triangulare*	Extracts	Inhibition of CYPs 3A4, 3A7	Agbonon et al. (2010)
Tanzanian medicinal plant *Cyphostemma ildebrandtii*	Extract	Inhibition of CYPs 3A4, 3A7	Van den Bout-van den Beukel et al. (2008)
Tanzanian medicinal plants *Acacia robusta* *Agauria salicifolia*	Extracts	Inhibition of CYPs 2C9, 2C19, 3A4	Van den Bout-van den Beukel et al. (2008)
Tanzanian medicinal plants *Elaeodendron buchananii* *Sclerocarya birrea*	Extracts	Inhibition of CYPs 2C9, 3A4	Van den Bout-van den Beukel et al. (2008)
Peppermint (*Mentha piperita*)	Oil	Inhibition of CYP 3A4	Dresser et al. (2002)
	Menthol		
	Menthyl acetate		
	Ascorbyl palmitate		
Sesamin (*Sesamum indicum*)	Not stated	Inhibition of CYPs 1A2, 2C9, 2C19, 2D6, 3A4	Yasuda and Sakaki (2012)
Tumeric (*Curcuma longa*)	Not stated	Inhibition of CYPs 1A2, 2C9, 3A4	Bahramsoltani et al. (2017)
St. John's wort (*Hypericum perforatum*)	Not stated	Inhibition of CYPS 1A2, 2C9, 3A4	Roby et al. (2000)
Ginkgo biloba	Extract	Inhibition of CYP2B6	Lau and Chang (2009) and Zhou et al. (2014)
Activation of the cytochrome P450 3A4 promoter by PXR-dependent mechanisms			
Gan Gao licorice (Radix et Rhizoma Glycyrrhizae)	Extract	Activation of the cytochrome P450 3A4 promoter by PXR-dependent mechanisms	Li et al. (2015) and Xu et al. (2016)
Huang Qi *Astragalus mebranaceus*	Extract	"	Li et al. (2015) and Xu et al. (2016)
Ji Xue Cao *Centella asiatica*	Extract	"	Li et al. (2015) and Xu et al. (2016)
Ban Lan Gen *Isatis indigotica*	Extract	"	Li et al. (2015) and Xu et al. (2016)
Jin Yin Hua *Lonicera japonica*	Extract	"	Li et al. (2015) and Xu et al. (2016)
Hong Jing Tian *Rhodiola crenulata*	Extract	"	Li et al. (2015) and Xu et al. (2016)
Da Huang-Rhubarb (Radix et Rhizoma Rhei)	Extract	"	Li et al. (2015) and Xu et al. (2016)
	Trans-resveratrol	"	

(continued)

Table 2 (continued)

Herbal supplement	Key compound or product form	Effects	References
Fu Ling *Poria cocos*	Extract	”	Li et al. (2015) and Xu et al. (2016)
Bai Shao *Paeonia lactiflora*	Extract	”	Li et al. (2015) and Xu et al. (2016)
Sang Qi *Panax notoginseng*	Extract	”	Li et al. (2015) and Xu et al. (2016)
Chuan Xiong *Ligusticum chuanxiong*	Extract	”	Li et al. (2015) and Xu et al. (2016)
Dang Gui-Chinese angelica *Angelicae sinensis*	Extract	”	Li et al. (2015) and Xu et al. (2016)
	Ligustilide	”	
Sheng Di Huang-Rehmannia root *Radix rehmanniae*	Extract	”	Li et al. (2015) and Xu et al. (2016)
Yin Yang Huo *Epimedium brevicornum*	Extract	”	Li et al. (2015) and Xu et al. (2016)
Di Gu Pi *Lycium chinense*	Extract	”	Li et al. (2015) and Xu et al. (2016)
Bai Zhu *Atractylodes macrocephala*	Extract	”	Li et al. (2015) and Xu et al. (2016)
Wu Wei Zi *Schisandra chinensis*	Extract	”	Li et al. (2015) and Xu et al. (2016)
	Schisantherin A	”	
Bai Shao *Paeonia lactiflora*	Extract	”	Li et al. (2015) and Xu et al. (2016)
Mai Dong *Ophiopogon japonicus*	Extract	”	Li et al. (2015) and Xu et al. (2016)
Hu Zhang *Polygonum multiflorum*	Extract	”	Li et al. (2015) and Xu et al. (2016)
Huang Lian *Coptis chinensis*	Extract	”	Li et al. (2015) and Xu et al. (2016)
	Berberine hydrochloride	”	
Yin Chen *Artemisia scoparia Herba artemisiae*	Extract	”	Li et al. (2015) and Xu et al. (2016)
Tian Hua Fen *Trichosanthes kirilowii*	Extract	”	Li et al. (2015) and Xu et al. (2016)
Shui Fei Ji *Silybum marianum*	Extract	”	Li et al. (2015) and Xu et al. (2016)
Zhi Zi-Gardenia fruit *Fructus gardeniae*	Extract	”	Kang et al. (1997), Li et al. (2015) and Xu et al. (2016)
Ren Shen Ginseng Radix et Rhizoma ginseng	Ginsenoside F2, protopanaxadiol	”	Gurley et al. (2005), Janetzky and Morreale (1997), Jones and Runikis (1987), Li et al. (2015) and Xu et al. (2016)
	Panaxatriol, Rg2, pseudoginsenoside F11, Rg1, ginsenodide, Rb3	”	
	Extract	”	
Black pepper *Piper nigrum*	Piperidine	”	Wang et al. (2013a, b)
St. John’s Wort *Hypericum perforatum*	Hyperforin	Also increased CYP2C9, CYP2C19 and CYP2E1	Godtel-Armbrust et al. (2007)
Gingko biloba	Extract	Activation of the cytochrome P450 3A4 promoter by PXR-dependent mechanisms	Gurley et al. (2005) and Zhou et al. (2014)
	Gingkolide A, Gingkolide B	Also increased CYP2B6 and CYP3A4	
Kava kava *Piper methysticum*	Not stated	Activation of the cytochrome P450 3A4 promoter by PXR-dependent mechanisms Also increased CYP2C19	Gurley et al. (2008)

(continued)

Table 2 (continued)

Herbal supplement	Key compound or product form	Effects	References
Echinacea purpurea	Extract	Activation of the cytochrome P450 3A4 promoter by PXR-dependent mechanisms Also CYP1A2	Awortwe et al. (2015), Gorski et al. (2004), Gurley et al. (2005) and Gurley et al. (2008)
Thyme *Thymus vulgaris*	Extract	Activation of the cytochrome P450 3A4 promoter by PXR-dependent mechanisms	Kluth et al. (2007)
Clove *Syzygium aromaticum*	Extract	”	Kluth et al. (2007)
Tumeric *Curcuma longa*	Curcumin	”	Kluth et al. (2007)
Red wine *Vitis vinifera*	Resveratrol	”	Kluth et al. (2007)
Southern African medicinal plant *Hypoxis hemerocallidea*	Extract	”	Monera et al. (2008)
	Rooperol	”	
	Stimasherol	”	
Southern African medicinal plant *Sutherlandia frutescens*	Extract	”	Monera et al. (2008)
Tanzanian medicinal plant *Cyphostemma hildebrandtii*	Extract	”	Van den Bout-van den Beukel et al. (2008)
Tanzanian medicinal plant *Agauria salicifolia*	Extract	”	Van den Bout-van den Beukel et al. (2008)
Tanzanian medicinal plant *Elaeodendron buchananii*	Extract	”	Van den Bout-van den Beukel et al. (2008)
Tanzanian medicinal plant *Sclerocarya birrea*	Extract	”	Van den Bout-van den Beukel et al. (2008)
Tanzanian medicinal plant *Sterculia africana*	Extract	”	Van den Bout-van den Beukel et al. (2008)
Tanzanian medicinal plant *Turraea holstii*	Extract	”	Van den Bout-van den Beukel et al. (2008)
Allspice *Pimenta dioica*	Extract	”	Kluth et al. (2007)
Grape seed *Vitis vinifera*	Extract	”	Kluth et al. (2007)
Garlic *Allium sativum*	Daillysulfide	”	Zhou et al. (2014)

Chaste tree (*Vitex agnus castus*) products have been associated with allergic reactions with pruritus, erythema, gastrointestinal symptoms and acneform facial inflammation (Daniele et al. 2005). Headache, weight gain and persistent gastroenteritis have also occurred in humans.

Grapes (*Vitis vinifera*) are a suspected cause of allergic-like reactions including oral syndrome, urticaria, angio-edema, hypotension and respiratory distress, anaphylaxis and exercise-induced anaphylaxis. The major allergens in grapes are endochitinases A and B, a lipid transfer protein and a thaumatin-like protein.

6 Adverse Effects on the Coagulation System

Consumption of *Ginkgo biloba* leaves and extracts has been associated with hemorrhagic events including subdural hematoma (Bebbington et al. 2005; Castellote Varona and Atienza Morales 2005; Meisel et al. 2003; Miller and Freeman 2002; Pedroso et al. 2011). The effects are reputedly associated with the antiplatelet activity of ginkgosides with ginkgolide B being the likely main terpenoid responsible for

these effects. Co-ingestion of *Ginkgo biloba* products with pharmaceuticals that act on coagulation (aspirin, ibuprofen and warfarin) appears to exacerbate these anticoagulation effects of this plant. Foods containing soybean and its isoflavones were responsible for bleeding when combined with estradiol.

7 Adverse Effects on the Central Nervous System

Ginkgo biloba-associated induction of CYP2C19 catabolism of phenytoin and valproic acid has been associated with fatal breakthrough seizures in humans (Kupiec and Raj 2005). Hypokalemic encephalopathy is a classical effect of liquorice toxicity. *Harpagophytum procumbens* (Devil's claw) is claimed to act as a cyclooxygenase 2 inhibitor (Grahame and Robinson 1981). Apart from the predictable adverse effects on the gut, products containing this material have been associated with throbbing frontal headache, tinnitus, anorexia and loss of taste for food. *Panax ginseng* (ginseng)-containing products have been associated with stimulant effects, such as nervousness and tremor and maniacal episodes (Gonzalvez-Seijo et al. 1995; Vasquez and Aguera-Ortiz 2002).

8 Effects on the Musculoskeletal System

Myopathy with severe asthenia and rhabdomyolysis in humans has been associated with *Cimicifuga racemosa* (black cohosh) use (Minciullo et al. 2006). Secondary rhabdomyolysis is also a noted effect of sympathomimetic substances, and the risk of this effect should always be recognised in cases of overdose with these substances. Rhabdomyolysis and hypokalemic paralysis are classical effects that occur with liquorice toxicity (Sundaram and Swaminathan 1981).

9 Endocrine and Reproductive System Effects

The estrogenic effects of soybean isoflavones are well-known. Associated side effects have been detected in both biological sexes and include pseudohormonal activity-related precocious thelarche; uterine fibroids; ureteral Müllerian carcinosarcoma associated with endometriosis, gynaecomastia, hypogonadism and erectile dysfunction; gastric cancer; testicular cancer; and reproductive disorders (Balk et al. 2005; Dinsdale and Ward 2010; Kwack et al. 2009; Martinez and Lewis 2008; Nagata et al. 2009; Nan et al. 2005; Noel et al. 2006). Hypothyroidism associated with excessive iodine intake has been associated with the consumption of soy milks (Bell and Ovalle 2001; Crawford et al. 2010). Fatal hypernatremia has also been associated with excessive consumption of soy milks (Furukawa et al. 2011). Hypochloremic alkalosis has occurred in infants fed with soy-based milk replacers (Linshaw et al. 1980).

Glycyrrhetic acid in liquorice is an inhibitor of 11-β-hydroxysteroid dehydrogenase type 2, the enzyme that is needed to inactivate cortisol before it binds the aldosterone receptor inside principal cells (Francini-Pesenti et al. 2008; Hukkanen et al. 2009; Russo et al. 2000; Sundaram and Swaminathan 1981). The net effect is an aldosterone-like effect which results in the suppression of the renin-angiotensin-aldosterone axis, blood volume expansion, hypertension, hypertension encephalopathy, hypokalemia and metabolic alkalosis. Chaste tree (*Vitex agnus castus*) products have been associated with intermenstrual bleeding or disorders (Daniele et al. 2005; Loch et al. 2000; Prilepskaya et al. 2006; Propping et al. 1991).

10 Concluding Remarks and Future Directions

Botanical nutraceutical products and supplements should not be regarded as clinically and toxicologically inert irrespective of whether or not the substance has a long history of dietary or cultural use. While, fortunately, severe reactions appear to be somewhat uncommon, fatalities and major adverse events have been recorded. However, what is extremely clear is that there is usually a very limited body of premarket and post-market pharmacovigilance data on many common botanical products. Given this context it is possible that many problems currently go unrecognised and the burden of adverse effects may be substantially underestimated. This situation will likely continue until a shift towards a regime premarket regulatory approval with mandatory post-market pharmacovigilance is applied in higher-risk and/or data-poor substances.

References

Aaronov D, Tasher D, Levine A et al (2008) Natural history of food allergy in infants and children in Israel. Ann Allergy Asthma Immunol 101:637–640

Abdel-Zaher AO, Farghaly HSM, El-Refaiy AEM et al (2017) Protective effect of the standardized extract of ginkgo biloba (EGB761) against hypertension with hypercholesterolemia-induced renal injury in rats: insights in the underlying mechanisms. Biomed Pharmacother 95:944–955

Agbonon A, Eklu-Gadegbeku K, Aklikokou K et al (2010) *In vitro* inhibitory effect of west african medicinal and food plants on human cytochrome P450 3A subfamily. J Ethnopharmacol 128:390–394

Agollo MC, Miszputen SJ, Diament J (2014) Hypericum perforatum-induced hepatotoxicity with possible association with copaiba (*Copaifera langsdorffii* Desf): case report. Einstein 12:355–357

Albassam AA, Mohamed ME, Frye RF (2015) Inhibitory effect of six herbal extracts on CYP2C8 enzyme activity in human liver microsomes. Xenobiotica 45:406–412

Aleksunes LM, Klaassen CD (2012) Coordinated regulation of hepatic phase i and ii drug-metabolizing genes and transporters using AhR-, CAR--, PPARα-, and Nrf2-null mice. Drug Metab Dispos 40:1366–1379

Ali N, Auerbach HE (2017) New-onset acute thrombocytopenia in hospitalized patients: pathophysiology and diagnostic approach. J Community Hosp Intern Med Perspect 7:157–167

Anderson IB, Mullen WH, Meeker JE et al (1996) Pennyroyal toxicity: measurement of toxic metabolite levels in two cases and review of the literature. Ann Intern Med 124:726–734

Appiah S, Revitt M, Jones H et al (2017) Antiinflammatory and hepatoprotective medicinal herbs as potential substitutes for bear bile. Int Rev Neurobiol 135:149–180

Armanini D, Lewicka S, Pratesi C et al (1996) Further studies on the mechanism of the mineralocorticoid action of licorice in humans. J Endocrinol Investig 19:624–629

Avorn J, Monane M, Gurwitz JH et al (1994) Reduction of bacteriuria and pyuria after ingestion of cranberry juice. JAMA 271:751–754

Awortwe C, Manda VK, Avonto C et al (2015) *Echinacea purpurea* up-regulates CYP1A2, CYP3A4 and MDR1 gene expression by activation of pregnane X receptor pathway. Xenobiotica 45:218–229

Baes M, Gulick T, Choi HS et al (1994) A new orphan member of the nuclear hormone receptor superfamily that interacts with a subset of retinoic acid response elements. Mol Cell Biol 14:1544–1552

Bagheri H, Broué P, Lacroix I et al (1998) Fulminant hepatic failure after herbal medicine ingestion in children. Therapie 53:77–83

Bahramsoltani R, Rahimi R, Farzaei MH (2017) Pharmacokinetic interactions of curcuminoids with conventional drugs: a review. J Ethnopharmacol 209:1–12

Bajad S, Bedi KL, Singla AK et al (2001) Antidiarrhoeal activity of piperine in mice. Planta Med 67:284–287

Bakerink JA, Gospe SM Jr, Dimand RJ et al (1996) Multiple organ failure after ingestion of pennyroyal oil from herbal tea in two infants. Pediatrics 98:944–947

Balk E, Chung M, Chew P et al (2005) Effects of soy on health outcomes. Evid Rep Technol Assess (Summ) 126:1–8

Barrett B (2003) Echinacea: a safety review. HerbalGram 57:36–39

Bebbington A, Kulkarni R, Roberts P (2005) *Ginkgo biloba*: persistent bleeding after total hip arthroplasty caused by herbal self-medication. J Arthroplast 20:125–126

Bell DSH, Ovalle F (2001) Use of soy protein supplement and resultant need for increased dose of levothyroxine. Endocr Pract 7:193–194

Benichou C (1990) Standardization of definitions and criteria of causality assessment of adverse drug reactions. Drug-induced liver disorders: report of an international consensus meeting. Int J Clin Pharmacol Ther Toxicol 28:317–322

Benichou C, Danan G, Flahault A (1993) Causality assessment of adverse reactions to drugs—II. An original model for validation of drug causality assessment methods: case reports with positive rechallenge. J Clin Epidemiol 46:1331–1336

Bessone F, Hernandez N, Lucena MI et al (2016) The latin american DILI registry experience: a successful ongoing collaborative strategic initiative. Int J Mol Sci 17:313

Bhopal JS (2001) St John's wort-induced sexual dysfunction. Can J Psychiatr 46:456–457

Bilgi N, Bell K, Ananthakrishnan AN et al (2010) Imatinib and *Panax ginseng*: a potential interaction resulting in liver toxicity. Ann Pharmacother 44:926–928

Borrelli F, Izzo AA (2009) Herb-drug interactions with St John's wort (*Hypericum perforatum*): an update on clinical observations. AAPS J 11:710–727

Bosisio E, Giavarini F, Dell'Agli M et al (2004) Analysis by high-performance liquid chromatography of teucrin A in beverages flavoured with an extract of Teucrium chamaedrys L. Food Addit Contam 21:407–414

Brewer CT, Chen T (2016) PXR variants: the impact on drug metabolism and therapeutic responses. Acta Pharm Sin B 6:441–449

Brown TM (2000) Acute St. John's wort toxicity. Am J Emerg Med 18:231–232

Bruno JJ, Ellis JJ (2005) Herbal use among us elderly: 2002 national health interview survey. Ann Pharmacother 39:643–648

Bye SN, Dutton MF (1991) The inappropriate use of traditional medicines in south africa. J Ethnopharmacol 34:253–259

Campbell TM, Neems R, Moore J (2008) Case report: severe exacerbation of rosacea induced by cinnamon supplements. J Drugs Dermatol 7:586–587

Canter PH, Ernst E (2004) Herbal supplement use by persons aged over 50 years in britain: frequently used herbs, concomitant use of herbs, nutritional supplements and prescription drugs, rate of informing doctors and potential for negative interactions. Drugs Aging 21:597–605

Carrasco MS, Vallejo JR, Pardo-de-Santayana M et al (2009) Interactions of *Valeriana officinalis* L. and *Passiflora incarnata* L. in a patient treated with Lorazepam. Phytother Res 23:1795–1796

Castellote Varona FJ, Atienza Morales MP (2005) *Ginkgo biloba* and cerebral hemorrhage. An Med Interna 22:199

Chaabane M, Bidat E, Chevallier B (2010) A new case of food protein-induced enterocolitis syndrome. Arch Pediatr 17:502–506

Chalasani N, Fontana RJ, Bonkovsky HL et al (2008) Drug induced liver injury network. Causes, clinical features, and outcomes from a prospective study of drug-induced liver injury in the united states. Gastroenterology 135:1924–1934

Chan TY, Tam HP, Lai CK et al (2005) A multidisciplinary approach to the toxicologic problems associated with the use of herbal medicines. Ther Drug Monit 27:53–57

Chanet A, Milenkovic D, Deval C et al (2012) Naringin, the major grapefruit flavonoid, specifically affects atherosclerosis development in diet-induced hypercholesterolemia in mice. J Nutr Biochem 23:469–477

Chen T (2008) Nuclear receptor drug discovery. Curr Opin Chem Biol 12:418–426

Chen D, Klesmer J, Giovanniello A et al (2002) Mental status changes in an alcohol abuser taking valerian and *Gingko biloba*. Am J Addict 11:75–77

Chen Y, Ferguson SS, Negishi M et al (2004) Induction of human CYP2C9 by rifampicin, hyperforin, and phenobarbital is mediated by the pregnane x receptor. J Pharmacol Exp Ther 308:495–501

Chen M, Suzuki A, Borlak J et al (2015) Drug-induced liver injury: interactions between drug properties and host factors. J Hepatol 63:503–514

Chen M, Li L, Zhong D et al (2016) 9-glutathionyl-6,7-dihydro-1-hydroxymethyl-5hpyrrolizine is the major pyrrolic glutathione conjugate of retronecine-type pyrrolizidine alkaloids in liver microsomes and in rats. Chem Res Toxicol 29:180–189

Cherian MT, Chai SC, Chen T (2015) Small-molecule modulators of the constitutive androstane receptor. Expert Opin Drug Metab Toxicol 11:1099–1114

Chiu NT, Tomlinson Guns ES, Adomat H et al (2014) Identification of human cytochrome P450 enzymes involved in the hepatic and intestinal biotransformation of 20(S)-protopanaxadiol. Biopharm Drug Dispos 35:104–118

Cianfrocca C, Pelliccia F, Auruti A et al (2002) *Ginkgo biloba*-induced frequent ventricular arrhythmia. Ital Heart J 3:689–691

Ciganda C, Laborde A (2003) Herbal infusions used for induced abortion. J Toxicol Clin Toxicol 41:235–239
Cingi C, Toros SZ, Gurbuz MK et al (2013) Effect of grapefruit juice on bioavailability of montelukast. Laryngoscope 123:816–819
Cirmi S, Maugeri A, Ferlazzo N et al (2017) Anticancer potential of citrus juices and their extracts: a systematic review of both preclinical and clinical studies. Front Pharmacol 8:420
Clare KE, Miller MH, Dillon JF (2017) Genetic factors influencing drug-induced liver injury: do they have a role in prevention and diagnosis? Curr Hepatol Rep 16:258–264
Coelho C, Tyler R, Ji H et al (2016) Survey on the effectiveness of dietary supplements to treat tinnitus. Am J Audiol 25:184–205
Cohen DM, Bhattacharyya I (2000) Cinnamon-induced oral erythema multiformelike sensitivity reaction. J Am Dent Assoc 131:929–934
Cohen DL, Del Toro Y (2008) A case of valerian-associated hepatotoxicity. J Clin Gastroenterol 42:961–962
Corey R, Werner KT, Singer A et al (2016) Acute liver failure associated with garcinia cambogia use. Ann Hepatol 15:123–126
Crawford BA, Cowell CT, Emder PJ et al (2010) Iodine toxicity from soy milk and seaweed ingestion is associated with serious thyroid dysfunction. Med J Aust 193:413–415
Crean AM, Abdel-Rahman SE, Greenwood JP (2009) A sweet tooth as the root cause of cardiac arrest. Can J Cardiol 25:e357–e358
Cuzzolin L, Benoni G (2009) Attitudes and knowledge toward natural products safety in the pharmacy setting: an Italian study. Phytother Res 23:1018–1023
Dag M, Ozturk Z, Aydnl M et al (2014) Postpartum hepatotoxicity due to herbal medicine teucrium polium. Ann Saudi Med 34:541–543
Dai J, Zhang F, Zheng J (2010) Retrorsine, but not monocrotaline, is a mechanism-based inactivator of P450 3A4. Chem Biol Interact 183:49–56
Danan G, Benichou C (1993) Causality assessment of adverse reactions to drugs—I. A novel method based on the conclusions of international consensus meetings: application to drug-induced liver injuries. J Clin Epidemiol 46:1323–1330
Danan G, Teschke R (2015) Rucam in drug and herb induced liver injury: the update. Int J Mol Sci 17:14
Daniele C, Thompson Coon J et al (2005) *Vitex agnus castus*: a systematic review of adverse events. Drug Saf 28:319–332
De la Garza AL, Etxeberria U, Haslberger A et al (2015) Helichrysum and grapefruit extracts boost weight loss in overweight rats reducing inflammation. J Med Food 18:890–898
De Maat MM, Hoetelmans RM, Math RA, van Gorp EC et al (2001) Drug interaction between St John's wort and nevirapine. AIDS 15:420–421
De Souza Grinevicius VM, Kviecinski MR, Santos Mota NS et al (2016) Piper nigrum ethanolic extract rich in piperamides causes ROS overproduction, oxidative damage in DNA leading to cell cycle arrest and apoptosis in cancer cells. J Ethnopharmacol 189:139–147
Deferme S, Kamuhabwa A, Nshimo C et al (2003) Screening of tanzanian plant extracts for their potential inhibitory effect on P-glycoprotein mediated efflux. Phytother Res 17:459–464
Den Braver MW, den Braver-Sewradj SP, Vermeulen NP et al (2016) Characterization of cytochrome P450 isoforms involved in sequential two-step bioactivation of diclofenac to reactive P-benzoquinone imines. Toxicol Lett 253:46–54
Dergal JM, Gold JL, Laxer DA et al (2002) Potential interactions between herbal medicines and conventional drug therapies used by older adults attending a memory clinic. Drugs Aging 19:879–886
Dinsdale EC, Ward WE (2010) Early exposure to soy isoflavones and effects on reproductive health: a review of human and animal studies. Nutrients 2:1156–1187
Dittmar FW, Bohnert KJ, Peeters M et al (1992) Syndrom, Behandlung mit einem Phytopharmakon. TW Gynakol 5:60–68
Domínguez Jiménez JL, Pleguezuelo Navarro M et al (2007) Hepatotoxicity associated with *Hypericum* (St. John's wort). Gastroenterol Hepatol 30:54–55
Dona M, Dell'Aica I, Calabrese F et al (2003) Neutrophil restraint by green tea: inhibition of inflammation, associated angiogenesis, and pulmonary fibrosis. J Immunol 170:4335–4341
Dresser GK, Wacher V, Wong S et al (2002) Evaluation of peppermint oil and ascorbyl palmitate as inhibitors of cytochrome P450 3A4 activity in vitro and in vivo. Clin Pharmacol Ther 72:247–255
Druckova A, Mernaugh RL, Ham AJ et al (2007) Identification of the protein targets of the reactive metabolite of teucrin a in vivo in the rat. Chem Res Toxicol 20:1393–1408
Emoto Y, Yoshizawa K, Kinoshita Y et al (2014) Green tea extract-induced acute hepatotoxicity in rats. J Toxicol Pathol 27:163–174
Enbom ET, Le MD, Oesterich L et al (2014) Mechanism of hepatotoxicity due to black cohosh (Cimicifuga racemosa): histological, immunohistochemical and electron microscopy analysis of two liver biopsies with clinical correlation. Exp Mol Pathol 96:279–283
Evans JR (2013) Ginkgo biloba extract for age-related macular degeneration. Cochrane Database Syst Rev 31:CD001775
Fau D, Lekehal M, Farrell G et al (1997) Diterpenoids from germander, an herbal medicine, induce apoptosis in isolated rat hepatocytes. Gastroenterology 113:1334–1346
Federico A, Tiso A, Loguercio C (2007) A case of hepatotoxicity caused by green tea. Free Radic Biol Med 43:474
Felix R, Martorell C, Martorell A et al (2013) Induced bronchospasm after handling of orange flavedo (zest). J Allergy Clin Immunol 131:1423–1425
Fisher CD, Augustine LM, Maher JM et al (2007) Induction of drug-metabolizing enzymes by garlic and allyl sulfide compounds via activation of constitutive androstane receptor and nuclear factor E2-related factor 2. Drug Metab Dispos 35:995–1000
Fisher K, Vuppalanchi R, Saxena R (2015) Drug-induced liver injury. Arch Pathol Lab Med 139:876–887
Fong TL, Klontz KC, Canas-Coto A et al (2010) Hepatotoxicity due to hydroxycut: a case series. Am J Gastroenterol 105:1561–1566
Foti RS, Pearson JT, Rock DA et al (2009) *In vitro* inhibition of multiple cytochrome P450 isoforms by xanthone derivatives from mangosteen extract. Drug Metab Dispos 37:1848–1855
Francini-Pesenti F, Puato M, Piccoli A et al (2008) Liquorice-induced hypokalaemia and water retention in the absence of hypertension. Phytother Res 22:563–565
Franco DL, Kale S, Lam-Himlin DM et al (2017) Black cohosh hepatotoxicity with autoimmune hepatitis presentation. Case Rep Gastroenterol 11:23–28
Furukawa S, Takaya A, Nakagawa T et al (2011) Fatal hypernatremia due to drinking a large quantity of shoyu (Japanese soy sauce). J Forensic Leg Med 18:91–92
Gagnier JJ, van Tulder MW, Berman B et al (2007) Herbal medicine for low back pain: a Cochrane review. Spine 32:82–92
Galati G, Lin A, Sultan AM et al (2006) Cellular and in vivo hepatoxicity caused by green tea phenolic acids and catechins. Free Radic Biol Med 40:570–580
Ganey PE, Luyendyk JP, Maddox JF et al (2004) Adverse hepatic drug reactions: inflammatory episodes as consequence and contributor. Chem Biol Interact 150:35–51
Ganey PE, Luyendyk JP, Newport SW et al (2007) Role of the coagulation system in acetaminophen-induced hepatotoxicity in mice. Hepatology 46:1177–1186
Gange CA, Madias C, Felix-Getzik EM et al (2006) Variant angina associated with bitter orange in a dietary supplement. Mayo Clin Proc 81:545–548
Gao LN, Zhang Y, Cui YL et al (2014) Evaluation of genipin on human cytochrome P450 isoenzymes and P-glycoprotein in vitro. Fitoterapia 98:130–136

Gardner-Stephen D, Heydel JM, Goyal A et al (2004) Human PXR variants and their differential effects on the regulation of human UDP-glucuronosyltransferase gene expression. Drug Metab Dispos 32:340–347

Gentry-Maharaj A, Karpinskyj C, Glazer C et al (2015) Use and perceived efficacy of complementary and alternative medicines after discontinuation of hormone therapy: a nested United Kingdom collaborative trial of ovarian cancer screening cohort study. Menopause 22:384–390

Ghannam M, Mansour S, Nabulsi A et al (2017) Anticonvulsant hypersensitivity syndrome after phenytoin administration in an adolescent patient: a case report and review of literature. Clin Mol Allergy 15:14

Glintborg B, Andersen SE, Dalhoff K (2005) Drug-drug interactions among recently hospitalised patients—frequent but mostly clinically insignificant. Eur J Clin Pharmacol 61:675–681

Gloro R, Hourmand-Ollivier I, Mosquet B et al (2005) Fulminant hepatitis during self-medication with hydroalcoholic extract of green tea. Eur J Gastroenterol Hepatol 17:1135–1137

Godtel-Armbrust U, Metzger A, Kroll U et al (2007) Variability in PXR-mediated induction of CYP3A4 by commercial preparations and dry extracts of St. John's wort. Naunyn Schmiedebergs Arch Pharmacol 375:377–382

Goksu E, Kilic T, Yilmaz D (2012) Hepatitis: a herbal remedy germander. Clin Toxicol 50:158

Gong H, Singh SV, Singh SP et al (2006) Orphan nuclear receptor pregnane X receptor sensitizes oxidative stress responses in transgenic mice and cancerous cells. Mol Endocrinol 20:279–290

Gonzalvez-Seijo JC, Ramos YM, Lastra I (1995) Manic episode and ginseng: report of a possible case. J Clin Psychopharmacol 15:447–448

Gordon P, Khojasteh SC (2015) A decades-long investigation of acute metabolism-based hepatotoxicity by herbal constituents: a case study of pennyroyal oil. Drug Metab Rev 47:12–20

Gordon WP, Forte AJ, McMurtry RJ et al (1982) Hepatotoxicity and pulmonary toxicity of pennyroyal oil and its constituent terpenes in the mouse. Toxicol Appl Pharmacol 65:413–424

Gordon WP, Huitric AC, Seth CL et al (1987) The metabolism of the abortifacient terpene, (R)-(+)-pulegone, to a proximate toxin, menthofuran. Drug Metab Dispos 15:589–594

Gorski JC, Huang S, Pinto A et al (2004) The effect of echinacea (*Echinacea purpurea* root) on cytochrome P450 activity in vivo. Clin Pharmacol Ther 75:89–100

Grahame R, Robinson BV (1981) Devils's claw (*Harpagophytum procumbens*): pharmacological and clinical studies. Ann Rheum Dis 40:632

Guesmi F, Prasad S, Tyagi AK et al (2017) Antinflammatory and anticancer effects of terpenes from oily fractions of teucruim alopecurus, blocker of IκBα kinase, through downregulation of nf-kappab activation, potentiation of apoptosis and suppression of NF-κB-regulated gene expression. Biomed Pharmacother 95:1876–1885

Gum SI, Jo SJ, Ahn SH et al (2007) The potent protective effect of wild ginseng (Panax ginseng C.A. Meyer) against benzo[α]pyrene-induced toxicity through metabolic regulation of CYP1A1 and GSTs. J Ethnopharmacol 112:568–576

Gurley BJ, Gardner SF, Hubbard MA et al (2002) Cytochrome P450 phenotypic ratios for predicting herb-drug interactions in humans. Clin Pharmacol Ther 72:276–287

Gurley BJ, Gardner SF, Hubbard MA et al (2004) *In vivo* assessment of botanical supplementation on human cytochrome P450 phenotypes: *Citrus aurantium, Echinacea purpurea*, milk thistle, and saw palmetto. Clin Pharmacol Ther 76:428–440

Gurley BJ, Gardner SF, Hubbard MA et al (2005) Clinical assessment of effects of botanical supplementation on cytochrome P450 phenotypes in the elderly: St John's wort, garlic oil, panax ginseng and ginkgo biloba. Drugs Aging 22:525–539

Gurley BJ, Swain A, Hubbard MA et al (2008) Clinical assessment of CYP2D6-mediated herb–drug interactions in humans: effects of milk thistle, black cohosh, goldenseal, kava kava, St. John's wort, and *Echinacea*. Mol Nutr Food Res 52:755–763

Gurley BJ, Steelman SC, Thomas SL (2015) Multi-ingredient, caffeine-containing dietary supplements: history, safety, and efficacy. Clin Ther 37(2):275–301

Haller CA, Benowitz NL, Jacob P 3rd (2005) Hemodynamic effects of ephedra-free weight-loss supplements in humans. Am J Med 118:998–1003

Haller CA, Kearney T, Bent S et al (2008) Dietary supplement adverse effects: report of a one-year poison center surveillance project. J Med Toxicol 4:84–92

Haqqi TM, Anthony DD, Gupta S et al (1999) Prevention of collagen-induced arthritis in mice by a polyphenolic fraction from green tea. Proc Natl Acad Sci USA 96:4524–4529

Hardeman E, Van Overbeke L, Ilegems S, Ferrante M (2008) Acute hepatitis induced by greater celandine (Chelidonium majus). Acta Gastroenterol Belg 71:281–282

Hasegawa A, Yoshino M, Nakamura H et al (2002) Identification of inhibitory component in cinnamon—O-methoxycinnamaldehyde inhibits CYP1A2 and CYP2E1. Drug Metab Pharmacokinet 17:229–236

Hayashi PH (2016) Drug-induced liver injury network causality assessment: criteria and experience in the United States. Int J Mol Sci 17:201

He YQ, Yang L, Liu HX et al (2010) Glucuronidation, a new metabolic pathway for pyrrolizidine alkaloids. Chem Res Toxicol 23:591–599

He X, Xia Q, Ma L et al (2016) 7-cysteine-pyrrole conjugate: a new potential DNA reactive metabolite of pyrrolizidine alkaloids. J Environ Sci Health C Environ Carcinog Ecotoxicol Rev 34:57–76

Herrera S, Bruguera M (2008) Hepatotoxicity induced by herbs and medicines used to induce weight loss. Gastroenterol Hepatol 31:447–453

Higashino S, Sasaki Y, Giddings JC et al (2014) Crocetin, a carotenoid from gardenia jasminoides ellis, protects against hypertension and cerebral thrombogenesis in stroke-prone spontaneously hypertensive rats. Phytother Res 28:1315–1319

Holmberg MT, Tornio A, Neuvonen M et al (2014) Grapefruit juice inhibits the metabolic activation of clopidogrel. Clin Pharmacol Ther 95:307–313

Holmes RO, Tavee J (2008) Vasospasm and stroke attributable to ephedra-free Xenadrine: case report. Mil Med 173:708–710

Honkakoski P, Zelko I, Sueyoshi T et al (1998) The nuclear orphan receptor CAR-retinoid X receptor heterodimer activates the phenobarbital-responsive enhancer module of the CYP2B gene. Mol Cell Biol 18:5652–5658

Hoshino M, Ikarashi N, Tsukui M et al (2014) Menthol reduces the anticoagulant effect of warfarin by inducing cytochrome P450 2C expression. Eur J Pharm Sci 56:92–101

Hoskyn J, Guin JD (2005) Contact allergy to cinnamal in a patient with oral lichen planus. Contact Dermatitis 52:160–161

Hu Z, Yang X, Ho PC et al (2005) Herb-drug interactions: a literature review. Drugs 65:1239–1282

Huang W, Zhang J, Chua SS et al (2003) Induction of bilirubin clearance by the constitutive androstane receptor (CAR). Proc Natl Acad Sci USA 100:4156–4161

Huang Y, Jiang B, Nuntanakorn P et al (2010) Fukinolic acid derivatives and triterpene glycosides from black cohosh inhibit CYP isozymes,

but are not cytotoxic to HEP-G2 cells in vitro. Curr Drug Saf 5:118–124
Hukkanen J, Ukkola O, Savolainen MJ (2009) Effects of low-dose liquorice alone or in combination with hydrochlorothiazide on the plasma potassium in healthy volunteers. Blood Press 18:192–195
Ingraffea A, Donohue K, Wilkel C et al (2007) Cutaneous vasculitis in two patients taking an herbal supplement containing black cohosh. J Am Acad Dermatol 56:S124–S126
Ioannides C, Lewis DF (2004) Cytochromes P450 in the bioactivation of chemicals. Curr Top Med Chem 4:1767–1788
Irefin S, Sprung J (2000) A possible cause of cardiovascular collapse during anesthesia: long-term use of St John's Wort. J Clin Anesth 12:498–499
Jacobsson I, Jönsson AK, Gerdén B et al (2009) Spontaneously reported adverse reactions in association with complementary and alternative medicine substances in Sweden. Pharmacoepidemiol Drug Saf 18:1039–1047
Janetzky K, Morreale A (1997) Probably interaction between warfarin and ginseng. Am J Health Syst Pharm 54:692–693
Jaradat NA, Ayesh OI, Anderson C (2016) Ethnopharmacological survey about medicinal plants utilized by herbalists and traditional practitioner healers for treatments of diarrhea in the west bank/palestine. J Ethnopharmacol 182:57–66
Jerome SV, Hughes TF, Friesner RA (2016) Successful application of the DBLOC method to the hydroxylation of camphor by cytochrome P450. Protein Sci 25:277–285
Jones BD, Runikis AM (1987) Interactions of Ginseng with phenelzine. J Clin Psychopharmacol 7:201–202
Kalogeromitros DC, Makris MP, Gregoriou SG et al (2005) Grape anaphylaxis: a study of 11 adult onset cases. Allergy Asthma Proc 26:53–58
Kanda T, Yokosuka O, Okada O et al (2003) Severe hepatotoxicity associated with Chinese diet product 'Onshidou-Genbi-Kounou'. J Gastroenterol Hepatol 18:354–355
Kang JJ, Wang HW, Liu TY et al (1997) Modulation of cytochrome P-450-dependent monooxygenases, glutathione and glutathione s-transferase in rat liver by geniposide from gardenia jasminoides. Food Chem Toxicol 35:957–965
Karalapillai DC, Bellomo R (2007) Convulsions associated with an overdose of St John's wort. Med J Aust 186:213–214
Kawamoto T, Sueyoshi T, Zelko I et al (1999) Phenobarbital-responsive nuclear translocation of the receptor CAR in induction of the CYP2b gene. Mol Cell Biol 19:6318–6322
Khawaja IS, Marotta RF, Lippmann S (1999) Herbal medicines as a factor in delirium. Psychiatr Serv 50:969–970
Khojasteh SC, Hartley DP, Ford KA et al (2012) Characterization of rat liver proteins adducted by reactive metabolites of menthofuran. Chem Res Toxicol 25:2301–2309
Khojasteh-Bakht SC, Chen W, Koenigs LL et al (1999) Metabolism of (R)-(+)-pulegone and (R)-(+)-menthofuran by human liver cytochrome P-450s: evidence for formation of a furan epoxide. Drug Metab Dispos 27:574–580
Kim E, Sy-Cordero A, Graf TN et al (2011) Isolation and identification of intestinal CYP3A inhibitors from cranberry (Vaccinium macrocarpon) using human intestinal microsomes. Planta Med 77:265–270
Kim HJ, Kim H, Ahn JH et al (2015) Liver injury induced by herbal extracts containing mistletoe and kudzu. J Altern Complement Med 21:180–185
Kimura Y, Ito H, Hatano T (2010) Effects of mace and nutmeg on human cytochrome P450 3A4 and 2C9 activity. Biol Pharm Bull 33:1977–1982
Kinoshita H, Okabayashi M, Kaneko M et al (2009) Shakuyaku-kanzo-to induces pseudoaldosteronism characterized by hypokalemia, rhabdomyolysis, metabolic alkalosis with respiratory compensation, and increased urinary cortisol levels. J Altern Complement Med 15:439–443
Kishida T, Onozato T, Kanazawa T et al (2012) Increase in covalent binding of 5-hydroxydiclofenac to hepatic tissues in rats co-treated with lipopolysaccharide and diclofenac: involvement in the onset of diclofenac-induced idiosyncratic hepatotoxicity. J Toxicol Sci 37:1143–1156
Kliewer SA, Moore JT, Wade L et al (1998) An orphan nuclear receptor activated by pregnanes defines a novel steroid signaling pathway. Cell 92:73–82
Kluth D, Banning A, Paur I et al (2007) Modulation of pregnane X receptor- and electrophile responsive element-mediated gene expression by dietary polyphenolic compounds. Free Radic Biol Med 42:315–325
Kocaman O, Hulagu S, Senturk O (2008) Echinacea-induced severe acute hepatitis with features of cholestatic autoimmune hepatitis. Eur J Intern Med 19:148–152
Kouzi SA, McMurtry RJ, Nelson SD (1994) Hepatotoxicity of germander (Teucrium chamaedrys L.) and one of its constituent neoclerodane diterpenes teucrin a in the mouse. Chem Res Toxicol 7:850–856
Kramlinger VM, von Weymarn LB, Murphy SE (2012) Inhibition and inactivation of cytochrome P450 2A6 and cytochrome P450 2A13 by menthofuran, beta-nicotyrine and menthol. Chem Biol Interact 197:87–92
Kumar BS, Chung BC, Kwon OS et al (2012a) Discovery of common urinary biomarkers for hepatotoxicity induced by carbon tetrachloride, acetaminophen and methotrexate by mass spectrometry-based metabolomics. J Appl Toxicol 32:505–520
Kumar S, Jin M, Ande A et al (2012b) Alcohol consumption effect on antiretroviral therapy and HIV-1 pathogenesis: role of cytochrome P450 isozymes. Expert Opin Drug Metab Toxicol 8:1363–1375
Kupiec T, Raj V (2005) Fatal seizure due to potential herb-drug interactions with *Ginkgo biloba*. J Anal Toxicol 29:755–758
Kwack SJ, Kim KB, Kim HS et al (2009) Risk assessment of soybean-based phytoestrogens. J Toxicol Environ Health A 72:1254–1261
Lamba V, Yasuda K, Lamba JK et al (2004) PXR (NR1I2): splice variants in human tissues, including brain, and identification of neurosteroids and nicotine as PXR activators. Toxicol Appl Pharmacol 199:251–265
Langhammer AJ, Nilsen OG (2014) *In vitro* inhibition of human CYP1A2, CYP2D6, and CYP3A4 by six herbs commonly used in pregnancy. Phytother Res 28:603–610
Lassila T, Mattila S, Turpeinen M et al (2016) Tandem mass spectrometric analysis of S- and N-linked glutathione conjugates of pulegone and menthofuran and identification of P450 enzymes mediating their formation. Rapid Commun Mass Spectrom 30:917–926
Lau AJ, Chang TK (2009 Sep) Inhibition of human CYP2B6-catalyzed bupropion hydroxylation by Ginkgo biloba extract: effect of terpene trilactones and flavonols. Drug Metab Dispos 37 (9):1931–1937
Lau G, Lo DS, Yao YJ et al (2004) A fatal case of hepatic failure possibly induced by nitrosofenfluramine: a case report. Med Sci Law 44:252–263
Lee Soon S, Crawford RI (2001) Recurrent erythema nodosum associated with Echinacea herbal therapy. J Am Acad Dermatol 44:298–299
Lee AJ, Chan WK, Harralson AF et al (1999) The effects of grapefruit juice on sertraline metabolism: an *in vitro* and *in vivo* study. Clin Ther 21:1890–1899
Lee JI, Cho BK, Ock SM, Park HJ (2010) Pigmented contact cheilitis: from green tea? Contact Dermatitis 62:60–61

Lee GH, Bhandary B, Lee EM et al (2011) The roles of ER stress and P450 2E1 in CCL(4)-induced steatosis. Int J Biochem Cell Biol 43:1469–1482

Lee WJ, Kim HW, Lee HY et al (2015) Systematic review on herb-induced liver injury in korea. Food Chem Toxicol 84:47–54

Lehmann JM, McKee DD, Watson MA et al (1998) The human orphan nuclear receptor PXR is activated by compounds that regulate CYP3A4 gene expression and cause drug interactions. J Clin Investig 102:1016–1023

Leitolf H, Dixit KCS, Higham CE et al (2010) Licorice—or more? Exp Clin Endocrinol Diabetes 118:250–253

Lekehal M, Pessayre D, Lereau JM et al (1996) Hepatotoxicity of the herbal medicine germander: metabolic activation of its furano diterpenoids by cytochrome P450 3A depletes cytoskeleton-associated protein thiols and forms plasma membrane blebs in rat hepatocytes. Hepatology 24:212–218

Letsyo E, Jerz G, Winterhalter P et al (2017) Incidence of pyrrolizidine alkaloids in herbal medicines from german retail markets: risk assessments and implications to consumers. Phytother Res 31 (12):1903–1909

Li L, Stanton JD, Tolson AH et al (2009) Bioactive terpenoids and flavonoids from ginkgo biloba extract induce the expression of hepatic drug-metabolizing enzymes through pregnane X receptor, constitutive androstane receptor, and aryl hydrocarbon receptor-mediated pathways. Pharm Res 26:872–882

Li M, Chen PZ, Yue QX et al (2013a) Pungent ginger components modulates human cytochrome P450 enzymes in vitro. Acta Pharmacol Sin 34(9):1237–1242

Li F, Lu J, Cheng J et al (2013b) Human PXR modulates hepatotoxicity associated with rifampicin and isoniazid co-therapy. Nat Med 19:418–420

Li L, Bonneton F, Chen XY, Laudet V (2015) Botanical compounds and their regulation of nuclear receptor action: the case of traditional Chinese medicine. Mol Cell Endocrinol 401:221–237

Lim TY, Considine A, Quaglia A et al (2013) Subacute liver failure secondary to black cohosh leading to liver transplantation. BMJ Case Rep 2013:bcr2013009325

Lin YP, Zhang MP, Wang KY et al (2016) Research achievements on ginsenosides biosynthesis from panax ginseng. Zhongguo Zhong Yao Za Zhi 41:4292–4302

Linshaw MA, Harrison HL, Gruskin AB et al (1980) Hypochloremic alkalosis in infants associated with soy protein formula. J Pediatr 96:635–640

Loch EG, Selle H, Boblitz N (2000) Treatment of premenstrual syndrome with a phytopharmaceutical formulation containing *Vitex agnus castus*. J Womens Health Gend Based Med 9:315–320

Lundgren H, Martinsson K, Cederbrant K et al (2017) HLA-DR7 and HLA-DQ2: transgenic mouse strains tested as a model system for ximelagatran hepatotoxicity. PLoS One 12:e0184744

Lunow M, Adam B, Seidel G (2011) Pseudo-Conn's syndrome with hypokalemic paralysis due to diuretics and licorice abuse. Fortschr Neurol Psychiatr 79:46–50

Lynch CR, Folkers ME, Hutson WR (2006) Fulminant hepatic failure associated with the use of black cohosh: a case report. Liver Transpl 12:989–992

Ma Y, Sachdeva K, Liu J et al (2004) Desmethoxyyangonin and dihydromethysticin are two major pharmacological kavalactones with marked activity on the induction of CYP3A23. Drug Metab Dispos 32:1317–1324

Ma X, Shah Y, Cheung C et al (2007) The pregnane X receptor gene-humanized mouse: a model for investigating drug-drug interactions mediated by cytochromes P450 3A. Drug Metab Dispos 35:194–200

MacVie OP, Harney BA (2005) Vitreous haemorrhage associated with *Gingko biloba* use in a patient with age related macular disease. Br J Ophthalmol 89:1378–1379

Madsen C, Wurtzen G, Carstensen J (1986) Short-term toxicity study in rats dosed with menthone. Toxicol Lett 32:147–152

Maeda J, Inoue K, Ichimura R et al (2015) Essential role of constitutive androstane receptor in ginkgo biloba extract induced liver hypertrophy and hepatocarcinogenesis. Food Chem Toxicol 83:201–209

Maglich JM, Parks DJ, Moore LB et al (2003) Identification of a novel human constitutive androstane receptor (CAR) agonist and its use in the identification of CAR target genes. J Biol Chem 278:17277–17283

Martinez J, Lewi JE (2008) An unusual case of gynecomastia associated with soy product consumption. Endocr Pract 14:415–418

Matthews HB, Lucier GW et al (1999) Medicinal herbs in the United States: research needs. Environ Health Perspect 107:773–778

Mazzanti G, Menniti-Ippolito F, Moro PA et al (2009) Hepatotoxicity from green tea: a review of the literature and two unpublished cases. Eur J Clin Pharmacol 65:331–341

Mazzanti G, Di Sotto A, Vitalone A (2015) Hepatotoxicity of green tea: an update. Arch Toxicol 89:1175–1191

McKenzie SC, Rahman A (2010) Bradycardia in a patient taking black cohosh. Med J Aust 193:479–481

Meier Y, Pauli-Magnus C, Zanger UM et al (2006) Interindividual variability of canalicular ATP-binding-cassette (ABC)-transporter expression in human liver. Hepatology 44:62–74

Meisel C, Johne A, Roots I (2003) Fatal intracerebral mass bleeding associated with *Ginkgo biloba* and ibuprofen. Atherosclerosis 167:367

Messer A, Raquet N, Lohr C et al (2012) Major furocoumarins in grapefruit juice ii: phototoxicity, photogenotoxicity, and inhibitory potency vs. Cytochrome P450 3A4 activity. Food Chem Toxicol 50:756–760

Meyer S, Vogt T, Obermann EC et al (2007) Cutaneous pseudolymphoma induced by *Cimicifuga racemosa*. Dermatology 214:94–96

Mihail RC (1992) Oral leukoplakia caused by cinnamon food allergy. J Otolaryngol 21:366–367

Miller LG, Freeman B (2002) Possible subdural hematoma associated with *Ginkgo biloba*. J Herb Pharmacother 2:57–63

Mills E, Foster BC, van Heeswijk R et al (2005) Impact of African herbal medicines on antiretroviral metabolism. AIDS 19:95–97

Minciullo PL, Saija A, Patafi M et al (2006) Muscle damage induced by black cohosh (*Cimicifuga racemosa*). Phytomedicine 13:115–118

Mingatto FE, Dorta DJ, dos Santos AB et al (2007) Dehydromonocrotaline inhibits mitochondrial complex I. A potential mechanism accounting for hepatotoxicity of monocrotaline. Toxicon 50 (5):724–730

Misaka S, Kawabe K, Onoue S et al (2013a) Effects of green tea catechins on cytochrome P450 2B6, 2C8, 2C19, 2D6 and 3A activities in human liver and intestinal microsomes. Drug Metab Pharmacokinet 28:244–249

Misaka S, Kawabe K, Onoue S et al (2013b) Green tea extract affects the cytochrome P450 3A activity and pharmacokinetics of simvastatin in rats. Drug Metab Pharmacokinet 28:514–518

Misaka S, Miyazaki N, Fukushima T et al (2013c) Effects of green tea extract and (−)-epigallocatechin-3-gallate on pharmacokinetics of nadolol in rats. Phytomedicine 20:1247–1250

Miwa H, Iijima M, Tanaka S et al (2001) Generalized convulsion after consuming a large amount of Gingko nuts. Epilepsia 42:280–281

Miyazawa M, Marumoto S, Takahashi T et al (2011) Metabolism of (+)- and (−)-menthols by CYP2A6 in human liver microsomes. J Oleo Sci 60:127–132

Mizutani T, Nomura H, Nakanishi K et al (1987) Effects of drug metabolism modifiers on pulegone-induced hepatotoxicity in mice. Res Commun Chem Pathol Pharmacol 58:75–83

Monera TG, Wolfe AR, Maponga CC et al (2008) *Moringa oleifera* leaf extracts inhibit 6beta-hydroxylation of testosterone by CYP3A4. J Infect Dev Ctries 2:379–383

Mooiman KD, Maas-Bakker RF, Moret EE et al (2013) Milk thistle's active components silybin and isosilybin: novel inhibitors of PXR-mediated CYP3A4 induction. Drug Metab Dispos 41:1494–1504
Moore GA (2001) Oranges and lemons: clues to the taxonomy of citrus from molecular markers. Trends Genet 17:536–540
Moore LB, Parks DJ, Jones SA et al (2000) Orphan nuclear receptors constitutive androstane receptor and pregnane X receptor share xenobiotic and steroid ligands. J Biol Chem 275:15122–15127
Mousa HA (2017) Prevention and treatment of influenza, influenza-like illness, and common cold by herbal, complementary, and natural therapies. J Evid Based Complementary Altern Med 22:166–174
Mukherjee T, Bhatt K, Sirsat R (2006) A young female with quadriparesis. J Assoc Physicians India 54:400–402
Mullins RJ, Heddle R (2002) Adverse reactions associated with echinacea: the Australian experience. Ann Allergy Asthma Immunol 88:42–51
Murray KF, Christie DL (1993) Dietary protein intolerance in infants with transient methemoglobinemia and diarrhea. J Pediatr 122:90–92
Nagata C, Nakamura K, Oba S et al (2009) Association of intakes of fat, dietary fibre, soya isoflavones and alcohol with uterine fibroids in Japanese women. Br J Nutr 101:1427–1431
Nakahashi H, Miyazawa M (2011) Biotransformation of (−)-camphor by salmonella typhimurium OY1002/2A6 expressing human CYP2A6 and NADPH-P450 reductase. J Oleo Sci 60:545–548
Nan HM, Park JW, Song YJ et al (2005) Kimchi and soybean pastes are risk factors of gastric cancer. World J Gastroenterol 11:3175–3181
Nasir JM, Durning SJ, Ferguson M et al (2004) Exercise-induced syncope associated with QT prolongation and ephedra-free Xenadrine. Mayo Clin Proc 79:1059–1062
Navarro VJ, Senior JR (2006) Drug-related hepatotoxicity. N Engl J Med 354:731–739
Nelson HD, Vesco KK, Haney E et al (2006) Nonhormonal therapies for menopausal hot flashes: systematic review and meta-analysis. JAMA 295:2057–2071
Nencini C, Galluzzi P, Pippi F et al (2014) Hepatotoxicity of Teucrium chamaedrys L. Decoction: role of difference in the harvesting area and preparation method. Indian J Pharmacol 46:181–184
Nierenberg AA, Burt T, Matthews J et al (1999) Mania associated with St. John's wort. Biol Psychiatry 46:1707–1708
Noel JC, Anaf V, Fayt I et al (2006) Ureteral mullerian carcinosarcoma (mixed mullerian tumor) associated with endometriosis occurring in a patient with a concentrated soy isoflavones supplementation. Arch Gynecol Obstet 274:389–392
O'Connell R, Parkin L, Manning P et al (2005) A cluster of thyrotoxicosis associated with consumption of a soy milk product. Aust N Z J Public Health 29:511–512
Oboh G (2006) Tropical green leafy vegetables prevent garlic-induced hepatotoxicity in the rat. J Med Food 9:545–551
Oladimeji PO, Lin W, Brewer CT et al (2017) Glucose-dependent regulation of pregnane x receptor is modulated by AMP-activated protein kinase. Sci Rep 7:46751
Pal D, Kwatra D, Minocha M et al (2011) Efflux transporters- and cytochrome P-450-mediated interactions between drugs of abuse and antiretrovirals. Life Sci 88:959–971
Palop-Larrea V, Gonzalvez-Perales JL, Catalan-Oliver C et al (2000) Metrorrhagia and ginseng. Ann Pharmacother 34:1347–1348
Pant P, Nadimpalli L, Singh M et al (2010) A case of severe hypokalemic paralysis and hypertension. Licorice-induced hypokalemic paralysis. Am J Kidney Dis 55:A35–A37
Patel S, Robinson R, Burk M (2002) Hypertensive crisis associated with St. John's Wort. Am J Med 112:507–508
Pedroso JL, Henriques Aquino CC, Escorcio Bezerra ML et al (2011) *Ginkgo biloba* and cerebral bleeding: a case report and critical review. Neurologist 17:89–90
Pennisi RS (2006) Acute generalized exanthematous pustulosis induced by the herbal remedy *Ginkgo biloba*. Med J Aust 184:583–584
Pilapil VR (1989) Toxic manifestations of cinnamon oil ingestion in a child. Clin Pediatr 28:276
Pillukat MH, Bester C, Hensel A et al (2014) Concentrated green tea extract induces severe acute hepatitis in a 63-year-old woman—a case report with pharmaceutical analysis. J Ethnopharmacol 155:165–170
Pitter MH, Schmidt K, Ernst E (2005) Adverse events of herbal food supplements for body weight reduction: systematic review. Obes Rev 6:93–111
Piyachaturawat P, Kingkaeohoi S, Toskulkao C (1995) Potentiation of carbon tetrachloride hepatotoxicity by piperine. Drug Chem Toxicol 18:333–344
Prasad S, Tyagi AK (2016) Historical spice as a future drug: therapeutic potential of piperlongumine. Curr Pharm Des 22:4151–4159
Prilepskaya VN, Ledina AV, Tagiyeva AV et al (2006) *Vitex agnus castus*: successful treatment of moderate to severe premenstrual syndrome. Maturitas 55:S55–S63
Propping D, Bohnert KJ, Peeters M et al (1991) *Vitex agnus castus*. Behandlung gynakologischer Krankheitsbilder. Therapeutikon 5:581–585
Radha Krishna Y, Mittal V, Grewal P et al (2011) Acute liver failure caused by 'fat burners' and dietary supplements: a case report and literature review. Can J Gastroenterol 25:157–160
Raederstorff DG, Schlachter MF, Elste V et al (2003) Effect of EGCG on lipid absorption and plasma lipid levels in rats. J Nutr Biochem 14:326–332
Raji MA, Kuo YF, Snih SA et al (2005) Ethnic differences in herb and vitamin/mineral use in the elderly. Ann Pharmacother 39:1019–1023
Rana SV, Pal R, Vaiphei K et al (2006) Garlic hepatotoxicity: safe dose of garlic. Trop Gastroenterol 27:26–30
Rashid NN, Grant J (2010) Hydroxicut hepatotoxicity. Med J Aust 192:173–174
Ratnatilaka A, Yakandawala D, Ratnayake J et al (2003) Poisoning with 'hondala' leaves due to misidentification as 'passion fruit' leaves. Ceylon Med J 48:23
Raucy JL (2003) Regulation of CYP3A4 expression in human hepatocytes by pharmaceuticals and natural products. Drug Metab Dispos 31:533–539
Reay JL, Scholey AB, Kennedy DO (2010) *Panax ginseng* (G115) improves aspects of working memory performance and subjective ratings of calmness in healthy young adults. Hum Psychopharmacol 25:462–471
Retamero C, Rivera T, Murphy K (2011) Ephedra-Free Diet pill-induced psychosis. Psychosomatics 52:579–582
Ricketts ML, Moore DD, Banz WJ et al (2005) Molecular mechanisms of action of the soy isoflavones includes activation of promiscuous nuclear receptors. A review. J Nutr Biochem 16:321–330
Ridker PM, Ohkuma S, McDermott WV et al (1985) Hepatic venocclusive disease associated with the consumption of pyrrolizidine-containing dietary supplements. Gastroenterology 88:1050–1054
Rietjens IMCM, Martena MJ, Boersma MG et al (2005) Molecular mechanisms of toxicity of important food-borne phytotoxins. Mol Nutr Food Res 49:131–158
Roby CA, Anderson GD, Kantor E et al (2000) Pharmacokinetics and drug disposition. St John's Wort: effect on CYP3A4 activity. Clin Pharmacol Ther 67:451–457
Romano C, Ferrara A (1998) Food allergy induced by grapes. Allergy 53:93
Rozenfeld P, Docena GH, Anon MC et al (2002) Detection and identification of a soy protein component that cross-reacts with caseins from cow's milk. Clin Exp Immunol 130:49–58

Russo S, Mastropasqua M, Mosetti MA et al (2000) Low doses of liquorice can induce hypertension encephalopathy. Am J Nephrol 20:145–148

Ryan CK, Reamy B, Rochester JA (2002) Ischemic colitis associated with herbal product use in a young woman. J Am Board Fam Pract 15:309–312

Sanfelix Genoves J, Palop Larrea V, Rubio Gomis E et al (2001) Consumption of medicinal herbs and medicines. Aten Primaria 28:311–314

Sarges P, Steinberg JM, Lewis JH (2016) Drug-induced liver injury: highlights from a review of the 2015 literature. Drug Saf 39:801–821

Sartippour MR, Shao ZM, Heber D et al (2002) Green tea inhibits vascular endothelial growth factor (VEGF) induction in human breast cancer cells. J Nutr 132:2307–2311

Sasaki T, Sato Y, Kumagai T et al (2017) Effect of health foods on cytochrome P450-mediated drug metabolism. J Pharm Health Care Sci 3:14

Schell J, Betts NM, Foster M et al (2017) Cranberries improve postprandial glucose excursions in type 2 diabetes. Food Funct 8:3083–3090

Scholey A, Ossoukhova A, Owen L et al (2010) Effects of american ginseng (Panax quinquefolius) on neurocognitive function: an acute, randomised, double-blind, placebo-controlled, crossover study. Psychopharmacology 212:345–356

Schwarz UI, Hanso H, Oertel R et al (2007) Induction of intestinal P-glycoprotein by St John's wort reduces the oral bioavailability of talinolol. Clin Pharmacol Ther 81:669–678

Schyschka L, Sanchez JJ, Wang Z et al (2013) Hepatic 3D cultures but not 2D cultures preserve specific transporter activity for acetaminophen-induced hepatotoxicity. Arch Toxicol 87:1581–1593

Seddik M, Lucidarme D, Creusy C et al (2001) Is exolise hepatotoxic? Gastroenterol Clin Biol 25:834–835

Senna G, Mistrello G, Roncarolo D et al (2001) Exercise-induced anaphylaxis to grape. Allergy 56:1235–1236

Sezer RG, Bozaykut A (2012) Pediatric hepatotoxicity associated with polygermander (*Teucrium polium*). Clin Toxicol 50:153

Shahbaz O, Mahajan S, Lewis JH (2017) Highlights of drug—and herb-induced liver injury in the literature from 2016: how best to translate new information into clinical practice? Expert Opin Drug Metab Toxicol 13:935–951

Sharma T, Wong L, Tsai N et al (2010) Hydroxycut® (herbal weight loss supplement) induced hepatotoxicity: a case report and review of literature. Hawaii Med J 69:188–190

Shaw D, Leon C, Kolev S, Murray V (1997) Traditional remedies and food supplements. A 5-year toxicological study (1991–1995). Drug Saf 17:342–356

Shenai JP, Jhaveri BM, Reynolds JW et al (1981) Nutritional balance studies in very low-birth-weight infants: role of soy formula. Pediatrics 67:631–637

Shim M, Saab S (2009) Severe hepatotoxicity due to Hydroxycut: a case report. Dig Dis Sci 54:406–408

Siegel MA (2006) Perioral dermatitis. J Am Dent Assoc 137:1121–1122

Siepmann T, Roofeh J, Kiefer FW et al (2011) Hypogonadism and erectile dysfunction associated with soy product consumption. Nutrition 27:859–862

Silano V, Coppens P, Larrañaga-Guetaria A et al (2011) Regulations applicable to plant food supplements and relative products in the European Union. Food Funct 2:710–719

Siqueira AS, Santos CCO, Cristino MR et al (2009) Intraoral contact mucositis induced by cinnamon-falvored chewing gum—a case report. Quintessence Int 40:719–721

Smejkal K, Rjaskova V (2016) Use of plant extracts as an efficient alternative therapy of respiratory tract infections. Ceska Slov Farm 65:139–160

Smith LW, Culvenor CC (1981) Plant sources of hepatotoxic pyrrolizidine alkaloids. J Nat Prod 44:129–152

Stephensen TA, Sarlay RJ (2009) Ventricular fibrilation associated with use of synephrine containing dietary supplement. Mil Med 174:1313–1319

Stickel F, Poschl G, Seitz HK et al (2003) Acute hepatitis induced by greater celandine (Chelidonium majus). Scand J Gastroenterol 38:565–568

Stjernberg L, Berglund J (2000) Garlic as an insect repellent. JAMA 284:831

Stjernberg L, Berglund J, Halling A (2006) Age and gender effect on the use of herbal medicine products and food supplements among the elderly. Scand J Prim Health Care 24:50–55

Stohs SJ (2017) Safety, efficacy, and mechanistic studies regarding citrus aurantium (bitter orange) extract and p-synephrine. Phytother Res 31(10):1463–1474

Stojanovic NM, Samardzic L, Randjelovic PJ et al (2017) Prevalence of self-medication practice with herbal products among non-psychotic psychiatric patients from southeastern serbia: a cross-sectional study. Saudi Pharm J 25:884–890

Su Y, Wu L, Wang Q et al (2014) New 9,19-cycloartenol glycosides isolated from the roots of cimicifuga simplex and their anti-inflammatory effects. Bioorg Med Chem Lett 24:5688–5691

Sueoka N, Suganuma M, Sueoka E et al (2001) A new function of green tea: prevention of lifestyle-related diseases. Ann N Y Acad Sci 928:274–280

Sueyoshi T, Green WD, Vinal K et al (2011) Garlic extract diallyl sulfide (DAS) activates nuclear receptor CAR to induce the sult1e1 gene in mouse liver. PLoS One 6:e21229

Sugatani J, Kojima H, Ueda A et al (2001) The phenobarbital response enhancer module in the human bilirubin UDP-glucuronosyltransferase UGT1A1 gene and regulation by the nuclear receptor CAR. Hepatology 33:1232–1238

Suk KT, Kim DJ, Kim CH et al (2012) A prospective nationwide study of drug-induced liver injury in Korea. Am J Gastroenterol 107:1380–1387

Sultan S, Spector J, Michell RM (2006) Ischemic colitis associated with use of a bitter orange-containing dietary weight-loss supplement. Mayo Clin Proc 81:1630–1631

Sun CL, Yuan JM, Arakawa K et al (2002) Dietary soy and increased risk of bladder cancer: the Singapore Chinese Health Study. Cancer Epidemiol Biomark Prev 11:1674–1677

Sunaga K, Ohkawa K, Nakamura K et al (2012) Mechanism-based inhibition of recombinant human cytochrome P450 3A4 by tomato juice extract. Biol Pharm Bull 35:329–334

Sundaram MB, Swaminathan R (1981) Total body potassium depletion and severe myopathy due to chronic liquorice ingestion. Postgrad Med J 57:48–49

Suzuki N, Irie M, Iwata K et al (2006) Altered expression of alkaline phosphatase (ALP) in the liver of primary biliary cirrhosis (PBC) patients. Hepatol Res 35:37–44

Sztajnkrycer MD, Otten EJ, Bond GR et al (2003) Mitigation of penny-royal oil hepatotoxicity in the mouse. Acad Emerg Med 10:1024–1028

Tanaka S, Uchida S, Miyakawa S et al (2013) Comparison of inhibitory duration of grapefruit juice on organic anion-transporting polypeptide and cytochrome P450 3A4. Biol Pharm Bull 36:1936–1941

Tannergren C, Engman H, Knutson L et al (2004) St John's wort decreases the bioavailability of R- and S-verapamil through induction of the first-pass metabolism. Clin Pharmacol Ther 75:298–309

Taylor JR, Wilt VM (1999) Probable antagonism of warfarin by green tea. Ann Pharmacother 33:426–428

Taylor JA, Weber W, Standish L et al (2003) Efficacy and safety of Echinacea in treating upper respiratory tract infections in children. JAMA 290:2824–2830

Teschke R (2010a) Black cohosh and suspected hepatotoxicity: inconsistencies, confounding variables, and prospective use of a

diagnostic causality algorithm. A critical review. Menopause 17:426–440
Teschke R (2010b) Kava hepatotoxicity—a clinical review. Ann Hepatol 9:251–265
Teschke R, Andrade RJ (2016) Drug, herb, and dietary supplement Hepatotoxicity. Int J Mol Sci 17(9):E1488
Teschke R, Glass X, Schulze J (2011) Herbal hepatotoxicity by greater celandine (*Chelidonium majus*): causality assessment of 22 spontaneous reports. Regul Toxicol Pharmacol 61:282–291
Teschke R, Frenzel C, Glass X et al (2012a) Greater celandine hepatotoxicity: a clinical review. Ann Hepatol 11:838–848
Teschke R, Wolff A, Frenzel C et al (2012b) Herbal hepatotoxicity: a tabular compilation of reported cases. Liver Int 32:1543–1556
Teschke R, Zhang L, Melzer L et al (2014) Green tea extract and the risk of drug-induced liver injury. Expert Opin Drug Metab Toxicol 10:1663–1676
Teschke R, Larrey D, Melchart D et al (2016) Traditional Chinese Medicine (TCM) and herbal hepatotoxicity: rucam and the role of novel diagnostic biomarkers such as microRNAs. Medicines 3:18
Thiolet C, Mennecier D, Bredin C et al (2002) Acute cytolysis induced by Chinese tea. Gastroenterol Clin Biol 26:939–940
Thomas JE, Munir JA, McLntyre PZ et al (2009) STEMI in a 24-year-old man after use of a synephrine-containing dietary supplement. Tex Heart Inst J 36:586–590
Thorup I, Wurtzen G, Carstensen J et al (1983a) Short term toxicity study in rats dosed with pulegone and menthol. Toxicol Lett 19:207–210
Thorup I, Wurtzen G, Carstensen J et al (1983b) Short term toxicity study in rats dosed with peppermint oil. Toxicol Lett 19:211–215
Tremblay S, Avon SL (2008) Contact allergy to cinnamon: case report. J Can Dent Assoc 74:445–461
Tsintis P, La Mache E (2004) Cioms and ich initiatives in pharmacovigilance and risk management: overview and implications. Drug Saf 27:509–517
Tucker GT, Houston JB, Huang SM (2001) Optimizing drug development: strategies to assess drug metabolism/transporter interaction potential-toward a consensus. Clin Pharmacol Ther 70:103–114
Tujios S, Fontana RJ (2011) Mechanisms of drug-induced liver injury: from bedside to bench. Nat Rev Gastroenterol Hepatol 8:202–211
Uc A, Bishop WP, Sanders KD (2000) Camphor hepatotoxicity. South Med J 93(6):596–598
Usia T, Watabe T, Kadota S et al (2005) Cytochrome P450 2D6 (CYP2D6) inhibitory constituents of catharanthus roseus. Biol Pharm Bull 28:1021–1024
Valentao P, Carvalho M, Carvalho F et al (2004) *Hypericum androsaemum* infusion increases tert-butyl hydroperoxide-induced mice hepatotoxicity in vivo. J Ethnopharmacol 94:345–351
Valla D, Benhamou JP (1988) Drug-induced vascular and sinusoidal lesions of the liver. Baillieres Clin Gastroenterol 2:481–500
Valli G, Giardina E-GV (2002) Benefits, adverse effects and drug interactions of herbal therapies with cardiovascular effects. J Am Coll Cardiol 39:1083–1095
Van den Bout-van den Beukel CJ, Hamza OJ, Moshi MJ et al (2008) Evaluation of cytotoxic, genotoxic and CYP450 enzymatic competition effects of tanzanian plant extracts traditionally used for treatment of fungal infections. Basic Clin Pharmacol Toxicol 102:515–526
Vannacci A, Lapi F, Gallo E et al (2009) A case of hepatitis associated with long-term use of *Cimicifuga racemosa*. Altern Ther Health Med 15:62–63
Vasquez I, Aguera-Ortiz LF (2002) Herbal products and serious side effects: a case of ginseng-induced manic episode. Acta Psychiatr Scand 105:76–77
Verma S, Thuluvath PJ (2007) Complementary and alternative medicine in hepatology: review of the evidence of efficacy. Clin Gastroenterol Hepatol 5:408–416
Vial T, Bernard G, Lewden B et al (2003) Acute hepatitis due to Exolise, a *Camellia sinensis*-derived drug. Gastroenterol Clin Biol 27:1166–1167
Vitalone A, Menniti-Ippolito F, Moro PA et al (2011) Suspected adverse reactions associated with herbal products used for weight loss: a case series reported to the Italian National Institute of Health. Eur J Clin Pharmacol 67:215–224
Walton EW (2014) Topical phytochemicals: applications for wound healing. Adv Skin Wound Care 27:328–332
Wang LS, Zhou G, Zhu B et al (2004a) St John's wort induces both cytochrome P450 3A4-catalyzed sulfoxidation and 2C19-dependent hydroxylation of omeprazole. Clin Pharmacol Ther 75:191–197
Wang LS, Zhu B, Abd El-Aty AM et al (2004b) The influence of St John's wort on CYP2C19 activity with respect to genotype. J Clin Pharmacol 44:577–581
Wang YM, Ong SS, Chai SC et al (2012) Role of CAR and PXR in xenobiotic sensing and metabolism. Expert Opin Drug Metab Toxicol 8:803–817
Wang YG, Liu HS, Zhang XX et al (2013a) Screening of pregnane X receptor activation from ginsenosides. Yao Xue Xue Bao 48:144–148
Wang YM, Lin W, Chai SC et al (2013b) Piperine activates human pregnane x receptor to induce the expression of cytochrome P450 3A4 and multidrug resistance protein 1. Toxicol Appl Pharmacol 272:96–107
Wang YM, Chai SC, Brewer CT et al (2014) Pregnane X receptor and drug-induced liver injury. Expert Opin Drug Metab Toxicol 10:1521–1532
Wang D, Wang Y, Wan X et al (2015) Green tea polyphenol (−)-epigallocatechin-3-gallate triggered hepatotoxicity in mice: responses of major antioxidant enzymes and the NRF2 rescue pathway. Toxicol Appl Pharmacol 283:65–74
Watkins RE, Maglich JM, Moore LB et al (2003) 2.1 a crystal structure of human PXR in complex with the St. John's wort compound hyperforin. Biochemistry 42:1430–1438
Wei J, Zhang F, Zhang Y et al (2014) Proteomic investigation of signatures for geniposide-induced hepatotoxicity. J Proteome Res 13:5724–5733
Werba JP, Giroli M, Cavalca V et al (2008) The effect of green tea on simvastatin tolerability. Ann Intern Med 149:286–287
Westra WH, McMurray JS, Califano J et al (1998) Squamous cell carcinoma of the tongue associated with cinnamon gum use: a case report. Head Neck 20:430–433
Whiting PW, Clouston A, Kerlin P (2002) Black cohosh and other herbal remedies associated with acute hepatitis. Med J Aust 177:440–443
WHO (2004) Guidelines on safety monitoring of herbal medicines in pharmacovigilance systems. World Health Organization, Geneva
Wiwanitkit V (2012) Excessive consumption of soybean milk and unexplained hepatitis. J Postgrad Med 58:226–227
Wiwanitkit V, Taungjaruwinai W (2004) A case report of suspected ginseng allergy. Med Gen Med 6:9
Woolbright BL, Jaeschke H (2012) Novel insight into mechanisms of cholestatic liver injury. World J Gastroenterol 18:4985–4993
Wu R, Tao W, Zhang H et al (2016) Instant and persistent antidepressant response of gardenia yellow pigment is associated with acute protein synthesis and delayed upregulation of BDNF expression in the hippocampus. ACS Chem Neurosci 7:1068–1076
Xia S-H, Fang DC (2007) Pharmacological action and mechanisms of ginkgolide B. Chin Med J 120:922–928
Xie Y, McGill MR, Dorko K et al (2014) Mechanisms of acetaminophen-induced cell death in primary human hepatocytes. Toxicol Appl Pharmacol 279:266–274
Xu C, Huang M, Bi H (2016) PXR- and CAR-mediated herbal effect on human diseases. Biochim Biophys Acta 1859(9):1121–1129

Yagmur E, Piatkowski A, Gröger A et al (2005) Bleeding complication under *Gingko biloba* medication. Am J Hematol 79:343–344

Yamaguchi Y, Akimoto I, Motegi K et al (2013) Synthetic models related to methoxalen and menthofuran-cytochrome P450 (CYP) 2A6 interactions. Benzofuran and coumarin derivatives as potent and selective inhibitors of CYP2A6. Chem Pharm Bull 61:997–1001

Yamano T, Tsujimoto Y, Noda T et al (1988) Hepatotoxicity of gardenia yellow color in rats. Toxicol Lett 44:177–182

Yamazaki F, Sone R (2017) Desensitization of menthol-activated cold receptors in lower extremities during local cooling in young women with a cold constitution. J Physiol Sci 67:331–337

Yang HJ, Fu MH, Wu ZL et al (2006) Experimental studies on hepatotoxicity of rats induced by Fructus gardeniae. Zhongguo Zhong Yao Za Zhi 31:1091–1093

Yang M, Ruan J, Fu PP et al (2016) Cytotoxicity of pyrrolizidine alkaloid in human hepatic parenchymal and sinusoidal endothelial cells: firm evidence for the reactive metabolites mediated pyrrolizidine alkaloid-induced hepatotoxicity. Chem Biol Interact 243:119–126

Yao HT, Hsu YR, Lii CK et al (2014a) Effect of commercially available green and black tea beverages on drug-metabolizing enzymes and oxidative stress in wistar rats. Food Chem Toxicol 70:120–127

Yao J, Li CG, Gong LK et al (2014b) Hepatic cytochrome P450s play a major role in monocrotaline-induced renal toxicity in mice. Acta Pharmacol Sin 35:292–300

Yasuda K, Sakaki T (2012 Jan) How is sesamin metabolised in the human liver to show its biological effects? Expert Opin Drug Metab Toxicol 8(1):93–102

Yeung EY, Sueyoshi T, Negishi M et al (2008) Identification of ginkgo biloba as a novel activator of pregnane x receptor. Drug Metab Dispos 36:2270–2276

Yin OQ, Tomlinson B, Waye MM et al (2004) Pharmacogenetics and herb-drug interactions: experience with ginkgo biloba and omeprazole. Pharmacogenetics 14:841–850

Yokotani K, Chiba T, Sato Y et al (2013) Effect of three herbal extracts on cytochrome P450 and possibility of interaction with drugs. Shokuhin Eiseigaku Zasshi 54:56–64

Yotsawimonwat S, Rattanadechsakul J, Rattanadechsakul P et al (2010) Skin improvement and stability of echinacea purpurea dermatological formulations. Int J Cosmet Sci 32:340–346

Yu C, Chai X, Yu L et al (2011) Identification of novel pregnane X receptor activators from traditional chinese medicines. J Ethnopharmacol 136:137–143

Yuste M, Sánchez-Estella J, Santos JC et al (2005) Síndrome de Stevens-Johnson/necrolisis epidérmica tóxica tratado com inmunoglobulinas intravenosas. Actas Dermosifiliogr 96:589–592

Zhang P, Noordine ML, Cherbuy C et al (2006) Different activation patterns of rat xenobiotic metabolism genes by two constituents of garlic. Carcinogenesis 27:2090–2095

Zhang HF, Huang LB, Zhong YB et al (2016) An overview of systematic reviews of ginkgo biloba extracts for mild cognitive impairment and dementia. Front Aging Neurosci 8:276

Zhou XW, Ma Z, Geng T et al (2014) Evaluation of *in vitro* inhibition and induction of cytochrome P450 activities by hydrolyzed ginkgolides. J Ethnopharmacol 158(Pt A):132–139

Ziment I (1991) History of the treatment of chronic bronchitis. Respiration 58:37–42

Zimmermann R, Witte A, Voll RE et al (2010) Coagulation activation and fluid retention associated with the use of black cohosh: a case study. Climacteric 13:187–191

Zollner G, Wagner M, Trauner M (2010) Nuclear receptors as drug targets in cholestasis and drug-induced hepatotoxicity. Pharmacol Ther 126:228–243

Zullino D, Borgeat F (2003) Hypertension induced by St. John's Wort—a case report. Pharmacopsychiatry 36:32

Part VI

Newer Trends in Nutraceutical Research and Product Development

Proteomics in the Evaluation of Nutraceuticals and Functional Foods

Christina Wilson-Frank

Abstract

Nutraceuticals and functional foods are used in veterinary medicine as preventative or supportive treatments for disease and to improve animal health. Although the purported health benefits of nutraceuticals and functional foods in animals is well-documented, understanding the molecular mechanisms involved in the nutritive health benefits and disease outcome on a cellular level is warranted. Proteomics-based techniques are evolving as a promising tool that may provide the scientific knowledge to link diet and disease in nutritional research. The use of proteomics in nutrition research has transformed into the disciplines of nutriproteomics and foodomics. Although more studies are warranted to garner a deeper understanding of the mechanisms responsible for the health benefits of these nutritive substances in animals, nutriproteomics and foodomics applications have offered new insights into the molecular mechanisms of action and changes in protein expression of a variety of nutritive substances.

Keywords

Foodomics · Functional Food · Nutraceutical · Nutriproteomics · Probiotic · Proteomic

1 Introduction

Proteomics studies encompass the qualitative and quantitative, global analysis and characterization of the entire protein complement in a cell, organism, or tissue that change in response to an external stimulus or due to a specific disease state. The measurable changes in the proteome have been used to identify potential biomarkers for specific disease states and to elucidate the mechanism of action of xenobiotics or other substances. In proteomics-based research, the first step in characterizing changes in the proteome requires separation of intact proteins or peptides. Adequately separating this complex, heterogeneous components have proven to be an analytical challenge, largely due to the large dynamic range of proteins and the microheterogeneity of protein expression in the samples. For example, a typical serum proteome can contain approximately 20,000 proteins, creating an analytical challenge when attempting to adequately isolate or separate them for identification (Anderson and Anderson 2002; Issaq et al. 2005). Emerging protein separation technologies, which include two-dimensional gel electrophoresis (2-DGE), two-dimensional in-gel electrophoresis (2-DIGE), and multidimensional high-performance liquid chromatography (HPLC), offer multidimensional levels of separation which increase resolution and isolation of proteins from complex mixtures (Görg et al. 2000; Hamdan and Righetti 2002; Issaq et al. 2005; Zhang et al. 2010). Therefore, the ability to better isolate proteins and peptides that change in complex biological samples with disease or in response to stimuli has improved and continue to evolve. In addition to the challenge of separating mixtures of proteins, the use of mass spectrometry-based instrumentation for identifying and quantifying changes in proteins has imposed significant advancements in that technology. Recent advancements in hybrid mass spectrometers have provided significant, high-throughput automation with exceptional sensitivity and improved resolving power of proteins. Specifically, advancements in matrix-assisted laser desorption/time-of-flight tandem mass spectrometry (MALDI/MS/MS) and electrospray ionization/tandem mass spectrometry (ESI/MS/MS) technologies have improved quantifying and isolating proteins and peptides significantly and are the two most common mass spectrometers used in proteomics investigations (Li et al. 2017).

C. Wilson-Frank (✉)
Department of Comparative Pathobiology, Animal Disease Diagnostic Laboratory, College of Veterinary Medicine, Purdue University, West Lafayette, IN, USA
e-mail: wilsonc@purdue.edu

R. C. Gupta et al. (eds.), *Nutraceuticals in Veterinary Medicine*, https://doi.org/10.1007/978-3-030-04624-8_52

Proteomics has been utilized in a wide variety of studies in biomedical research. However, many new disciplines are exploiting the utility of proteomic investigations. For example, the interaction of functional foods and nutraceuticals with medicine and pharmacology has presented a new challenge for the nutraceutical industry. Therefore, new disciplines such as "nutriproteomics" and "foodomics" aim to exploit advances in proteomics-based technologies in order to understand how nutraceuticals, functional foods, or other dietary nutrients influence protein expression. Scientific studies assessing the efficacy and safety of these nutraceuticals and functional foods, in addition to identifying any potential drug interactions, are lacking. As the use of nutraceuticals and functional foods in humans and animals continues to evolve, it will become important to have a complete understanding of how these nutritive components affect health and disease outcome. Therefore, nutriproteomics and foodomics aim to accomplish that need through characterizing nutritive bioactive proteins, identifying biomarkers important for disease regulation, assessing the safety of current and new nutraceuticals, and identifying mechanisms by which nutraceuticals and functional foods impact health outcomes. In veterinary medicine, there are several nutraceuticals and functional foods being used in animals in order to prevent disease and promote health or quality of life. This chapter describes proteomics investigations that have been conducted in order to elucidate the mechanism of action of nutraceuticals and functional foods used in veterinary medicine.

2 Nutriproteomics

2.1 *S*-Adenosylmethionine (SAM-e)

SAM-e is a naturally occurring, methyl donor involved in methyltransferase reactions, and supplementation has been shown to restore hepatic glutathione levels and reduce liver injury in animals and humans (Guo et al. 2015). Hepatic glutathione concentrations decrease in cats and dogs with liver disease (Center et al. 2002). Therefore, SAM-e supplementation has been recommended as a supportive treatment in canines and felines with liver disease or hepatotoxicity. SAM-e has been used as supportive treatment in animals with hepatotoxicity originating from acetaminophen toxicosis (Wallace et al. 2002; Webb et al. 2003), blue-green algae toxicosis (Bautista et al. 2015; Sebbag et al. 2013), and xylitol intoxication (Schmid and Hovda 2016). Although SAM-e has been used as a hepatoprotectant, the exact mechanism by which it protects the liver had not been explicitly defined. However, recent proteomic-based research of the effects of SAM-e on the liver after ethanol exposure have shed some light on the potential molecular targets involved in hepatoprotective effects of SAM-e. In two studies conducted in Sprague-Dawley rats, changes in the liver mitochondrial proteome were evaluated after exposing the rats to ethanol, with and without SAM-e supplementation (Andringa et al. 2010; Bailey et al. 2006). In one study, rats were fed control or ethanol diets, supplemented or not supplemented with SAM-e, for 5 weeks (Bailey et al. 2006). Using SDS-PAGE and immunoblot analysis, the investigators were able to show that SAM-e prevented alcohol-mediated decreases in hepatic mitochondrial respiration and cytochrome c oxidase. Metabolic stress has been associated with mitochondrial protein synthesis inhibition causing upregulation of the protein prohibitin (Bailey et al. 2006). Ethanol exposure caused a significant increase in this protein; whereas the control group and the group fed ethanol and SAM-e had no increase in this protein. This suggested that SAM-e protected the hepatic mitochondria from protein synthesis inhibition by ethanol. In another study, rats were fed control or ethanol diets, also supplemented or not supplemented with SAM-e, for 31 days. Using 2-DGE and MALDI/MS, 30 proteins had significantly changed with ethanol exposure or SAM-e or exposure to both. The types of proteins include chaperones, beta-oxidation proteins, sulfur metabolism proteins, and dehydrogenase enzymes. Ethanol was shown to decrease key mitochondrial proteins GRP78, Hspd1, and PDI/T3BP. SAM-e prevented ethanol-dependent decreases in these chaperone proteins thus preserving mitochondrial function. Therefore, these proteomic studies suggest that the heptoprotective effect of SAM-e against ethanol toxicity may be mediated through protecting proteins that are key to mitochondrial biosynthetic pathways and energy. Proteomics studies using human hepatoma cells in order to assess SAM-e's hepatoprotective effects in hepatocellular carcinoma have revealed a potential molecular target for SAM-e in the liver (Schroder et al. 2012). Using 2D-DIGE and HPLC/MS/MS, 128 proteins were identified that were involved in apoptosis, cell proliferation, and cell survival. However, human DEAD-box protein 3 (DDX3X) was downregulated when the enzyme that synthesizes SAM-e was downregulated. The investigators concluded that DDX3X may be a primary target of SAM-e and a chief intermediate of its antitumor effect. Therefore, reduced SAM-e in the liver may cause upregulation of DDX3X and contribute to the pathogenic process (Schroder et al. 2012). In these studies, proteomic evaluations suggest that SAM-e supplementation may prevent oxidative damage to hepatic mitochondria and downregulate proteins key to pathogenesis of hepatocellular carcinoma.

2.2 Glucosamine and Chondroitin

There are several nutraceuticals being used to treat osteoarthritis in animals which may include, but are not limited to, glucosamine, omega-3 fatty acids, chondroitin sulfate, and

green-lipped mussel powder (Gupta 2016). These nutraceuticals are used as an alternative treatment for osteoarthritis due to their purported anti-inflammatory benefits. In animals, the most common nutraceuticals being used are glucosamine and chondroitin. Glucosamine is an aminosaccharide that is a natural component in cartilage and is commonly sold as glucosamine sulfate or glucosamine hydrochloride as a supplement (Blum et al. 2006). Chondroitin sulfate is a glycosaminoglycan and is a functional component in proteoglycans in cartilaginous tissue (Pomin 2015). It is a common nutraceutical that is used in humans and animals with arthritis and osteoarthritis. While giving these nutraceuticals can be beneficial to the animal, the efficacy of these supplements and how they affect biological processes have not entirely been defined. One group of researchers conducted a review of 22 studies and the efficacy of nutraceuticals to alleviate clinical signs of osteoarthritis in horses, dogs, and cats (Vandeweerd et al. 2012). The nutraceuticals considered in this review included the use of glucosamine hydrochloride, chondroitin sulfate, omega-3 fatty acids, green-lipped mussel powder, and a variety of other types of treatments. Upon completion of the literature review, this group concluded that the evidence for efficacy was low for all nutraceuticals except for omega-3 fatty acids. However, since this review, proteomics research has been able to elucidate the potential targets of some of these nutraceuticals, which ultimately highlighted their anti-inflammatory potential. For example, in one study using 2-DIGE, protein microarrays and HPLC/MS, changes in the serum proteome of nine men and nine women taking glucosamine and chondroitin for osteoarthritis were assessed (Navarro et al. 2015). In this study, individuals took three capsules of 500 mg glucosamine hydrochloride and 400 mg chondroitin sulfate; whereas the control group was administered capsules containing crystalline cellulose for 28 days. Proteomic analysis revealed that C-reactive protein concentrations were significantly lowered in patients taking glucosamine and chondroitin when compared to the placebo group. The protein microarray results also revealed that the effects of glucosamine and chondroitin reduced proteins involved in the cytokine activity pathway. Based on their results, they concluded that glucosamine and chondroitin supplementation can lower systemic inflammation. Interestingly, some of the proteins and pathways impacted by these nutraceuticals were also suggested to be associated with reducing the risk of colorectal and lung cancer.

2.3 Omega-3 Fatty Acids

Omega-3 fatty acids, such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), have been used as a nutraceutical supplement in the treatment of a variety of canine diseases, including cancer (Bauer 2011). DHA supplementation in dogs with stage III lymphoma has been associated with producing longer survival times and periods of remission when used in combination with doxorubicin (Bauer 2011; Ogilvie et al. 2000). DHA and EPA supplementation in dogs given for nasal, malignant carcinomas has been shown to decrease harmful inflammatory eicosanoids as well as suppress some of the detrimental effects of radiation therapy (Hansen et al. 2011). In order to better understand the mechanism by which omega-3 fatty acids exert their anticancer properties, a proteomic evaluation of C6 glioma and SH-Y5Y cancer cells lines was compared to normal primary astrocytes after treatment with 100 μM DHA (Das and Das 2016). Two-dimensional in-gel electrophoresis and MALDI/MS/MS analyses identified six proteins that were altered with DHA treatment. The proteins annexin A2, pyruvate kinase M2, and annexin A2 were downregulated with DHA treatment, whereas aldo-keto reductase 1B8 and glutathione *S*-transferase P1 were upregulated in the cancer cells. These results suggest that DHA induces apoptosis in cancer cells by regulating the expression of the proteins identified in this study in order to activate apoptotic pathways promoting cancer cell death.

3 Foodomics

3.1 Probiotics

Probiotics are microorganisms that confer health benefits to humans and animals by promoting digestion, strengthening the gastrointestinal epithelium, and modulating gut microbiota and immune response (Wang et al. 2018a, b). The use of probiotics in veterinary medicine is becoming more prevalent, particularly with regard to preventative and supportive treatment for gastrointestinal disorders and infectious diseases (Bybee et al. 2011; Mudronova et al. 2018; Wang et al. 2018a, b). The most widely used probiotics include *Bifidobacterium* and *Lactobacillus* spp. (Cho et al. 2015; Solano-Aguilar et al. 2018). Although probiotics are well-recognized for their health benefits in animals, identification of molecular markers of their health-promoting activity and elucidation of the processes by which probiotic bacteria can exert health benefits in animals are still warranted. However, recent proteomics-based research has been rewarding and offers some insight into the molecular mechanisms of probiotic action and the role of bacterial proteins.

In the past few years, the genomes of some probiotic species have been sequenced. Having these genome sequences available has made protein identification possible through the use of proteomics-based technologies. For example, 2-DGE with MALDI/TOF MS and HPLC/MS/MS have been used to elucidate the molecular mechanisms of

probiotics with respect to how they adapt to the gastrointestinal tract environment and to identify proteins that may help explain the health benefits of probiotic use. One of the major challenges of probiotics is surviving the adverse environmental conditions in the gastrointestinal tract, such as acidic pH and exposure to bile. Proteomics has been able to offer some insight into how *Bifidobacterium spp*. and *Lactobacillus spp*. counteract and overcome these challenges.

Bifidobacteria and *Lactobacillus* are commonly added to fermented dairy products, which are acidic in nature. These bacteria have to cope with acidic stress during production of the functional food and also upon ingestion when exposed to the acidic conditions in the stomach. Proteomics investigations have been able to shed some light on how these bacteria adapt to these acidic conditions. For example, proteomics studies have revealed that *Bifidobacterium longum* overexpress the proteins AtpA and AtpD and induce H^+-F_1F_0-ATPase when in acidic conditions (Matsumoto et al. 2004; Cotter and Hill 2003). It is known that these proteins, particularly H^+-F_1F_0-ATPase, play a major role in the acid resistance of gram-positive bacteria. This enzyme is a proton pump that can extrude protons from the cell cytoplasm rendering the bacteria acid-tolerant. The effect of acidic pH on protein expression in *Lactobacillus reuteri* has also been investigated using proteomics. Approximately 40 proteins were identified that changed with exposure to an acidic environment and encompassed proteins involved in transport and binding, pH homeostasis and stress, carbon energy metabolism, and amino acid biosynthesis (Lee et al. 2007). Proteins overexpressed by *L. reuteri* in response to low pH included glyceraldehye-3-phosphate dehydrogenase, phosphoglycerate mutase, and pyruvate kinase. This overexpression is likely due to the fact that cells modify their energy metabolism and carbohydrate transport in acidic environments. Similar to *Bifidobacterium*, *L. reuteri* was also found to overexpress F_1-ATPase to enhance acid tolerance.

These probiotic bacteria have also adapted bile resistance mechanisms in order to survive exposure to bile salts in the gastrointestinal tract. Deconjugated bile salts are toxic to bacteria and can damage to cell membranes, cause protein denaturation, or induce DNA and RNA damage (Begley et al. 2005). Understanding some of the underlying mechanisms by which these probiotic bacteria have become tolerant of bile stress conditions has been accomplished through proteomics. The effect of bile salts on the stress tolerance of *Bifidobacterium longum* has been examined using 2-DGE and MALDI/TOF MS (Sanchez et al. 2005). This proteomic study focused on identifying changes in expression of cytosolic protein patterns under bile salt stress. Thirty-four proteins changed with bile salt stress with xylulose 5-phosphate/fructose 6-phosphate phosphoketolase significantly upregulated. This protein is a key enzyme in the bifidobacterial shunt. The other proteins that changed were primarily associated with bile exposure are involved in stress response, metabolism, protein synthesis and glycolysis, and pyruvate catabolism. This suggests that activation of proteins in these pathways was intended to protect the bacteria from oxidative stress, nucleic acid damage, and pH acidification. This study also highlighted that multiple proteins involved in many pathophysiological pathways are responsible for bile salt tolerance in *Bifidobacterium longum*. In order to protect the cell membrane from bile salt damage, proteomic evidence has shown that *Bifidobacterium* also increase production of exopolysaccharides (Ruas-Madiedo et al. 2009). Exopolysaccharides are surface-associated polysaccharides that cover bacterial cells and form the "capsule" (Nwodo et al. 2012). Proteomic evidence has revealed that bile-tolerant strains of *Bifidobacterium* increase production of exopolysaccharides to make it more tolerant of bile stress and the acidic environment in the gastrointestinal tract. Proteome levels were also investigated in *Lactobacillus rhamnosus* GG in response to bile salt stress (Koskenniemi et al. 2011). Forty-two proteins were differentially expressed, of which, 14 proteins increased under bile stress and also correlated with changes in the transcriptome. The proteins identified include those involved in pathways associated with cell envelope properties, active removal of bile compounds from the bacterial cell, stress responses, and metabolic processes. Interestingly, although proteins involved in thickening the exopolysaccharide layer were identified, the authors noted that a protein dedicated to the active removal of bile compounds from the cell walls was significantly upregulated providing a means by which this bacterium is bile tolerant. Although more research needs to be done with respect to the molecular mechanisms of probiotic health effects, proteomics has been instrumental in enhancing our understanding of some of the underlying molecular mechanisms of probiotic bacteria and how they overcome challenges when used to treat gastrointestinal disorders.

4 Concluding Remarks and Future Directions

Nutraceuticals and functional foods hold significant promise in ameliorating animal health and preventing disease. Although the clinical applications and health benefits of these nutritional disciplines is well-assessed, there is still a need to garner a deeper understanding of the molecular mechanisms responsible for their purported health benefits.

Proteomics is evolving as a promising tool that may provide this scientific knowledge to link diet and disease in nutritional research. Additionally, more long-term studies may be warranted to better understand their role and benefits for medical conditions and overall animal health.

References

Anderson NL, Anderson NG (2002) The human plasma proteome. Mol Cell Proteomics 1:311–326

Andringa NK, King AL, Eccleston HB et al (2010) Analysis of the liver mitochondrial proteome in response to ethanol and S-adenosylmethionine treatments: novel molecular targets of disease and hepatoprotection. Am J Physiol Gastrointest Liver Physiol 298: G732–G745

Bailey SM, Robinson G, Pinner A et al (2006) S-adenosylmethionine prevents chronic alcohol-induced mitochondrial dysfunction in the rat liver. Am J Physiol Gastrointest Liver Physiol 291: G857–G867

Bauer JE (2011) Therapeutic use of fish oils in companion animals. J Am Med Assoc 239:1441–11451

Bautista AC, Moore CE, Lin Y et al (2015) Hepatopathy following consumption of a commercially available blue-green algae supplement in a dog. BMC Vet Res 11:136. https://doi.org/10.1186/s12917-015-0453-2

Begley M, Gahan CG, Hill C (2005) The interaction between bacteria and bile. FEMS Microbiol Rev 15(8):625–651

Blum K, Meshkin B, Downs BW (2006) DNA based customized nutraceutical "gene therapy" utilizing a genoscore: a hypothesized paradigm shift of a novel approach to the diagnosis, stratification, prognosis and treatment of inflammatory processes in the human. Med Hypotheses 66(5):1008–1018

Bybee SN, Scorza AV, Lappin MR (2011) Effect of the probiotic E. Faecium SF68 on the presence of diarrhea in cats and dogs housed in an animal shelter. J Vet Intern Med 25(4):856–860

Center SA, Warner KL, Erb HN (2002) Liver glutathione concentrations in dogs and cats with naturally-occurring liver disease. Am J Vet Res 63:1187–1197

Cho JG, Geghart CJ, Furrow E et al (2015) Assessment of *in vitro* oxalate degradation by *Lactobacillus* species cultured from veterinary probiotics. Am J Vet Res 76(9):801–806

Cotter PD, Hill C (2003) Surviving the acid test: responses of gram-positive bacteria to low pH. Microbiol Mol Biol Rev 67:429–453

Das M, Das S (2016) Identification of cytotoxic mediators and their putative role in the signaling pathways during docosahexaenoic acid (DHA)-induced apoptosis of cancer cells. Apoptosis 21(12): 1408–1421

Görg A, Obermaier C, Boguth G et al (2000) The current state of two-dimensional electrophoresis with immobilized pH gradients. Electrophoresis 21:1037–1053

Guo T, Chang L, Xiao Y et al (2015) S-adenosyl-L-methionine for the treatment of chronic liver disease: a systematic review and meta-analysis. PLoS One 10(3):e0122124

Gupta RC (2016) Nutraceuticals in arthritis. In: Gupta RC (ed) Nutraceuticals: efficacy, safety and toxicity. Academic Press/Elsevier, Amsterdam, pp 161–176

Hamdan M, Righetti PG (2002) Modern strategies for protein quantification in proteome analysis: advantages and limitations. Mass Spectrom Rev 21:287–302

Hansen RA, Anderson C, Fettman MJ et al (2011) Menhaden oil administration to dogs treated with radiation for nasal tumors demonstrates lower levels of tissue eicosanoids. Nutr Res 31: 929–936

Issaq HJ, Chan KC, Janini GM et al (2005) Multidimensional separation of peptides for effective proteomic analysis. J Chromatogr B 817: 35–47

Koskenniemi K, Laakso K, Koponen J et al (2011) Proteomics and transcriptomics characterization of bile stress response in probiotic *Lactobacillus rhamnosus* GG. Mol Cell Proteomics 10(2). https://doi.org/10.1074/mcp.M110.002741–2

Lee K, Lee H, Pi K et al (2007) The effect of low pH on protein expression by the probiotic bacterium *Lactobacillus reuteri*. Proteomics 8:1624–1630

Li X, Wang W, Chen J (2017) Recent progress in mass spectrometry proteomics for biomedical research. Sci China Life Sci 60: 1093–1114

Matsumoto M, Ohishi H, Benno Y (2004) H^+-ATPase activity in *Bifidobacterium* with special reference to acid tolerance. Int J Food Microbiol 93:109–113

Mudronova D, Karaffova V, Csank T et al (2018) Systemic immune response of gnotobiotic mice infected with porcine circovirus type 2 after administration of *Lactobacillus reuteri* L26 Biocenol™. Benef Microbes 20:1–12

Navarro SL, White E, Kantor ED et al (2015) Randomized trial of glucosamine and chondroitin supplementation on inflammation and oxidative stress biomarkers and plasma proteomics profiles in healthy humans. PLoS One 10(2):e0117534. https://doi.org/10.1371/journal.pone.0117534

Nwodo UU, Green E, Okoh AI (2012) Bacterial exopolysaccharides: functionality and prospects. Int J Mol Sci 13(11):14002–14015

Ogilvie GK, Fettman MJ, Mallinckrodt CH et al (2000) Effect of fish oil, arginine, and doxorubicin chemotherapy on remission and survival time for dogs with lymphoma: a double-blind, randomized placebo-controlled study. Cancer 88:1916–1928

Pomin VH (2015) Medical gains of chondroitin sulfate upon fucosylation. Curr Med Chem 22(35):4166–4176

Ruas-Madiedo P, Gueimonde M, Arigoni F et al (2009) Bile affects the synthesis of exopolysaccharides by *Bifidobacterium animalis*. Appl Environ Microbiol 75:1204–1207

Sanchez B, Champonier-Verges MC, Anglade P et al (2005) Proteomic analysis of global changes in protein expression during bile salt exposure of *Bifidobacterium longum* NCIMB 8809. J Bacteriol 187(16):5799–5808

Schmid RD, Hovda LR (2016) Acute hepatic failure in a dog after xylitol ingestion. J Med Toxicol 12(2):201–205

Schroder PC, Fernandez-Irigoyen J, Bigaud E et al (2012) Proteomic analysis of human hepatoma cells expressing methionine adenosyltransferase I/III, characterization of DDX3X as a target of S-adenosylmethionine. J Proteomics 75:2855–2868

Sebbag L, Smee N, van der Merwe D et al (2013) Liver failure in a dog following suspected ingestion of blue-green algae (Microcystis spp.): a case report and review of the toxin. J Am Anim Hosp Assoc 49(5):342–346

Solano-Aguilar G, Shea-Donahue T, Madden KB et al (2018) *Bifidobacterium animalis subspecies lactis* modulates the local immune response and glucose uptake in the small intestine of juvenile pigs infected with the parasitic nematode *Ascaris suum*. Gut Microbes 9(5):422–436. https://doi.org/10.1080/19490976.2018.1460014

Vandeweerd JM, Coisnon C, Clegg P et al (2012) Systematic review of efficacy of nutraceuticals to alleviate clinical signs of osteoarthritis. J Vet Intern Med 26:448–456

Wallace KP, Center SA, Hickford FH et al (2002) S-adenosyl-L-methionine (SAMe) for the treatment of acetaminophen toxicity in a dog. J Am Anim Hosp Assoc 38:246–254

Wang J, Ji H, Wang S et al (2018a) Probiotic Lactobacillus plantarum promotes intestinal barrier function by strengthening the epithelium and modulating gut microbiota. Front Microbiol 9:1953. https://doi.org/10.3389/fmicb.2018.01953

Wang J, Zeng Y, Liu H et al (2018b) Swine-derived probiotic *Lactobacillus plantarum* inhibits growth and adhesion of entertoxicgenic escherichia coli and mediates host defense. Front Microbiol 9:1364. https://doi.org/10.3389/fmicb.2018.01364

Webb CB, Twedt DC, Fettman MJ et al (2003) S-adenosylmethionine (SAMe) in a feline acetaminophen model of oxidative injury. J Feline Med Surg 5:69–75

Zhang X, Fang A, Riley CP et al (2010) Multi-dimensional liquid chromatography in proteomics—a review. Anal Chem Acta 664 (2):101–113

Nanoparticles and Molecular Delivery System for Nutraceuticals Bioavailability

Gianfranco Risuleo and Camillo La Mesa

Abstract

This contribution discusses methods for transferring exogenous materials and drugs, particularly, into biological tissues. The focus is on matrices such as micelles, vesicles, and oil-based dispersions as well as carbon nanotubes. An *ensemble* of physical forces takes a fundamental role in drug dispersion and includes van der Waals (vdW), steric (ST), double layer, (DL), osmotic (OS), etc. Combination of these forces is responsible for drug uptake in matrices and for their release in tissues. Uptake of exogenous either macro- or small molecules into cargo particles and their transfer to recipient cells is the result of complex processes, concomitant to drug partition among supramolecular aggregates and the bulk. Similar conclusions apply to drug release, mostly as to the kinetic features are concerned; therefore, adsorption of nutraceuticals and release within target organs are particularly relevant. These complex features can be accounted for on thermodynamic grounds and expressed as the combination of different forces. In what follows some details on the energies to be considered are outlined. These include terms controlling the fate of transfectants. We will consider first the forces responsible for the formation of such supramolecular entities on physicochemical grounds and the drug uptake; finally, we will review the actual possibility of transfecting cargo-mediated aggregates of nanoparticle/drug complexes to cells or tissues of interest and their bioactivity upon release within the cell matrix.

Keywords

Nanoparticles · Adsorption/partition/release · Thermodynamics · Biological action · Cytotoxicity · Genotoxicity · Cultured cells · Living organisms

1 Introduction

Due to the complexity of the topics this chapter deals with, the authors have decided to introduce no figures and/or tables within the text. Two are the reasons at the basis of this choice: first, the figures referring specifically to the chemico-physical part would have been, possibly, not promptly intelligible for the general reader, who in the vision of the authors is rather interested in the more general features of nanoparticles and in their fabrication. The second reason is that figures illustrating very specific aspects and experimental results obtained either in cultured cells or in living animal model systems, would have not added more general information about their potential usage in human and veterinary medicine. As a matter of fact, also in this chapter, the authors intentionally tried to adopt a very plain language, meant to reach an as broad as possible audience. In other words, we aimed at composing a text comprehensible both to scientists active in biomolecular research on natural compounds, where the biochemical/physical part is fundamental, and to the layman interested in alternative ways to look at nutrition and health care in humans and other animals. The authors feel, as a matter of fact, the exploding interest in emerging habits of nutrition and health care should be everybody's endowment.

One final word of warning, the review may seem somewhat repetitious, but this was done on purpose. The reader may skip some parts considered too specialized or out of the

Gianfranco Risuleo is on retirement.

Obituary: The chapters authored by C. La Mesa and G. Risuleo are dedicated to the memory of Adalberto Bonincontro, an outstanding collaborator but, mainly, a lifelong friend. Most of the work reviewed here would have not been possible without his continuous, active participation, and support. Many works co-authored by the three of us are to be found among the references.

G. Risuleo (✉)
Department of Biology and Biotechnologies "Charles Darwin", Sapienza University of Rome, Rome, Italy
e-mail: gianfranco.risuleo@uniroma1.it

C. La Mesa
Department of Chemistry "Stanislao Cannizzaro", Sapienza University of Rome, Rome, Italy

R. C. Gupta et al. (eds.), *Nutraceuticals in Veterinary Medicine*, https://doi.org/10.1007/978-3-030-04624-8_53

field of interest. The same information will be reiterated in different points of the chapter. In any case, the abundant references given at the end of the main text will allow the reader to deepen the subjects of interest.

2 Advanced Matrices for Drug Vehiculation: Chemical-Physical Aspects of the Nanomaterials for Molecular Delivery

The uptake of drugs into cells and biological tissues is the result of a complex balance between many physical forces jointly contributing to these processes; their role is hierarchically controlled by the related energy contributions. From a thermodynamic viewpoint, one must consider drug uptake in a given tissue in analogy with partitioning of a species between immiscible fluids such as water and oil, for instance (Leo et al. 1971; Bolhassani and Rafati 2011; Florence and Attwood 1988). This oversimplified view of what effectively takes place in real systems is extremely useful in current formulation strategies.

Also, drug absorption in an organ is the result of a complex behavior, dictated by the fact that the former is never dispersed uniquely in that tissue. Partition depends on the similarity (in terms of polarity, for instance) of the species with the tissue. This implies that, for a drug to be effective toward a specific organ, chemical structure, specific dispersion, and transfer strategies are needed. Given the complex hydrophilic-lipophilic balance inherent to all biological tissues (Porter 1993; Barkat et al. 2011), we must rely on strategies capable to substantially disperse the drug to be transferred in the target organ, thus minimizing its partition into undesired tissues and increasing its topical efficiency. These strategies will increase the dose in the organ, still decreasing the overall amount of drug in the formulation (that is, the products result less toxic to the human body).

Whatever the carrier is, its features are optimized when it behaves in analogy with a bullet hitting an organ. In other words, the formulation containing the drug shall be selective toward a given tissue, termed target. This hypothesis is never fulfilled in practice, irrespective of the care used to optimize the formulation and on the drug quality, as well. Think to oil-based dispersions, for instance; these dissolve lipophilic drugs in more or less substantial amounts. Available oil-based dispersions do not easily disperse in biological matrices, when their viscosity, partly responsible for drug mobility, is high. It is not surprising, thus, that oil-based dispersions are intended for long-medium-term transfer procedures. In addition, one must consider if such dispersions are to be used for injection or skin treatment.

On this line, we must be aware of the preferred strategies to be fulfilled in practice. In what follows, we report on some aspects related to drug partition in selected carriers (Cammasab et al. 1997) and explain which contributions must be optimized to make the uptake effective. In a first approximation, we remind that a drug is partitioned among two immiscible fluids according to what is dictated by the Gibbs energy of transfer among two phases. Such relation quantifies the drug partition coefficient by equalizing the chemical potential of the chemical dissolved in immiscible liquids. The physical properties of the liquids (i.e., dipole moment, dielectric permittivity, viscosity, interfacial tension, hydrogen bonding capacity, and so forth) are relevant. Water and nontoxic octan-1-ol are jointly reference solvents; they represent highly polar and strongly nonpolar media, respectively (Andersson and Schräder 1999; Moriguchi et al. 1992). It is well known, in fact, that the partition coefficient of a drug between two media depends on the gradient in dielectric permittivity among two fluids and is rationalized by the Born equation (Monk 1961). Originally developed for ion partitioning between two fluids, the equation relates the Gibbs energy of transfer from the aqueous phase to oil to the gradient of dielectric permittivity among them, eWa and eO, respectively, according to

$$\Delta \ln K_{\mathrm{P,wa,O}} = \Delta(1/\varepsilon) = K[(\varepsilon_{\mathrm{Wa}} - \varepsilon_{\mathrm{O}})/\varepsilon_{\mathrm{Wa}}\varepsilon_{\mathrm{O}}]. \quad (1)$$

The above relation refers to the bare physical properties of the above media and does not explicitly account for temperature, T, ionic strength, I, and pH effects (although these, or the respective gradients, can be somehow inserted in the original theory). The model works well for immiscible liquids such as water and octan-1-ol, if the temperature of the two phases is the same. Although octan-1-ol is considered a reference solvent, it is rarely used in formulation.

What strange methods are possible in drug delivery? All these must guarantee the uptake of a significant amount of the drug in the target tissue; the latter can be polar or not. This fact has consequences on the best way required to dissolve the drug therein. In some instances, drugs are transformed in acid salts (in the form of hydrochlorides, usually) when target tissues are strongly polar. Alternatively, they may form complexes with crown ethers (Muzzalupo et al. 2007; Vintiloiu and Leroux 2008) or species of the like and with cyclodextrins, as well (Loftsson et al. 2005). The latter are saccharide units joined together to form a hole, whose width depends on the number of sugar units in the molecule. When crown ethers are intended for ion transport, cyclodextrins find preferential use with small hydrophobic species. More refined methods rely on drug transport by gels (Qiu and Park 2012), dendrimers, carbon nanotubes (Patri et al. 2005; Bianco et al. 2005), and many other supramolecular entities, some of which are discussed below.

We focus on two selected dispersants, representative of liquid-like and solid-like behavior, respectively. Cat-anionic

vesicles (Pucci et al. 2014a–c) and carbon nanotubes, CNTs, are representatives of nearly spherical and strongly anisometric entities, in turn. Vesicles retain hydrophilic species in the interior and outer surface but hydrophobic in the bilayer, whereas CNTs adsorb polar species on the outer surface only when they are properly functionalized. The amount of small-sized chemicals that can be uptake in the nanotubes' interior is moderate. Thus, CNTs retain very small amounts of molecules in the inside polar region. What is more, vesicles have significant curvature elasticity, when CNTs are relatively stiff. In some sense, thus, vesicles and CNTs are at the extreme limits of the drug dispersant class.

The stability of drug-based formulations depends on the dispersing matrices. In particular, surface energy terms become more and more significant as the size of the drug/carrier adducts decreases. In fact, the surface tension of such systems, g, is expressed as

$$d\Delta G/dA = \gamma \tag{2}$$

Equation (2) explains how the surface area can be controlled, and an increase/decrease of it is at the basis of an optimal working strategy.

These facts offer the opportunity to extend the application to colloid-like carriers; among them, carbon nanotubes, micelles, polymer-surfactant systems, vesicles, or liposomes are the most appealing (Weinstein and Leserman 1984; La Mesa 2005; Fadel and Fahmy 2014; Grumezescu 2018).

3 Vesicles and Related Aggregates

Such entities are jointly characterized by peculiar surface energy terms. However, carriers based on the above species largely differ each other in solvent capacity toward drugs. In such systems, hydrophobic and surface energy terms overlap and combine with electrostatic ones. Micelles, polymer-surfactant systems, liposomes, and vesicles are characterized by the presence of charges on the respective surfaces. This fact implies many outstanding effects, which jointly contribute to:

(i) Micelle/vesicle stabilization
(ii) The formation of an electrical double layer around such entities
(iii) Size modulation
(iv) Conformation of the polymer chain in case of polymer-surfactant systems and so forth

Macromolecules as those indicated in (iv) are surfactant adjuvants in modern drug transfer procedures. For instance, tablets contain sodium dodecyl sulfate, SDS, or potassium carboxylates, with polyethylene oxide, PEO, and/or polyvinylpyrrolidone, PVP, (to mention but a few) (Karthik 2016). The two chemicals in each of these formulations operate in associated form (as polymer-surfactant complexes) as dispersants and viscosity modulators (La Mesa 2005). We, deliberately, do not consider here the use of polymer-surfactant systems in baby diapers although putative usage of these systems is at times advertised in commercials. As a matter of fact, the scientific literature in support or discouragement of these aids is not corroborated by evidence (Degouy et al. 2014).

Turning back to the supramolecular association modes, let us point out that similarly charged polar heads, located at interfaces, repel each other. That process counteracts the hydrophobic effect; it is somehow modulated from counterions partly adsorbing at the interface and reducing the net charge on the aggregates. In this way, a denser/looser polar head packing is favored and the surface area optimized. Indirectly the volume contributions to the system energy (which are opposite in sign to surface ones) are also modulated. The surfactant self-assembly mode obtained in this way is defined as "packing constraint" (PC theory, see below, as discussed in Tanford 1980; Safran et al. 1990; Nagarajan 2002). Originally defined by Israelachvili et al. (1976), it has some points in common with the nucleation of a particle, be it a solid or a fluid. Association of surfactants is determined by the balance of surface and volume energy terms, of opposite sign. The chemical potential of the species in question depends on such contributions and defines the aggregate size better compatible with the minimum Gibbs energy. This fact finds analogy with the nucleation of oil droplets dispersed in a given medium (Morris et al. 1997); they start to form and grow in size only when a critical radius is attained. Surfactant aggregation, conversely, is determined by the balance of the (attractive) transfer of hydrocarbon chains in the micelle interior and is counteracted by the combination of surface tension and surface charge density terms.

The PC theory has significant advantages compared to the classical nucleation theory. In particular, it dictates the optimal size a surfactant molecule/ion must have to pack into entities of a given curvature, be it positive or negative. In doing so we combine surface energy terms and volume effects (jointly contributing to the chemical potential of a given species) with the preferred geometry of the aggregates we would get. Advantages due to the above considerations are horrific in modern formulation strategies and can be obtained by proper experimental actions, by adding salts or co-surfactants, for instance. Among many other advantages, the PC theory explains why added salt favors the formation of rod-like micelles with respect to spherical ones (Hayashi and Ikeda 1980; Alargova et al. 1997; Zoeller and Blankschtein 1998). It also accounts for the residual surface charge density

that controls the formation of thermodynamically stable cat-anionic vesicles (Bonincontro et al. 2007, 2008). The acronym indicates that such mixtures contain both cationic and anionic species in variable amounts. The latter vesicles are appealing transfectants for a series of reasons (Jung et al. 2001; Lo et al. 2010; Barbetta et al. 2011; Coey et al. 2011; Louzao and van der Hest 2013; Pucci et al. 2014a–c), namely:

(i) The surface charge density modulation favors an optimal vesicle size.
(ii) An optimal packing of the alkyl chains into bilayers is possible.
(iii) Drug uptake in the vesicle interior, or in the outer surface, is favored.
(iv) An optimal bilayer and curvature elasticity can be obtained.

As to the biological consequences of using vesicles of the cat-anionic type, a relevant one finds origin in the low cytotoxicity of such vesicles (orders of magnitude lower than the surface-active agents from which they are made of) (Moroi and Matuura 1988; Muzzalupo et al. 2006). Consider that when SDS is strongly cytotoxic and cetyltrimethylammonium bromide, CTAB, or didodecyldimethylammonium bromide, DDAB, even more, vesicles formed by blends of such substances are poorly toxic, if not at all (Kuo et al. 2005; Aiello et al. 2010). This point is quite counter-intuitive to the common sense, unless one is aware of the fact that surfactant toxicity toward biological tissues depends on its solubility in molecular form. It is not surprising, therefore. In fact, the concentration of surfactant(s) in molecular form coexisting with cat-anionic vesicles is orders of magnitude lower than that pertinent to aggregates made by a single surfactant. This behavior is rationalized by the so-called surfactant-surfactant interaction parameter (Muzzalupo et al. 2006), determined by comparison with the behavior expected from ideal mixing of the given species. Cooperativity is concomitant to a decrease in the concentration of molecular surfactant in the bulk and is proportional to b. In other words, the mentioned process finds analogy with metathesis. Further refinements are possible if alkanols or sterols or else are added to cat-anionic formulations; in particular, the latter chemicals favor a denser alkyl chain packing and a more robust character to the bilayers. In selected cases polymers adsorb on the vesicle surface and anchor thereon thanks to the combined action of ionic interactions and alkyl chain uptake, if hydrophobically modified polymers, HMPs, are used (Sallustio et al. 2004). The latter possibility has been used to modulate vesicle size (Pucci et al. 2014a–c) and, eventually, to favor its faceting. More advantages are on the possibility to pack different vesicles in superstructures.

Drugs can be selectively partitioned on the outer/inner vesicle surface, on its interior, and in the inner aqueous pool. Therefore, different formulation possibilities (such as surface adsorption, selective uptake in hydrophobic, or, conversely, polar matrices) are in our hands, depending on the chemical nature of the drug to be transferred. This is the reason for a wide-spread use of cat-anionic formulations in many different biomedical fields. Biological implications and suggestions for an optimal and selective use of the most common (and most promising too) cat-anionic mixtures are outlined below. What has been considered in case of the cat-anionic species mentioned above can be extended to many more species. For sure it holds for phospholipid mixtures (Lozano et al. 2009). More recently, efforts have been made by collaborating research groups active in the field. The basic idea underlying the working hypothesis is that surfactants having amino acids as polar head group are characterized by a very low cytotoxicity (Pinazo et al. 2011; Colomer et al. 2012; Mezei et al. 2012). If we consider, in addition, that the amount of surfactant in molecular form coexisting with vesicles is, as a rule, extremely low (in the micromole range), it is reasonably assumed that catatonic vesicles built up accordingly have very low, if any, cytotoxicity (Muzzalupo et al. 2017; Tavano et al. 2017). Furthermore, such a hypothesis was substantially demonstrated, mostly when pH controls the vesicle state of charge. At the best of our knowledge, these are among the most promising results in the field.

4 Carbon Nanotubes

Many systems made of extended carbon moieties are potential candidates for transfection technologies. However, when almost nothing is actually known on the potentialities offered by graphene derivatives, those based on functionalized fullerenes (Nakamura and Isobe 2003) have got some success. Even more successful are those based on carbon nanotubes, CNTs. These species have potentialities of substantial interest in biomedical applications. First, they have inner cavities; second, their outer surfaces can be properly functionalized, when it is required (as we will see below, this procedure is nearly compulsory). In addition, they have outstanding physical-mechanical properties such as elasticity and conductive/semi-conductive behavior. In terms of what is stated in many books and reviews, their properties are mostly engineering-intended (Hone et al. 1999; Arroyo and Belytschko 2004); we include in the category the preparation of new hard materials for tissue engineering. However, substantial efforts have been recently made by diverse scientists to make them suitable for refined bio-intended applications (Popov et al. 2007; Heister et al. 2013; Amenta and

Aschberger 2015). To proceed along this line, one must be aware of the fact that the high aspect ratios (i.e., the tube to diameter ratio) pertinent to CNTs and, mostly, the strongly hydrophobic character of their outer surfaces substantially hinder the preparation of biomedically relevant advanced materials. In particular, high axial ratios imply that long filaments of CNTs (several nm's long) easily form scaffolds by simply pressing or in other ways. As a rule, reticulation procedures are not strictly required, since the strong hydrophobic character of CNTs ensures a substantial interaction energy and significant aggregation.

Nanotubes are obtained since very specific five-/six-carbon ring ratios ensure them a proper surface curvature. Carbon monolayer rolling may stop to give single-walled carbon nanotubes, SWCNTs, or, later, multi-walled ones, MWCNTs. As to surface properties, there is no substantial difference among two such classes, which, however, largely differ in elasticity, rigidity, and so forth. Their chemical reactivity is mostly active on the outer surfaces, properly functionalized by drastic oxidation procedures, using HNO3/H2SO4 mixtures or H2O2 or other agents (Ajayan et al. 1993; Tsang et al. 1993). In the above cases, carboxylate or OH group attach on the surface, whereas the overall CNT length is drastically reduced. In this way the elastic properties of CNTs are minimized, whereas chemical reactivity largely increases. Such duality gives the opportunity to distinguish among engineering-intended, the former, and bio-inspired CNTs. Surface functionalization, in fact, is the key strategy leading to biocompatibility.

Pristine CNTs are poorly stabilized in aqueous media and associate by vdW interactions to form bundles. This fact strongly reduces possible applications and must be severely minimized. This is the reason why surface coverage is relevant. That process can be obtained by covalent functionalization procedures, as outlined above, or by non-covalent ones. Among the most used ones let us mention surfactant (Bandyopadhyaya et al. 2002; Islam et al. 2003; Wang 2009; Vaisman et al. 2006) or polymer (Liu 2005; Spitalsky et al. 2010; Huang and Terentjev 2012) adsorption. The former category implies the adsorption of medium-long-chain surfactants, be they ionic or not. It is well acquainted, however, that the efficiency in surfactant adsorption is inferiorly and superiorly limited. The rationale for that unexpected behavior arises by depletion phenomena (Tardani et al. 2012). The surfactant, to put it simply, partitions between the surface state and the bulk, where it may also form micellar aggregates, that is, ions, micelles, and surface bound states. The chemical potential of the surface-active species depends on the balance between all such states. As a result of unbalanced osmotic effects in the bulk, the surface-adsorbed species tend to detach from the nanotubes and form more micelles. Thus, non-more surface-covered CNTs directly interact to form bundles and will easily separate out from the solution. Note that the latter effect is non-peculiar to CNTs and has been brilliantly explained some years ago by De Gennes (1981).

An alternative to surfactants relies on polymers. Most of them do suffer from the same drawbacks outlined above; in many instances, adsorption is moderate and superiorly limited, mostly in the case of small proteins (Bomboi et al. 2013). There are exceptions to these general rules; the most prominent is that observed when single-stranded DNA adsorbs onto CNTs. For those non-explicitly aware of that method, we remind that the stable form of double-stranded DNA can be transformed in two filaments by the action of heat. At 95.0 °C, on average, the double helix melts into two filaments, which can remain for long time in such state if the solution is thermally quenched. Addition of CNTs allows efficient surface coverage. The difference between printing, surfactant-stabilized, and single-stranded covered nanotubes is astonishing. The solubility of the former two states is in the mg kg^{-1} range, when the latter can be as high as some wt%. This fact allowed to determine in some detail the phase diagram of ss-DNA/CNT systems and to visualize the formation of nematic order therein (Tardani et al. 2012, 2013; Tardani and Sennato 2014). The only possibility to use such formulations for biomedical purposes is that due to the possible adsorption of drugs on functionalized nanotubes. In other words, CNTs would act as surfaces on which polymer-drug adducts are adsorbed!

The only significant advantages of formulations based on biopolymers and CNTs are due to their high biocompatibility, as demonstrated by some of us in selected cases (Muzi et al. 2016a, b; Risuleo and La Mesa 2016). Thus, even though the quantity of vehiculated drug is moderate, the whole strategy is effective. Alternatively, we may use CNT-based formulations by taking into account the presence of a cavity therein; the latter can be filled with small drugs, as it has been demonstrated in the case of anticancer therapy based on cis-platinum (Muzi et al. 2015a, b). The latter species enters the nanotube cavities, the whole process being controlled by the sizes of the respective species. Once the inner complexes with cis-platinum are released in the infected tissue, a slow drug release occurs. The driving force for it is the result of a combination of diffusion, concentration gradients, and ion concentration in the target organ.

5 Nanomaterials as Cargos of Biological Molecules: Potential Usage in Advanced Therapeutic Strategies

The number, nature, and sophistication of molecular carriers for the delivery of exogenous material within a living cell are diversified and, in many cases, serve different purposes.

Therefore, for the sake of conciseness, we will focus mainly on three different nanoparticles, i.e.:

- Cationic liposomes
- Cat-anionic vesicles
- Single-walled carbon nanotubes

The largest part or the results discussed here were obtained in our or closely collaborating laboratories: as a matter of fact, nanoscience, though in a still quickly developing stage, requires a multidisciplinary effort where scientist with diverse but complementary expertises participates. However, achievements of other research groups will be also taken into account.

6 Liposomes

Due to their biocompatibility, liposomes are among the most studied drug delivery systems. Also, the possibility of targeting them in a selective manner to different tissues and the favorable cost/production facility plays an important role in their application as therapeutic agents in advanced medicine. Several physicochemical parameters determine the validity of liposomes as cargo particles for the transport of specific molecules be they: macromolecule such as DNA, RNA, and proteins or small molecules like hormones, natural compounds, and/or drugs in general. The type and amount of lipid in their formulation are crucial. For instance, liposomes based on cationic lipids are not found in nature but are synthesized in the chemistry laboratory. Since they are endowed of a net positive charge, they may promptly interact with negatively charged cell membrane and nucleic acids (Simberg et al. 2004). Relatively, recent works have evidenced their ability as carriers of nucleic acids and vaccine carrier/adjuvants (Joseph et al. 2006; Lonez et al. 2008). The possibility of using this type of liposome as drug carriers for neoplastic and other diseases has also been examined; in particular, cationic liposomes are able to deliver specifically their payload to embryologically different tissues such as tumors of endothelial origin, to the lungs and liver and gastrointestinal tract. Therefore, they have become a very attractive tool for oral administration of therapeutic agents in cancer therapy. A further important observation is the interaction of this type of liposome does not interfere with the morpho-functional features of the target cells as observed by optical and SFM imaging as well as NMR-metabolomic studies (Piccioni et al. 2007).

Finally, the antibacterial action of cationic liposomes has been also examined in Gram-negative or antibiotic-resistant pathogenic microorganisms: as a matter of fact, liposomes may increase the bacterial membrane permeability, which consequently causes a higher susceptibility to drug uptake (Hamblin and Hasan 2004; Bombelli et al. 2008; Cosimati et al. 2013; Stefanutti et al. 2014). For an insight into the role played by the cell membrane permeability in the uptake of exogenous molecules, the routes of internalization and the intracellular trafficking the reader should also address a previous chapter of this book written by the same authors and references therein. For the potential usage of liposomes in antiproliferative (anticancer) therapy, the reader is advised to address the following recent reviews: Heidarli et al. 2017; Olusanya et al. 2018; Deshantri et al. 2018.

7 Cat-Anionic Vesicles

Cat-anionic vesicles are supramolecular aggregates formed by mixing in non-stoichiometric ratios cationic and anionic surfactant species (Letizia et al. 2007). Surfactants of opposite charge tend to aggregate in aqueous polar solvents. The electrostatic interactions between the polar heads and hydrophobic tails favor the formation of self-assembled and organized supramolecular structures. An extensive analysis of the parameters governing the formation and morphology can be found in the classical work by Israelachvili and collaborators (Israelachvili et al. 1977). Vesicles may easily interact with DNA, RNA, or other biopolymers, resembling in this case the above discussed liposomes. In this case also, the complexes vesicle/macromolecule, commonly defined lipoplexes, assume a fundamental importance in biotechnological and biomedical applications: as a matter of fact, their intrinsic structural and functional properties attribute them the capacity to deliver genetic material across the cell membrane via plasma membrane fusion and/or endocytosis. Work from our laboratory showed that it is possible to form complexes between DNA and vesicles with an excess positive charge at the surface, which could be potentially delivered within the cell (Bonincontro et al. 2008). But concerning their biocompatibility, some *caveats* are to be considered: vesicles may show a cytotoxic effect, which is directly related to time of exposure and dose of administration as well as to the nature of the composing cat-anionic moieties. Literature on this specific aspect exists (Kuo et al. 2005; Vlachy et al. 2009; Lozano et al. 2011), and work from our laboratory showed that SDS-DDAB are far more cytotoxic than those formed with SDS and CTAB as hydrophobic counterion (Russo et al. 2013) ; furthermore, these latter ones (SDS-CTAB) are able to "hit" tumor cells more efficiently than normal mouse fibroblasts which show a higher survival/proliferation rate as compared to the previous cell population (Aiello et al. 2010). However, despite their cytotoxicity, it is possible to adjust the experimental conditions such as time and dose of treatment, to allow a successful vesicle-mediated transfection with subsequent expression of exogenous genetic material (Loftsson et al. 2005; Muzzalupo et al.

2007). With respect to this crucial aspect, one main interest in the use of nanoparticles as molecular carriers of genetic material consists in ascertaining the fate of this material, be it DNA or RNA, upon entry in the recipient cell: i.e., is the transfected macromolecule stable? Does it maintain its functional features? Can the genetic features encoded within the macromolecule be transformed into the mature functional product? We addressed this question by transfecting messenger RNA into recipient cells and measuring the level of the encoded gene product by standard CAT-assays. The sharp and clear-cut result is that the transfected cat-anionic/RNA lipoplex is efficiently metabolically translated into protein which exhibits functional epitopes promptly recognized by the cognate antibody as shown by ELISA assays (for a study of the effects of nanoparticle-mediated gene transfer on the overall cell metabolism, see Aiello et al. 2010; Russo et al. 2013; Cosimati et al. 2013; Stefanutti et al. 2014). Finally, pilot studies indicate that vesicles may be utilized in anticancer therapy (for recent reviews, see Wright 2008; Freire et al. 2015; Han et al. 2017).

8 Nanotubes

To recapitulate quickly, carbon nanotubes (CNTs) are rolled graphene sheets forming cylinders or quasi-cylindrical structures; these are found in single- (SWCNTs) or multi-walled concentric CNTs (MWCNTs) shapes. Nanotubes have very numerous potential applications in biochemistry, nanomedicine, pharmacology, and industry such as avionics as well as space engineering (see, for instance, Prato et al. 2008; Ménard-Moyon et al. 2010; Venkatesan et al. 2014; Shtansky et al. 2018, Krishna et al. 2018; Mohajeri et al. 2018).

Curiously enough, graphene was recently implied in the development of ultrathin highly resistant condoms: research was funded/sponsored by a very well-known head of an informatics company, but we will not expand on this aspect though will provide a useful link for the reader interested in this spearhead research (https://www.extremetech.com/extreme/171417-bill-gates-funds-creation-of-thin-light-impenetrable-graphene-condoms).

We will restrict, instead, our discussion to drug delivery and potential therapy, including diagnosis when appropriate. The development of biotechnologies based on nanotubes requires the evaluation of a few preliminary but essential aspects. In fact, as with other nanoparticles, the CNT biocompatibility must be carefully assessed both in cell culture systems and animal models: in this latter case, for instance, the ascertainment that they are not toxic and do not elicit immune responses is chiefly important. Also, since the cell membrane is the first barrier encountered by the nanotube upon cell entry, a further problem should be considered. CNTs are poorly dispersible in aqueous solution; thus, they establish van der Waals interactions (see also above, in this chapter) which cause their aggregation in large bundles which may be detrimental to membrane integrity and/or may hinder their passage across it (Holt et al. 2010). Initially, bundled, non-purified, and long SWCNTs have been associated with toxicity and negative cellular effects. Several dispersant molecules improve dispersibility, albumins in particular. Recent investigations conducted in our laboratories demonstrated that non-covalent carbon nanotubes/BSA complexes did not alter the cell viability. These nanocomposites were administered to different cell line murine fibroblasts (NH3T6), human embryonic kidney cells (HEK-293), or murine macrophages (RAW-294). No significant changes in the cell viability was monitored in 3T6 and HEK 293 cells after exposure to different concentrations of pristine or BSA-CNTs. A cytotoxic effect, on the contrary, was observed in the case of murine macrophages treated with both pristine and BSA-stabilized CNTs. Toxicity was diminished when CNTs were coated with BSA; this evidences that this protein enhances the biocompatibility. In any case, the good dispersion quality of these BSA-CNT complexes caused a good internalization both phagocytic (macrophages) and non-phagocytic (fibroblasts) cell types. Observations performed by transmission electron microscopy showed that the complexes penetrate the cells by a passive diffusion or endocytosis which is an active energy-dependent mechanism of assumption. However, crossing of the plasma membrane caused no alterations of the dielectric parameters of the cell membrane measured by a biophysical approach: electrorotation (Bonincontro and Risuleo 2015; Muzi et al. 2015a, b, 2016a, b).

As a second recapitulation, we will discuss in this section the role of graphene. Graphene is at the basis of the nanotube technology and consists of a one-atom-thick planar sheet of carbon atoms densely packed in a honeycomb crystal lattice. Graphene can display different forms which are conventionally combined under the so-called graphene family materials and include the few-layer graphene (FLG), graphene oxide, reduced graphene oxide, graphene nanosheets, ultrafine graphite, graphene ribbons, and graphene dots. In any case, FLG is constituted by 2–10 graphene layers (Bianco et al. 2013) and shares the basic structural element of other carbon allotropes, including carbon nanotubes and fullerenes, but their history is quite recent. However, from its discovery, graphene has been the object of exciting interest in many fields of basic science and industry: this is mainly due to graphene's extraordinary features. As a matter of fact, graphene-based nanotechnology represents nowadays an area of scientific research and industrial applications in full expansion (Liang and Chen 2010). Graphene was firstly

exploited in material sciences but recently has become a very good tool in electronics, photonics, composite materials, energy generation, energy storage, and sensors as well as biological applications (see also the web-link cited above). Last but not least, FLG is easily produced in high yield and at a relatively low cost (Novoselov et al. 2012). A new *caveat* is that the rapid increase in production and applications of FLG implies its potential release into the environment, especially into the aquatic compartment generally considered as a fragile and sensitive macro-sphere. With respect to this, the release of FLG could occur more or less accidentally from inadequate or uncontrolled disposal of such commercial products and the chemical modification and degradation following the waste: possible ecosystem risks induced by FLG were examined and are found in literature (Hu and Zhou 2013); also, a recent review discusses the toxicity of GFMs toward the aquatic environment including bacteria, crustaceans, nematodes, and fish (Zhao et al. 2014). The idea is that the toxicity of different aquatic organisms and the impact of FLG against aquatic organisms may not be as high as carbon nanomaterials such as fullerenes and carbon nanotubes. However, results are controversial. In fact, the biological response often depends on the intrinsic structural nature of graphene, such as number of layers, rigidity, hydrophobicity, dose and purity of the material under scrutiny, and, finally, the use of diverse cell/organism models (Bianco 2013). Based on the results from our laboratory on *Xenopus laevis* larvae, FLG is substantially nontoxic (Muzi et al. 2016a, b). These data are consistent with the literature available on FLG ecotoxicity on different organisms like *Pseudomonas aeruginosa*, *Caenorhabditis elegans*, and *Vibrio fischeri* (Gollavelli and Ling 2012; Zanni et al. 2012; Guo et al. 2013; Pretti et al. 2014). Analogous studies on crustaceans such as *Artemia salina* produced similar results as far as oxidative stress and life span and showed that FLG did not induce oxidative stress in the bacteria, but 70% of bacterial viability was lost after 5 h of exposure to 250 mg L^{-1} of the material. They also showed no effect on the lifespan is concerning. In our case, only the Xenopus larvae growth rate seemed to be affected by the material to some extent. Indeed, the larval size was decreased by about 40% only upon exposure to the highest concentrations of FLG. This profile response is usually observed in Xenopus larvae exposed under the same conditions to carbon nanotubes (Muzi et al. 2016a, b). Several hypotheses have been proposed to explain this phenomenon. For instance, the uptake of carbon-based nanomaterial may lead to digestive and respiratory obstructions causing exchange gas dysfunctions (Mouchet et al. 2011).

9 Concluding Remarks and Future Directions

In this multifaceted overview of the large and diversified group of carbon nanoparticles, we discussed the fabrication and the effects of liposomes, vesicles, and nanotubes. The effects of the administration of these nanocomposites were examined both in cultured cells and, in the case of few-layer graphene sheets, also in living animals: namely, *Xenopus laevis* larvae. Results were, when appropriate, compared to published literature data, and no relevant discrepancies could be detected between data from our studies and work conducted in other laboratories. The final conclusion is that, in general, nanoparticles can be considered a safe, though very efficient, tool to use in a number of different biomedical fields. These range from diagnostics to potential therapy of proliferative and/or immunosuppressive diseases in humans and other animals. Nanoparticles are internalized within the target cells where they deliver their molecular cargo. According to the presented data discussed in this work, very little, if any, permanent damage is inflicted to the animal assuming the exogenous material: therefore, one can safely conclude that neither the cell membrane, first barrier encountered by nano-complex upon cell entry, nor the overall cell metabolism suffers a significant damage. This renders nanoparticles very attractive in advanced biomedicine, which is the field of our main interest. Also, toxicity does not seem to be a primary concern in the usage of such particles. As very often repeated, the nanotechnological field is going through a process of ever-growing expansion: it is no easy task to predict what the developments will be in a medium to long time span. Who would have envisaged only a few decades ago the development of advanced imaging techniques, of noninvasive microsurgery, telemedicine: science fiction? The answer is negative! Science proceeds through inspiration but, as T.A. Edison[1] (*chapeau!*) put it, also through a lot of perspiration. Therefore, our auspices and ambition are that this review will serve mainly young scientist to find a spurt for their work.

In any case, a final consideration is needed: a very important side of the works discussed in this review regards the interdisciplinary character of the nanotechnological approach. A number of diversified expertises are required, ranging from basic science, molecular biology, physical chemistry, and biophysics, to economy and marketing. With respect to this, however, some socioeconomical aspects cannot be disregarded. A few words should be spent, namely, as far as their usage on field is concerned; that is, in spite of the relatively simple laboratory techniques required for their

[1] "Genius is one percent inspiration, ninety-nine percent perspiration." Oral statement reported by Harper's Monthly, September 1932.

fabrication and the relatively low cost, will it be possible, as things stand now, to apply these technologies also in disadvantaged situations? Will personnel be available to carry out these procedures in those unfavorable conditions? These questions deserve a prompt answer and when required a remedy.

References

Aiello C, Andreozzi P, La Mesa C et al (2010) Biological activity of SDS-CTAB cat-anionic vesicles in cultured cells and assessment of their cytotoxicity ending in apoptosis. Colloids Surf B Biointerfaces 78:149–154

Ajayan PM, Ebbesen TW, Ichihashi T et al (1993) Opening carbon nanotubes with oxygen and implications for filling. Nature 362:522–525

Alargova RG, Danov KD, Petkov JT et al (1997) Sphere-to-rod transition in the shape of anionic surfactant micelles determined by surface tension measurements. Langmuir 13:5544–5551

Amenta V, Aschberger K (2015) Carbon nanotubes: potential medical applications and safety concerns. Interdiscip Rev Nanomed Nanobiotechnol 7:371–386

Andersson JT, Schräder W (1999) A method for measuring 1-octanol/water partition coefficients. Anal Chem 71:3610–3614

Arroyo M, Belytschko T (2004) Finite crystal elasticity of carbon nanotubes based on the exponential Cauchy-Born rule. Phys Rev B 69:115415–115420

Bandyopadhyaya R, Nativ-Roth E, Regev O (2002) Stabilization of individual carbon nanotubes in aqueous solutions. Nano Lett 2:25–28

Barbetta A, Pucci C, Tardani F et al (2011) Size and charge modulation of surfactant-based vesicles. J Phys Chem B 115: 12751–12758

Barkat A, Barkat AK, Naveed A et al (2011) Basics of pharmaceutical emulsions: a review. Afr J Pharm Pharmacol 525:2715–2725

Bianco A (2013) Graphene: safe or toxic? The two faces of the medal. Angew Chem Int Ed Engl 52:4986–4997

Bianco A, Kostarelos K, Prato M (2005) Applications of carbon nanotubes in drug delivery. Curr Opin Chem Biol 9:674–679

Bianco A, Cheng H-M, Enoki T et al (2013) All in the graphene family—A recommended nomenclature for two-dimensional carbon materials. Carbon 65:1–6

Bolhassani A, Rafati S (2011) Non-viral delivery systems in gene therapy and vaccine developments. In: Xu-bo Y (ed) Non-viral gene therapy. Intech, Rijeka, Croatia, pp 27–50

Bombelli C, Bordi F, Ferro S, Giansanti L et al (2008) New cationic liposomes as vehicles of m-tetrahydroxyphenylchlorin in photodynamic therapy of infectious diseases. Mol Pharm 5:672–679

Bomboi F, Tardani F, Gazzoli D et al (2013) Lysozyme binds onto functionalized carbon nanotubes. Colloids Surf B Biointerfaces 108:16–22

Bonincontro A, Risuleo G (2015) Electrorotation: a spectroscopic imaging approach to study the alterations of the cytoplasmic membrane. Adv J Mol Imaging 5:1–15

Bonincontro A, La Mesa C, Proietti C, Risuleo G (2007) A biophysical investigation on the binding and controlled DNA release in a cetyltrimethylammonium bromide-sodium octyl sulfate cat-anionic vesicle system. Biomacromolecules 8:1824–1829

Bonincontro A, Falivene M, La Mesa C, Risuleo G (2008) Dynamics of DNA adsorption on and release from SDS-DDAB cat-anionic vesicles: a multitechnique study. Langmuir 24:1973–1978

Cammasab S, Suzuki K, Sone C et al (1997) Thermo-responsive polymer nanoparticles with a core-shell micelle structure as site-specific drug carriers. J Control Release 48:157–164

Coey AT, Sahu ID, Gunasekera TS et al (2011) Reconstitution of KCNE1 into lipid bilayers: comparing the structural, dynamic, and activity differences in micelle and vesicle environments. Biochemistry 50:10851–10859

Colomer A, Pinazo A, García MT et al (2012) pH-sensitive surfactants from lysine: assessment of their cytotoxicity and environmental behavior. Langmuir 28:5900–5912

Cosimati R, Milardi GL, Bombelli C, Bonincontro A et al (2013) Interactions of DMPC and DMPC/gemini liposomes with the cell membrane investigated by electrorotation. Biochim Biophys Acta 1828:352–356

De Gennes PG (1981) Polymer solutions near an interface. Adsorption and depletion layers. Macromolecules 14:1637–1642

Degouy A, Gomez-Berrada MP, Ferret PJ (2014) Baby care product development: artificial urine in vitro assay is useful for cosmetic product assessment. Toxicol In Vitro 28:3–7

Deshantri AK, Varela Moreira A, Ecker V et al (2018) Nanomedicines for the treatment of hematological malignancies. J Control Release 287:194–215

Fadel TR, Fahmy TM (2014) Immunotherapy applications of carbon nanotubes: from design to safe applications. Trends Biotechnol 32:198–209

Florence AT, Attwood D (1988) Physicochemical principles of pharmacy, II edn. MacMillan, London

Freire JM, Gaspar D, Veiga AS et al (2015) Shifting gear in antimicrobial and anticancer peptides biophysical studies: from vesicles to cells. J Pept Sci 21:178–185

Gollavelli G, Ling YC (2012) Multi-functional graphene as an in vitro and in vivo imaging probe. Biomaterials 33:2532–2545

Grumezescu AM (2018) Vesicle-based drug carriers: liposomes, polymersomes, and niosomes. In: Dan N (ed) Design and development of new nanocarriers. Elsevier, Oxford,. Chapt. 1, pp 1–55

Guo X, Dong S, Petersen EJ et al (2013) Biological uptake and depuration of radio-labeled graphene by Daphnia magna. Environ Sci Technol 47:12524–12531

Hamblin MR, Hasan T (2004) Photodynamic therapy: a new antimicrobial approach to infectious disease? Photochem Photobiol Sci 3:436–450

Han L, Xu J, Xu Q et al (2017) Extracellular vesicles in the tumor microenvironment: therapeutic resistance, clinical biomarkers, and targeting strategies. Med Res Rev 37(6):1318–1349

Hayashi S, Ikeda SJ (1980) Micelle size and shape of sodium dodecyl sulfate in concentrated sodium chloride solutions. J Phys Chem 84:744–751

Heidarli E, Dadashzadeh S, Haeri A (2017) State of the art of stimuli-responsive liposomes for cancer therapy. Iran J Pharm Res. 16:1273–1304

Heister E, Brunner EW et al (2013) Are carbon nanotubes a natural solution? Applications in biology and medicine. ACS Appl Mater Interfaces 5:1870–1891

Holt BD, Short PA, Rape AD et al (2010) Carbon nanotubes reorganize actin structures in cells and ex vivo. ACS Nano 4:4872–4878

Hone J, Whitney M, Piskoti C et al (1999) Thermal conductivity of single-walled carbon nanotubes. Phys Rev B 59:R2514–R2516

Hu X, Zhou Q (2013) Health and ecosystem risks of graphene. Chem Rev 113:3815–3835

Huang YY, Terentjev EM (2012) Dispersion of carbon nanotubes: mixing, sonication, stabilization, and composite properties. Polymers 4:275–295

Islam MF, Rojas E, Bergey DM et al (2003) High weight fraction surfactant solubilization of single-wall carbon nanotubes in water. Nano Lett 3:269–273

Israelachvili J, Mitchell DJ, Ninham BWJ (1976) Theory of self-assembly of hydrocarbon amphiphiles into micelles and bilayers. J Chem Soc Faraday Trans 72:1525–1568
Israelachvili JN, Mitchell DJ, Ninham BW (1977) Theory of self-assembly of lipid bilayers and vesicles. Biochim Biophys Acta 470:185–201
Joseph A, Itskovitz-Copper N, Samira S et al (2006) A new intranasal influenza vaccine based on a novel polycationic lipid—ceramide carbamoyl-spermine (CCS): I. Immunogenicity and efficacy studies in mice. Vaccine 24:3990–4006
Jung HT, Coldren B, Zasadzinski JA et al (2001) The origins of stability of spontaneous vesicles. Proc Natl Acad Sci USA 98:1353–1357
Karthik VV (2016) Excipients used in the formulation of tablets. Res Rev J Chem 5:143–154
Krishna VD, Wu K, Su D et al (2018) Nanotechnology: of concepts and potential application of sensing platforms in food safety. Food Microbiol 75:47–54
Kuo JH, Jan MS, Chang CH et al (2005) Cytotoxicity characterization of catanionic vesicles in RAW 264.7 murine macrophage-like cells. Colloids Surf B Biointerfaces 41:189–196
La Mesa C (2005) Polymer-surfactant and protein-surfactant interactions. J Colloid Interface Sci 286:148–157
Leo A, Hansch C, Elkins D (1971) Partition coefficients and their uses. Chem Rev 71:525–616
Letizia C, Andreozzi P, Scipioni A et al (2007) Protein binding onto surfactant-based synthetic vesicles. J Phys Chem B 111:898–908
Liang F, Chen B (2010) A review on biomedical applications of single-walled carbon nanotubes. Curr Med Chem 17:10–24
Liu P (2005) Modifications of carbon nanotubes with polymers. Eur Polym J 41:2693–2703
Lo CT, Jahn A, Locascio LE et al (2010) Controlled self-assembly of monodisperse niosomes by microfluidic hydrodynamic focusing. Langmuir 26:8559–8566
Loftsson T, Jarho P, Másson M et al (2005) Cyclodextrins in drug delivery. Expert Opin Drug Deliv 2:335–351
Lonez C, Vandenbranden M, Ruysschaert J-M (2008) Cationic liposomal lipids: from gene carriers to cell signaling. Progr Lipid Res 47:340–347
Louzao I, van der Hest JCM (2013) Permeability effects on the efficiency of antioxidant nanoreactors. Biomacromolecules 14:2364–2372
Lozano N, Pinazo A, La Mesa C et al (2009) Catanionic vesicles formed with arginine-based surfactants and 1,2-dipalmitoyl-sn-glycero-3-phosphate monosodium salt. Phys Chem B 113:6321–6327
Lozano N, Perez L, Pons R, Pinazo A (2011) Diacyl glycerol arginine-based surfactants: biological and physicochemical properties of catanionic formulations. Amino Acids 40:721–729
Ménard-Moyon C, Kostarelos K, Prato M et al (2010) Functionalized carbon nanotubes for probing and modulating molecular functions. Chem Biol 17:107–115
Mezei A, Pérez L, Pinazo A et al (2012) Self-assembly of pH-sensitive cationic lysine based surfactants. Langmuir 28:16761–16771
Mohajeri M, Behnam B, Sahebkar A (2018) Biomedical applications of carbon nanomaterials: drug and gene delivery potentials. J Cell Physiol. https://doi.org/10.1002/jcp.26899
Monk CB (1961) Electrolytic dissociation. Academic, New York
Moriguchi I, Shuichi Hirono S, Qian Liu Q et al (1992) Simple method of calculating octanol/water partition coefficient. Chem Pharm Bull 40:127–130
Moroi Y, Matuura RJ (1988) Thermodynamics of solubilization into surfactant micelles: effect of hydrophobicity of both solubilizate and surfactant molecules. J Colloid Interface Sci 125:456–462
Morris J, Olsson U, Wennerström H (1997) Homogeneous nucleation in a mono-disperse oil-in-water emulsion. Langmuir 13:606–608
Mouchet F, Landois P, Datsyuk V et al (2011) International amphibian micronucleus standardized procedure (ISO 21427-1) for in vivo evaluation of double-walled carbon nanotubes toxicity and genotoxicity in water. Environ Toxicol 26:136–145
Muzi L, Ménard-Moyon C, Russier J et al (2015a) Diameter-dependent release of a cisplatin pro-drug from small and large functionalized carbon nanotubes. Nanoscale 7:5383–5394
Muzi L, Ménard-Moyon C, Russier J et al (2015b) A comparative study on the anticancer efficacy of two types of functionalized multi-walled carbon nanotubes filled with a cisplatin prodrug. Nanoscale 7:5383–5394
Muzi L, Tardani F, La Mesa C et al (2016a) Interactions and effects of BSA-functionalized single-walled carbon nanotubes on different cell lines. Nanotechnology 15:155704
Muzi L, Mouchet F, Cadarsi S et al (2016b) Examining the impact of multi-layer graphene using cellular and amphibian models. 2D Mater 3:1–10
Muzzalupo R, Gente G, La Mesa C et al (2006) Micelles in mixtures of sodium dodecyl sulfate and a bolaform surfactant. Langmuir 22:6001–6009
Muzzalupo R, Nicoletta FP, Trombino S et al (2007) A new crown ether as vesicular carrier for 5-fluorouracil: synthesis, characterization and drug delivery evaluation. Colloids Surf B Biointerfaces 58:197–202
Muzzalupo R, Pérez L, Pinazo A et al (2017) Pharmaceutical versatility of cationic noises derived from amino acid-based surfactants: skin penetration behavior and controlled drug release. Int J Pharm 29:245–252
Nagarajan R (2002) Molecular packing parameter and surfactant self-assembly: the neglected role of the surfactant tail. Langmuir 18:31–38
Nakamura F, Isobe H (2003) Functionalized fullerenes in water. The First 10 Years of their chemistry, biology, and nanoscience. Acc Chem Res 36:807–815
Novoselov KS, Fal'ko VI, Colombo L et al (2012) A roadmap for graphene. Nature 490:192–200
Olusanya TOB, Haj Ahmad RR, Ibegbu DM et al (2018) Liposomal drug delivery systems and anticancer drugs. Molecules 23:907–911
Patri AK, Kukowska-Latallo JF, Baker JR Jr (2005) Targeted drug delivery with dendrimers: comparison of the release kinetics of covalently conjugated drug and non-covalent drug inclusion complex. Adv Drug Deliv Rev 57:2203–2214
Piccioni F, Borioni A, Delfini M, Del Giudice MR et al (2007) Metabolic alterations in cultured mouse fibroblasts induced by an inhibitor of the tyrosine kinase receptors fibroblast growth factor receptor 1. Anal Biochem 367(1):111–121
Pinazo A, Lozano N, Perez L et al (2011) Arginine diacyl-glycerolipid conjugates as multifunctional biocompatible surfactants. Compt Rend Chim 14:726–735
Popov AM, Lozovik YE, Fiorito S et al (2007) Biocompatibility and applications of carbon nanotubes in medical nanorobots. Int J Nanomed 2:361–372
Porter WL (1993) Paradoxical behavior of antioxidants in food and biological systems. Toxicol Ind Health 9:93–122
Prato M, Kostarelos K, Bianco A (2008) Functionalized carbon nanotubes in drug design and discovery. Acc Chem Res 41:60–68
Pretti C, Oliva M, Di Pietro R et al (2014) Ecotoxicity of pristine graphene to marine organisms. Ecotoxicol Environ Saf 101:138–145
Pucci C, Barbetta A, Sciscione F et al (2014a) Ion distribution around synthetic vesicles of the cat-anionic Type. J Phys Chem B 118:557–566
Pucci C, Pérez L, La Mesa C et al (2014b) Characterization and stability of catanionic vesicles formed by pseudo-tetraalkyl surfactant mixtures. Soft Matter 10:9657–9667
Pucci C, Scipioni A, La Mesa C (2014c) Albumin binding onto synthetic vesicles. Soft Matter 10:9669–9675

Qiu Y, Park K (2012) Environment-sensitive hydrogels for drug delivery. Adv Drug Deliv Rev 64:49–60

Risuleo G, La Mesa C (2016) Dispersibility of carbon nanotubes in biopolymer-based fluids and their potential biotechnological applications. Trends Nanotechnol Mater Sci 1:1–7

Russo L, Berardi V, Tardani F, Risuleo G (2013) Delivery of RNA and its intracellular translation into protein mediated by SDS-CTAB vesicles: potential use in nanobiotechnology. Biomed Res Int 734596:1–6

Safran SA, Pincus P, Andelman D (1990) Theory of spontaneous vesicle formation in surfactant mixtures. Science 248:354–356

Sallustio S, Galantini L, Gente G et al (2004) Hydrophobically modified pullulans: characterization and physicochemical properties. J Phys Chem B 108:18876–18883

Shtansky DV, Firestein KL, Golberg DV (2018) Fabrication and application of BN nanoparticles, nanosheets and their nanohybrids. Nanoscale 10:17477–17493

Simberg D, Weisman S, Talmon Y et al (2004) DOTAP (and other cationic lipids): chemistry, biophysics, and transfection. Crit Rev Ther Drug Carrier Syst 21:257–319

Spitalsky Z, Tasis D, Papagelis K et al (2010) Carbon nanotube–polymer composites: chemistry, processing, mechanical and electrical properties. Prog Polym Sci 35:357–401

Stefanutti E, Papacci F, Sennato S et al (2014) Cationic liposomes formulated with DMPC and a gemini surfactant traverse the cell membrane without causing a significant bio-damage. Biochim Biophys Acta 1838:2646–2655

Tanford C (1980) The hydrophobic effect; formation of micelles, vesicles and biological membranes. Wiley-Interscience, New York

Tardani F, Sennato S (2014) Phase behavior of DNA-stabilized carbon nanotubes dispersions: association with oppositely-charged additives. J Phys Chem 118:9268–9274

Tardani F, La Mesa C, Poulin P et al (2012) Phase behavior of DNA-based dispersions containing carbon nanotubes: effects of added polymers and ionic strength on excluded volume. J Phys Chem C 2012(116):9888–9894

Tardani F, Strobbia P, Scipioni A (2013) Encapsulating carbon nanotubes in aqueous ds-DNA anisotropic phases: shear orientation and rheological properties. RSC Adv 3:25917–25923

Tavano L, Mazzotta E, Muzzalupo R (2017) Nanovesicular formulations for cancer gene therapy. Curr Pharm Des 23:5327–5335

Tsang SC, Harris PJF, Green MLH (1993) Thinning and opening of carbon nanotubes by oxidation using carbon dioxide. Nature 362:520–522

Vaisman L, Wagner HD, Marom G (2006) The role of surfactants in dispersion of carbon nanotubes. Adv Colloid Interface Sci 128–130:37–46

Venkatesan J, Pallela R, Kim SK (2014) Applications of carbon nanomaterials in bone tissue engineering. J Biomed Nanotechnol 10:3105–3123

Vintiloiu A, Leroux J-CJ (2008) Organogels and their use in drug delivery—a review. J Control Release 125:179–192

Vlachy N, Touraud D, Heilmann J et al (2009) Determining the cytotoxicity of catanionic surfactant mixtures on HeLa cells. Colloids Surf B Biointerfaces 70:278–280

Wang H (2009) Dispersing carbon nanotubes using surfactants. Curr Opin Colloid Interface Sci 14:364–371

Weinstein JN, Leserman LD (1984) Liposomes as drug carriers in cancer chemotherapy. Pharmacol Ther 24:207–233

Wright PK (2008) Targeting vesicle trafficking: an important approach to cancer chemotherapy. Recent Pat Anticancer Drug Discov 3 (2):137–147

Zanni E, De Bellis G, Bracciale MP et al (2012) Graphite nanoplatelets and *Caenorhabditis elegans*: insights from an *in vivo* model. Nano Lett 12:2740–2744

Zhao J, Wang Z, White JC, Xing B (2014) Graphene in the aquatic environment: adsorption, dispersion, toxicity and transformation. Environ Sci Technol 48:9995–10009

Zoeller N, Blankschtein D (1998) Experimental determination of micelle shape and size in aqueous solutions of dodecyl ethoxy sulfates. Langmuir 14:7155–7165

Nanosupplements and Animal Health

Alessia Bertero, Leon J. Spicer, Teresa Coccini, and Francesca Caloni

Abstract

Nanosupplements have raised great attention in recent years, particularly in animal production industry and veterinary science where they are constantly being developed and expanded because of their peculiar characteristics largely due to their dimensions that go from approximately 1 up to 100 nm. Interestingly, in this nanodimensional size range, substances acquire new characteristics, different from those of the bulk material from which they originate, leading, in some cases, to an increased suitability of these materials for specific uses. In this scenario, the regular inclusion of nanosupplements in animal feed or water to benefit the quality and the quantity of the product obtained, as well as the production cycle, is likely possible in the near future. These compounds have many potential applications: as growth promotants, enhancers of the ruminal flora, anti-inflammatories, immune system stimulants, etc. Research in this field has made considerable progress, but there are still many gaps to be filled, given that, for example, the mechanisms of action of nanoparticles remain unknown as well as the health and environmental implications of a widespread use of this particular type of dietary supplement, especially but not only in terms of reproductive/developmental toxicity and environmental pollution.

Keywords

Nanosupplements · Veterinary nutraceuticals

A. Bertero · F. Caloni (✉)
Department of Veterinary Medicine, Università degli Studi di Milano, Milan, Italy
e-mail: alessia.bertero@unimi.it; francesca.caloni@unimi.it

L. J. Spicer
Department of Animal and Food Sciences, Oklahoma State University, Stillwater, OK, USA
e-mail: leon.spicer@okstate.edu

T. Coccini
Laboratory of Clinical and Experimental Toxicology, Toxicology Unit, ICS Maugeri SpA-BC, IRCCS, Pavia, Italy
e-mail: teresa.coccini@icsmaugeri.it

1 Introduction

1.1 Nanoparticles

Nanoparticles (NPs) are particles with a size range that goes from approximately 1 to 100 nm and derive from both natural and anthropogenic sources (Klaine et al. 2008). Fumes originating from volcanoes, the fine fraction of desert sand, and aquatic colloids (Ostiguy et al. 2006) are some examples of NPs naturally produced, while anthropogenic ones could be produced intentionally or unintentionally, like NPs generated from welding fumes (Nowack and Bucheli 2007; Ostiguy et al. 2006). The intentionally produced NPs are also designated as engineered NPs (Maurer-Jones et al. 2013), and the technology that deals with NPs production and use is referred to as nanotechnology. NPs properties (Ostiguy et al. 2006; Batley and McLaughlin 2010; Schodek et al. 2009) allow their use in a wide range of applications in different areas from chemical industry to medical applications (Farré et al. 2009; De Berardis et al. 2010).

1.1.1 Nanoparticle Classification

NPs can be classified in relation to their composition, dimensionality, morphology, uniformity, and state of agglomeration (Buzea et al. 2007). NPs can be synthesized from different materials and characterized in function of their composition: carbon NPs, metal NPs, metal oxide NPs, non-metal NPs, and others (Burda et al. 2005; Berube et al. 2011). Dimensionality and morphology play an important role in relation to NPs toxicity (Buzea et al. 2007), and the chemical and electromagnetic properties of NPs may influence their agglomeration state (Corbierre et al. 2005; Han et al. 2009;

R. C. Gupta et al. (eds.), *Nutraceuticals in Veterinary Medicine*, https://doi.org/10.1007/978-3-030-04624-8_54

Shen et al. 2009), losing some of their typical characteristics and behaving like larger particles (Buzea et al. 2007).

2 Nanosupplement Delivery

Nutraceutical NPs and NPs as delivery systems are areas of growing interest in veterinary medicine and the feed industry (Hill and Li 2017). The use of nano-nutritional supplement delivery systems adds several advantages compared to the traditional forms of nutritional supplements. Nanocarrier delivery systems as well as nano-molecules are more efficient in reaching the site of action; thus, the influence in the target sites can be optimized, also minimizing the undesirable side effects. Many nanostructures, including liposomes, polymers, etc., have been studied as nanodelivery systems. The two main groups are the nanodevices and nanomaterials. Nanoscale devices include biosensors and various detectors, the latter of which are characterized by surface modification, due to the nanodimension, or coatings, that enhance their biocompatibility.

Nanomaterials can also be divided into nanocrystalline and nanostructured materials. Nanocrystallines are directly obtained from bulk materials and are characterized by higher performance in terms of absorption and biological actions. They are employed, for example, in drug encapsulation for drug delivery. Nanostructured materials can be made from raw nanomaterials to obtain certain shapes with a specific purpose such as quantum nanotubes (Bhatia 2016). Another categorization identifies four groups: metals, polymers, natural compounds, and nanostructured materials (Hill and Li 2017).

Independently from the form in which the NPs are administered, there are the following mechanisms of action: NPs can act as the functional unit, as a delivery vehicle for materials that can be conjugated to their surface or encapsulated within (Hill and Li 2017).

For nutritional supplement delivery the most used NPs are:

2.1 Metallic NPs

This type of NP is characterized by a very small size (<100 nm), resulting in a higher surface area and so a high functional area. This form of nanodelivery is emerging as a good delivery carrier and biosensor. Moreover, some metallic NPs proved to have antimicrobial properties and to be a useful tool in mineral nutrition since the nanomineral particles show higher potential than their conventional counterpart and thus the quantity required is reduced (Sri Sindhura et al. 2014).

Another advantage of NPs administration is that a lot of nanominerals can efficiently and easily be synthesized with physical, chemical, and biological methods (Swain et al. 2015) which are not expensive. Moreover, they are stable and can be modified via conjugation with different functional groups (Jain et al. 2007). Despite their great potential and advantages, metallic NPs show a wide range of toxic effects that cannot be ignored. Various metals have been used to prepare NPs. Copper, silver, gold, and zinc are most commonly used (Ghosh et al. 2008; Mishra et al. 2010). Among them, one of the most used metallic NP in animal science is zinc oxide. It is a good alternative to conventional zinc as an animal feed supplement, because of its higher bioavailability, but studies have also pointed out many other effects of zinc oxide such as growth promotion, antibacterial actions, and immunomodulatory effects (Partha et al. 2016). Other nanometals used are selenium, chromium, and calcium, which showed positive effects on various apparatuses and physiological activities (Hosnedlova et al. 2018; Hill and Li 2017).

2.2 Natural NPs

Some NPs can be defined as naturally occurring NPs. For example, some casein isoforms are able to assemble around calcium, proteins, and other hydrophobic substances (Haham et al. 2012). These micelles can be used to incorporate hydrophobic nutrients (Semo et al. 2006), such as vitamin D, increasing in vivo vitamin bioavailability after the stomach's proteolytic cleavage of casein occurs, leading to the release of encapsulated vitamins.

Another molecule potentially useful in nutrient delivery is cruciferin, a promising nanocarrier for the delivery of nutraceutical compounds. Studies were performed using this NP made from the canola protein cruciferin, which could encapsulate both hydrophobic and hydrophilic substances, protecting them from an in vitro simulated acid environment like that of the stomach and releasing them in an in vitro simulated intestinal environment (Akbari and Wu 2016).

2.3 Polymeric Micelles

Polymeric micelles are nano-aggregates usually characterized by a spheroidal shape in which the hydrophobic molecules constitute the core of the structure, surrounded by a hydrophilic shell. The hydrophilic phase can be made of phospholipid micelles, pluronic micelles (which consist of the hydrophilic polyethylene oxide and the hydrophobic polypropylene oxide), polyamino acid micelles, and polyester micelles surrounded by biocompatible polymers (Koo et al. 2005).

This nanodelivery system is produced through self-assembly of amphiphilic polymers in an aqueous environment, so they are easy to prepare. Moreover, the micelles are characterized by a low toxicity and great versatility due to the available combinations. Polymeric micelles are mainly used for the delivery of therapeutic agents, above all anticancer drugs.

2.4 Polymeric NPs

Polymeric NPs are made combining a biodegradable polymer (that has to be biocompatible and nontoxic) with the active substance. The latter can be adsorbed on the surface of the polymer, encapsulated within the polymeric NP or even dissolved on it. The polymer can be natural or synthetic. Among natural polymers, the most widely used is chitosan (a linear polysaccharide) but also gelatin, albumin, and sodium alginate. These natural compounds were introduced to overcome some toxicological problems showed by synthetic polymers (Vyas and Khar 2002).

Polymeric NPs are a huge group that include a variety of NPs such as nanocapsules (which are characterized by a vesicular structure) and nanospheres (which are a matrix system and the matrix is uniformly dispersed). More specifically, nanocapsules are structures with an inner cavity in which the active substance is confined, and this core cavity is surrounded by single polymeric membrane, whereas in the nanosphere, the active substance is disseminated throughout the polymer matrix. The characteristics of this type of nanodelivery system can be varied and optimized in order to obtain the desired kinetics and dynamic properties. These delivery systems and in particular natural polymer-based NPs are mainly used in cancer therapy, delivery of vaccines, drug, and targeted antibiotics where they lead to a significant increase of the delivery efficiency.

2.5 Nanocapsules

Minute micelles (nanocapsules) can be used as carriers for essential oils, polyphenols, antioxidant, coenzyme Q10, vitamins, minerals, and micronutrients reaching a better bioavailability of these compounds (El Amin 2006). The encapsulation protects them from oxidation without any undesirable off-tastes.

2.6 Liposomes

Liposomes are lipid vesicles synthesized by hydration of dry phospholipids, which can consist of one or more lipid bilayers (five or even more) concentrically arranged around an aqueous core. This structure resembles those of cell membranes allowing a ready fusion with them. The high biocompatibility of liposomes and their capability to preserve, dissolve, and deliver water- or fat-soluble molecules make them a powerful and flexible tool especially as a drug delivery system. The type of lipid composition and the number of phospholipid bilayers can be modified changing their charge and their physical-chemical properties in order to optimize the suitability of this system for a specific use. Despite the flexibility of liposomes, they are mainly suitable for topical, intravenous, and intramuscular administration and have a limited oral bioavailability due to their susceptibility to gastrointestinal degradation. So far, liposomes have been studied for drug, vaccine, and gene delivery, and as imaging agents, with encouraging results.

However, their application in animal science is limited by their short shelf life, the complex production techniques, and their high costs (Underwood and van Eps 2012). Recently, in a study performed by Colom et al. (2015), the efficacy of bacteriophages encapsulated in liposomes in reducing *Salmonella* in poultry was investigated. In broilers experimentally infected with *Salmonella*, the mix containing liposome-encapsulated bacteriophages was administered orally every day for 6 days post-infection. This formulation prevents the sedimentation in the drinking water and simplifies the addition to the feed; moreover, the results showed a prolonged intestinal residence time for the encapsulated bacteriophages and a good release of the phage in the intestine with a strong and long-lasting protection against *Salmonella* colonization.

2.7 Nanoemulsions

Nanoemulsions are a multiphase colloidal dispersion of two immiscible liquids, typically oil and water, using a surface film made of surfactant. In this system, the active substance is usually carried by the dispersed phase. The manufacturing techniques are cheap and simple; moreover, nanoemulsions are usually characterized by a great stability. By varying the aqueous solution, the type of oils, and surfactants, a large variety of nanoemulsions with peculiar properties can be prepared, adapting the formulation to the molecule to be encapsulated, thus optimizing the delivery system (Vandamme and Anton 2010). El-Sherbiny et al. (2016) performed a study on a new type of supplement made of nanoemulsified oils (soybean, fish, and rapeseed oils). The oils were used either in a raw form or in a nanoemulsified form and were tested using rumen batch cultures. Results proved that the nanoemulsified oil mixture was more effective than the raw oils in preserving polyunsaturated fatty acids in the biohydrogenation environment of the culture, showing that the oil-in-water nanoemulsions may represent an efficient and manageable approach to the supplementation

of unsaturated lipids that could be easily administered with the drinking water.

2.7.1 Lipid NPs (LNPs)

These delivery systems are carrier systems characterized by a solid lipid matrix (that can be made of highly purified triglycerides, complex glyceride mixtures, and waxes) (Müller and Lucks 1996), usually prepared with an oil-in-water emulsion technique. This structure of physiological lipids, usually stabilized by surfactants, allows it to obtain a protective action toward core materials and to increase the oral bioaccessibility of lipophilic materials such as flavonoids (Ban et al. 2015). This is obtained thanks to a higher residence time because of their capability to adhere to the gastrointestinal wall and enter the intervillar spaces. Moreover, this system protects the core material from the physical and biochemical damage that can occur during its passage in the gastrointestinal tract (Li et al. 2015). When LNPs enter the small intestine, duodenal and bile juices, together with digestive enzymes, separate LNPs into monoglycerides and fatty acids which are taken up into micelles, containing the nutraceuticals, that contact the surface of the enterocytes where absorption occurs (McClements and Xiao 2012). Another innovative application of LNPs is linked to their ability to be delivered into the central nervous system, leading to an improved activity of the encapsulated drug because of the protecting action of the nanolipids and the increased absorption surface. In a study performed by Valdes et al. (2018), two antibiotics, minocycline and ciprofloxacin, were incorporated in LNPs of solid phase and intrathecally injected in rats. The encapsulated antibiotics showed to be at least 50% more efficient than the antibiotic alone proving the efficiency of this nanodelivery system (Valdes et al. 2018).

LNPs have also been investigated as an mRNA delivery system. Since the main concern related to the mRNA technology is its immunogenic properties and its poor stability, the use of LNPs seemed to be suitable to avoid these critical points. In a recent study performed by Sedic et al. (2018), modified mRNA, encoding for human erythropoietin, was formulated in LNPs and administered intravenously in rats and monkeys. Successively, a significant increase in red blood cell parameters was recorded, demonstrating the possibility to induce, via LNPs, therapeutic levels of proteins with modified mRNA (Sedic et al. 2018).

2.8 Nanocrystals

Nanocrystals are aggregates of many particles of the active substance in crystalline form which are coated with one or a combination of surfactants in order to achieve the stabilization of the structure. This type of nanosystem is used to overcome absorption and bioavailability issues related to the administration of certain drugs. In a study performed by Pensel et al. (2018), albendazole nanocrystals (ABZ-NCs) were used to treat mice experimentally infected with *E. multilocularis*, and this nanoform of the drug had greater chemoprophylactic and clinical efficacy than classic albendazole, probably due to an increased systemic availability of albendazole sulfoxide (Pensel et al. 2018).

3 Animal Production

3.1 Growth

Nowadays, nanosupplements are raising more and more attention and interest, particularly in animal production industry where they are constantly being developed and expanded (Thornton 2010; Hill and Li 2017; Pinar et al. 2018). The regular inclusion of nanosupplements in animal feed or water to benefit the quality (and the quantity) of the product obtained, as well as the production cycle, is likely possible in the near future. Indeed, many studies have been performed, in several species, to investigate the action of NPs in animal production and verify their potential role in enhancing animal growth and, as a consequence, meat production.

3.1.1 Pigs

Copper is a microelement routinely added to all swine diets in order to enhance growth. Indeed, copper fed at 125–250 ppm had a positive effect on growth, particularly of the weaned pigs (Holden et al. 2002). The mechanism of action by which copper exerts its effect lies in its antibacterial properties (Cromwell 2001) and in its ability to stimulate the immune capacity. Despite all these positive effects, copper supplementation had some disadvantages such as an increased environmental contamination because when fed at a high level, copper digestibility and absorption drop. As a consequence, there is a high copper excretion in feces increasing environmental pollution. In this context, supplementation with nano-copper has been studied in order to enhance digestibility and therefore increase absorption and decrease fecal excretion of this important growth promotant.

The effect of nano-copper supplementation on digestibility and bioavailability of this element in pigs and the effects of nano-copper on growth and serum parameters have been investigated. Findings showed that nano-copper (administered at 50 mg/kg) enhanced growth performance as well as the digestibility and bioavailability in the nano-copper-supplemented pigs compared with pigs fed with copper sulfate (at the same dose). Furthermore, copper excretion was reduced. Regarding the serum parameters, IgG, γ-globulin, total globulin protein levels, and superoxide

dismutase activity (the activity of this enzyme is reduced in pigs with copper deficiency) were increased by nano-copper supplementation (Eguia et al. 2009).

Silver is another element that has had been investigated to assess its potential as a NP additive in diets for weanling pigs. When weanling pigs were fed a diet supplemented with 0, 20, or 40 mg nano-silver/kg, in the second week after weaning, the daily growth was increased linearly with dose of silver NPs. Moreover, the ileal concentration of total bacteria was decreased with increasing concentration of nano-silver in the diet, also showing a linear relation (Fondevila et al. 2009).

Regarding the productive performance, feed intake was higher in animals fed a diet supplemented with 20 mg nano-silver/kg with no silver retention detected in kidneys or skeletal muscles although traces were found in the liver (1.354 and 2.445 μg silver/g dry liver for 20 and 40 mg nano-silver/kg diets, respectively). Taken together, these results indicate that the effect of the nano-silver on feed intake and growth performance could be related also, as for copper, to its antimicrobial properties.

Another NP studied as dietary supplement in pigs is chromium, and its effects on growth, carcass characteristics, pork quality, and tissue presence were investigated in finishing pigs. This element increases insulin activity thus being fundamental in the maintenance of normal glucose tolerance as well as protein synthesis and nucleic acid and lipid metabolism (Anderson 1987).

In swine, feeding chromium picolinate increases lean percentage in meat and lowers fat deposition in the longissimus muscle (Xi et al. 2001). In this scenario, chromium NP may bring advantages in the use of this element as a dietary supplement because of its unique properties.

The addition of nano-chromium (200 μg/kg) to the diet for 35 days (Wang and Xu 2004) decreased the feed/gain ratio by 3.56% compared with animals fed a control diet without integration of chromium, suggesting a positive effect on growth performance. Pigs that ate the supplemented diet had an increased carcass lean percentage (14.06%), longissimus muscle area (19.96%), longissimus muscle (16.33%), and semimembranosus (14.87%) weight, while the carcass fat percentage (25.53%) and the backfat thickness (18.22%) were decreased. Simultaneously, an increment of the chromium content was found in many organs (liver, kidney, and heart) and in the longissimus muscle. These findings suggest that the dietary supplementation of 200 μg/kg chromium NP could be convenient because of the proven effect on growth performance and carcass characteristics. Nevertheless, the increase in chromium concentrations in longissimus muscle and other organs has to be taken in account for potential human health implications (Wang and Xu 2004).

3.1.2 Ruminants

Selenium plays important roles as antioxidant and in reproductive, endocrine, and immune systems. Selenium deficiency has been reported to cause white muscle disease and many other pathological symptoms (Rock et al. 2001). Since diets for ruminants are almost exclusively based on plants and selenium concentrations in plants can be extremely variable (Juniper et al. 2009), a supplementation of this element may be necessary, especially in particular geographical areas characterized by selenium-deficient soils, such as China.

The bioavailability of selenium is related to its form. Sodium selenite is the usual selenium source used as a supplement in animal feeds, while the selenium-enriched yeast is the most common organic form used. Recently, nano-selenium has gained increased attention due to its high bioavailability and low toxicity (Zhang et al. 2008). Studies have evaluated effects of elemental nano-selenium on selenium retention, growth performance, selenium concentrations in blood and tissues, and serum oxidant status of goats. The effect of feeding inorganic (0.3 mg/kg selenium as sodium selenite), organic (selenium yeast), and elemental nano-selenium on growing male goats (from weaning to maturity) has been investigated (Shi et al. 2011b). In this study, body weight was increased in bucks supplemented with selenium, with higher values detected for the nano-selenium and selenium yeast supplementations. Serum GSH-Px, SOD, and CAT and selenium retention of whole blood, serum, and organs were higher in the animals fed with the nano-selenium supplementation compared to the animals that received selenium yeast and sodium selenite as supplementation, thus demonstrating that the dietary supplementation of elemental nano-selenium could be more efficient when compared to inorganic or organic selenium (Shi et al. 2011b).

3.1.3 Chickens

Chickens reared for meat are often kept in large groups characterized by high animal densities and raised rapidly to slaughter. In this difficult farming condition, antibiotics have been used as growth promoters/feed additives because of their ability to stimulate immunity thus favoring better growth performance, but this has fostered antibiotic resistance (Wegener 2003). In the context new growth promoter research, biodegradable polymer NPs have received increased attention.

Chitosan, a nontoxic and biodegradable carbohydrate polymer, has many actions such as immuno-enhancing and antibacterial activities. Copper-loaded chitosan NPs improve growth performance and have action on microbiota in cecal digesta (Han et al. 2010). Specifically, the effects of dietary copper-loaded chitosan NP supplementation on growth performance, hematological and immunological values, and

cecal microbiota in broiler chickens have been evaluated, demonstrating that copper-loaded chitosan NP administration could enhance growth performance, immune system, and protein synthesis, as well as have a beneficial action on the cecal microbiota, especially when administered at a dose of 100 mg/kg. At this concentration, the average daily gain, the contents of IgA, IgG, IgM, and complement C3-C4, and serum protein and albumin were increased, together with populations of *Lactobacillus* and *Bifidobacterium* in cecal digesta, and importantly, the population of coliforms was decreased. The concentration of urea nitrogen in serum was also decreased. These promising results seem to indicate the copper-loaded chitosan as a possible substitute for chlortetracycline in dietary supplementation (Wang et al. 2011a).

Another element studied in chickens in its nanoform is chromium. Its trivalent (more stable) and hexavalent states are biologically active. Trivalent chromium appears to be involved in the activity of glucose tolerance factor (GTF) that facilitates cellular binding and enhances the action of insulin. Dietary supplementation of trivalent chromium decreased the negative impact of high environmental temperatures on the broiler performance (Sahin et al. 2002b). Whether chickens receive adequate supplementation of chromium or not depends on their stress status, since it has been demonstrated that stress is responsible for the depletion of the body reserves of chromium (Ahmed et al. 2005). Moreover, it should be considered that most of the poultry diets are almost entirely composed of ingredients of plant origin, which are usually characterized by low contents of chromium (Giri et al. 1990). It is already known that chromium from organic complexes (i.e., chromium picolinate) and high-chromium yeast is better absorbed compared to chromium from inorganic compounds such as chromium chloride, but recently nano-chromium was found to exhibit greater absorption and bioavailability in comparison with chromium picolinate and chromium chloride (Zha et al. 2007, 2008).

A comparative investigation on the effects of a 6-week supplementation with three different forms of chromium (nano-chromium, chromium picolinate, and chromium chloride at a dosage of 500 μg/kg) has been performed on 1-day-old male broiler chickens. The supplementation of nano-chromium and chromium picolinate was found to significantly increase the average daily gain, feed efficiency, carcass yields, and lean muscles while decreasing the abdominal fat. In particular, nano-chromium significantly increased protein content of breast and thigh muscles and decreased fat and cholesterol levels in thigh muscles. Moreover, all the chromium supplementation (nano-chromium, chromium picolinate, and chromium chloride) resulted in significant increases of chromium content in the serum, liver, and kidney with nano-chromium inducing also significant increments of chromium in breast and thigh muscles. Based on these results, it can be stated that the supplementation of chromium, particularly if used in the nanoform, may help improve the growth performance and carcass traits of broiler chickens (Zha et al. 2009), but further research is needed.

3.2 Gastrointestinal Function

3.2.1 Ruminants

Selenium, in the form of selenocysteine, plays an important role in animal nutrition as component of more than 30 selenoenzymes and acts also as an antioxidant. Several kinds of selenium sources have been used as supplements in ruminants. Despite this, little is known about the influence of nano-selenium as a ruminant nutritional supplement on ruminant metabolism and performance.

Evaluation of the effects of nano-selenium (at a dosage of 0.3, 3, and 6 g of nano-selenium/kg dry matter, for a period of 20 days) on feed digestibility, rumen fermentation, and excretion of purine was performed on ruminally cannulated sheep (Shi et al. 2011a). Mean ruminal pH and ammonia nitrogen content were quadratically decreased with increasing nano-selenium supplementation, while total ruminal volatile fatty acid concentration was linearly and quadratically increased with increasing nano-selenium supplementation. Mean ruminal pH remained in the physiologic range for cellulolytic bacterial activity with all the dosages of selenium administration. Regarding the in situ ruminal digestion kinetics of *Leymus chinensis* and soybean meal, with increase of nano-selenium supplementation, the soluble fraction, fractional degradation rate, and the effective degradability of *Leymus chinensis* were linearly and quadratically increased. Conversely, slowly degradable fraction was quadratically decreased. Likewise, nutrient digestibility in the total tract and urinary excretion of purine derivatives were quadratically increased by increasing nano-selenium supplementation. All these digestive parameters taken together indicated that nano-selenium supplementation improves rumen fermentation and thus feed utilization. It also enhanced rumen microbial activity and digestive microorganism and enzyme activity. According to this data, the optimum dose in sheep appeared to be about 3.0 g/kg dietary dry matter (Shi et al. 2011a).

Another nano-element studied for its action on rumen fermentation pattern was zinc. The in vitro effect of five levels of nano-zinc oxide supplementation, namely, 0 (control), 50, 100, 200, and 400 mg/kg of dry matter on rumen fermentation, was investigated (Chen et al. 2011). These researchers found that supplementation of nano-zinc oxide did not affect the rumen pH, but it enhanced the growth of ruminal microorganisms and increased ruminal microbial protein synthesis and the energy utilization efficiency in the early phase (6–12 h) of incubation (Chen et al. 2011). Moreover, an increase in concentrations of volatile fatty acids,

microbial crude protein production, and the fermentation of organic matter was found. Conversely, the concentration of ammonia nitrogen and the ratio of acetate to propionate were significantly lowered with the supplementation of 100 and 200 mg/kg of zinc oxide NP at the 6th and 12th hours of in vitro culture. Thus, supplementation of nano-zinc oxide may enhance the growth of ruminal microorganisms and increase the ruminal microbial protein synthesis and the energy utilization efficiency in vitro, but additional studies are needed.

3.2.2 Chickens

Nanominerals have proven to act on the cecal microbiota of chickens, with promising effects. The administration of dietary copper-loaded chitosan to broilers at dosages of 0 (negative control group), 50, 100, and 150 mg/kg and, at positive control, 50 mg/kg of chlortetracycline was compared. Nano-copper addition increased the populations of *Lactobacillus* and *Bifidobacterium*, while it decreased the population of coliforms in cecal digesta, and these effects were stronger at a dose of 100 mg/kg. Compared with the positive control, the populations of *Lactobacillus* and *Bifidobacterium* were increased by 3.31 and 3.85%, respectively, and the population of coliforms was lowered by 5.71%. The mechanism underneath the growth of *Lactobacillus* and *Bifidobacterium* is not clear, but it has been supposed that nano-copper inhibits some of microbiota establishing a better environment for proliferation of *Lactobacillus* and *Bifidobacterium* (Wang et al. 2011a). The impact on broiler immune system in this study is summarized in Sect. 5.1.

Another NP potentially useful for its gastrointestinal action in poultry is silver. The effects of low dose of silver NPs were investigated as a potential replacement for antibiotic coccidiostats. Coccidiosis is a big issue in poultry farms, since it has been responsible for poor production performance for many years. Moreover, signs of resistance development have been evident recently, raising the need for the research of new ways to control this parasite.

The effect of low-dose supplementation (15 mg/l) of silver NPs in the drinking water of broilers was evaluated as a substitute for coccidiostats. The results showed that the coccidiostat treatment group had a significantly lower weight gain compared to unmedicated controls but had the lowest lesion score of cecal lesions. The silver NP group had, numerically, a slightly better score than the untreated group, and both the silver NP group and the coccidiostat group had 50% less oocysts in the fecal samples compared to the control group. These results demonstrate that the administration of silver NPs did not alter growth performance but may be suitable to control coccidia in broiler intestines in terms of oocyst excretion (Chauke and Siebrits 2012).

3.3 Reproduction

Some NPs have been proven to enhance fertility and protect spermatozoa. The mechanisms of action are not fully understood but appear to be related to the functional groups that they carry and that characterize them.

3.3.1 Ruminants

The effects of the oral administration of nano-selenium (ration supplemented with 0.3 mg/kg selenium for 12 weeks, namely, the duration needed to reach to sexual maturity) on reproductive parameters such as testicular ultrastructure, semen quality, and semen glutathione peroxidase activity of male goats have been evaluated (Shi et al. 2011b). Findings showed a significant increase in testicular selenium levels, semen glutathione peroxidase, and ATPase activity in animals supplemented with nano-selenium compared with the control animals (fed with an unsupplemented ration which was present 0.06 mg/kg of selenium). Conversely, the nanosupplementation did not influence the semen quality parameters (volume, density, motility, and pH) although sperm abnormality rate of the control group was significantly higher than those of the supplemented group. Transmission electron microscopy examination was also performed, and while the spermatozoa from goats in the control group displayed marked midpiece abnormality, the supplemented group did not, pointing out that the selenium deficiency affected the sperm tail midpiece. Moreover, in samples from the control group, the mitochondria pattern was less tight than normal with gaps between organelles that showed also an abnormal shape and extensive vacuolation (Shi et al. 2010).

Nano-antioxidants represent a field with great potential in animal reproduction, and they could be used to prevent and treat infertility problems (Partha et al. 2016). Among nano-antioxidants, nano-zinc could be a suitable tool since it has a high antioxidant feature. Studies show that a zinc deficiency can be responsible for poor sperm quality and that this element is involved in the mechanisms that control sperm motility via its action on the ATP system involved in contraction and through the regulation of phospholipid energy reserves. In goats, zinc has also an influence on the development of the sperm tail. In this context, poor zinc supply in diets can be a discriminating risk factor for poor-quality semen and male infertility (Croxford et al. 2011; Colagar et al. 2009). In females, zinc deficiency has been reported to be responsible for high incidence of abortions and stillbirths (Campbell and Mills 1979), thus nanosupplementation of antioxidants should be investigated in order to prevent reproductive problems before and during pregnancy and after calving such as retained placenta.

The effect of different concentrations (1, 10, and 100 μg/ml) of titanium oxide NPs (size <100 nm) on buffalo spermatozoa in terms of viability, membrane integrity, capacitation ability, and DNA integrity has been examined. The results showed a significant decrease in cell viability and membrane integrity after 6 h of exposure to the titanium oxide NPs and a dose-dependent increase in the DNA fragmentation. These findings, together with the demonstration of the presence of titanium oxide NPs inside the head and plasma membrane of spermatozoa, suggest that these NPs may impair sperm functionality (Pawar and Kaul 2014).

3.3.2 Chickens

In poultry, supplemental dietary chromium picolinate increases production at low temperatures, and higher doses of chromium picolinate can increase egg production (Yildiz et al. 2004). Moreover, organic chromium supplementation, particularly at 1200 ppb, increased performance criteria and egg quality (Sahin et al. 2002a). The effects of different levels of NPs of chromium picolinate (0 ppb, 500 ppb, and 3000 ppb of nano-chromium integration for 60 days) on performance, egg quality, and mineral retention of layer hens have also been evaluated. These researchers found that supplemental nano-chromium significantly improved egg quality and retention of chromium and zinc but decreased shell ratio in 60th day eggs (Sirirat et al. 2013). Thus, feeding chromium picolinate improved egg quality in all three studies, but whether nano-chromium picolinate can be more effective at lower doses will require further study.

3.3.3 Other Animals

Several studies have shown that selenium in nanosize has better biocompatibility and efficacy in comparison to inorganic and organic selenocompounds (Wang et al. 2007). For these reasons, together with the antioxidant properties and the lower toxicity exerted by this compound, nanosized selenium is considered to be a good choice for replacing other forms of selenium in nutritional supplements.

Selenium is an important regulator of the male reproductive system in rats (Behne et al. 1982), and deficiency of this element has been associated to decreased fertility, low sperm motility, and alteration of the midpiece of the spermatozoa. These alterations are responsive to selenium supplementation, but, interestingly, they do not respond to vitamin E supplementation, highlighting the importance of this element in male reproduction in rats (Wu et al. 1979). Nevertheless, providing additional selenium sources to animals that already have adequate selenium levels and maximal selenoprotein activity (i.e., the phospholipid-hydroperoxide glutathione peroxidase of the testis) could potentially expose them to a risk of toxicosis.

The effects of the oral administration of selenium NPs (0.08, 0.2, 0.4, 0.8, 2.0, 4.0, and 8.0 mg selenium/kg body weight, for 2 weeks) on male reproductive performance of rats have been assessed. The results showed that selenium NPs administered in a supranutritional dose had a positive effect on the reproductive function of the rats. The supplementation promoted concentration, vitality, and motility parameters of spermatozoa. The concentration of serum testosterone was significantly higher in the rats that received selenium (0.8 mg selenium/kg body weight) in comparison with those in the control group. Moreover, dosages of 4.0 and 8.0 mg selenium/kg body weight significantly improved phospholipid-hydroperoxide glutathione peroxidase in the testis. Damaging effects were only observed at 8.0 mg selenium/kg body weight (Liu et al. 2017).

3.4 Reproductive and Developmental Consequences of NP Formulations

Reproductive and developmental consequences of NP formulations have to be taken into consideration, especially in breeding animals. Considering the potential reproductive toxicity of NPs, it should be underlined that the biocompatibility (and thus the toxic potential) is influenced not only by the type of material and the dosages but also by the results of the interactions of a number of complex factors. Besides chemical composition, surface, shape, and polarity, other characteristics have to be considered, such as the suspending medium that strongly influences the surface potential (SP) of the nanoparticles (Taylor et al. 2012). The effect of the SP had been well illustrated in a work by Ding et al. (2010). The SP is often referred to as zeta potential, and it is known that NPs with higher SP showed a faster cell uptake and enhanced cell nuclear targeting. On the other hand, NPs with too high SP may exert cytotoxic effects destabilizing the cell membrane and thus leading to cell damage. Specifically, an increased loss of cell viability was described after exposure cell culture (human gastric carcinoma cell line BGC 823) to gold NPs with a zeta potential of 40 mV, compared with 20 and 30 mV (Ding et al. 2010). A further effect is added by the surface modifications. Massich et al. (2010) observed a cytotoxic effect of gold NPs in the presence of citrate (a stabilizing agent used during NP production), but no cytotoxic effect was detected when other substances were used during the production process (e.g., bovine serum albumin).

3.4.1 NP Transport into Reproductive Tissues

Mammalian gametes and embryos, despite being protected by biological barriers, are highly sensitive to the effect of NPs. In fact, several studies reported that most NPs have the ability to effectively cross biological barriers including those of the reproductive tract. NP-induced disruption of the blood-testis barrier has been reported and could lead to significant

reproductive consequences. Kim et al. (2006) synthesized a biocompatible silica-overcoated magnetic NP with 50 nm thickness to use as a model of nanomaterial. Then this compound was administered intraperitoneally for 4 weeks to mice at dosages of 100, 50, and 25 mg/kg. After this period NPs were detected in testes and uterus as well as in many other organs, demonstrating that NPs of 50 nm diameter, although in this study did not show apparent toxicity, can penetrate the blood-testis barrier (Kim et al. 2006). In addition, polymethyl (2-14C) methacrylate NPs (Araujo et al. 1999) and gold NPs (Balasubramanian et al. 2010) were found in rat testes after oral and intravenous administration, respectively. Also, the toxicity of silver NPs (size of 60 nm) was studied, using rats and feeding them for 28 days with 30, 300, and 1000 mg/kg of silver NPs. Then silver distribution was investigated demonstrating that after exposure to silver NPs a clear accumulation was detected in various organs including the brain and testis (Kim et al. 2008).

Regarding the female gonads, to date, no studies have been performed to investigate whether NPs have the ability to penetrate into ovaries, but considering the abovementioned data, this possibility has to be considered. Conversely, several studies have been performed, using rodent models, to verify the ability of NPs to cross the placental barrier, but the results reported are far from conclusive. NPs were found to cross the placenta barrier in some studies (Semmler-Behnke et al. 2007; Takahashi and Matsuoka 1981), but in other studies this ability was not seen (Challier et al. 1973; Sadauskas et al. 2007). For example, in mice, 10 nm silver NPs did not appear to across the placenta in pregnant mice (Austin et al. 2016). Other in vivo studies (Takeda et al. 2009; Yamashita et al. 2011) were performed on pregnant mice proving the ability of titanium dioxide NPs to cross the placental barrier after intravenous injection with embryotoxic effects.

Taken together, these results seem to indicate that the placental barrier could be crossed by some NPs but only in certain conditions that are still unknown, so further studies should be performed to clarify the mechanisms that regulate this phenomenon particularly in farm animals.

3.4.2 Embryonic and Developmental Effects of NPs

Developmental defects in association with metallic NPs have been described. The majority of studies on embryo toxicology have concentrated on using mice and zebrafish (*Danio rerio*) embryos as a model to assess NP toxicity in vertebrates since it is an established system for NP toxicity assay (Chakraborty et al. 2016). Heiden et al. (2007) using zebrafish embryos evaluated developmental toxicity of low-generation G3,5-G4 (full-generation dendrimers are amine-terminated, while half-generation dendrimers terminate with carboxylic acid groups) Starburst™ polyamidoamine dendrimers (a highly branched peptide dendrimer), and Arg-Gly-Asp-conjugated dendrimers (dendrimers bioconjugated with selective ligands—Arg-Gly-Asp—which are produced to target cells that express integrin receptors and show potential use as drug delivery system). Their results showed that G4 dendrimers, characterized by amino functional groups, were toxic and caused decreased development and growth in zebrafish embryos at sublethal concentrations. Conversely, G3,5 dendrimers, characterized by carboxylic acid terminal functional groups, did not show any toxic effect in zebrafish embryos.

The effects and distribution pattern in zebrafish embryos of silver NPs have also been assessed (Asharani et al. 2008), with results showing a concentration-dependent increase in mortality and hatching delay in the embryos exposed to silver NPs. Moreover, abnormal body axes, twisted notochord, decreased blood flow, pericardial edema, and cardiac arrhythmia were described in embryos treated with silver NPs together with an increased apoptosis, demonstrating a concentration-dependent toxicity for this nanocompound. Conversely, silver ions and stabilizing agents did not show any significant effects on the embryos. Silver NPs were found in several embryo organs such as the brain, heart, yolk, and blood (Asharani et al. 2008).

Another target of NP toxicity in offspring is the respiratory system, as observed by Fedulov et al. (2008). Fedulov and coworkers demonstrated that neonates of mice exposed intranasally to titanium dioxide NPs at day 14 of pregnancy had a higher risk to develop airway hyperresponsiveness and allergic inflammation, thus demonstrating that allergic susceptibility was increased in prenatally exposed mice (see Sect. 4).

Effects of Embryonic Exposure to NPs in Chickens

Research in the area of nanoreprotoxicology has also been conducted on chicken embryos. In this species, the injection of NPs made of gold (Zielinska et al. 2009), silver (Sikorska et al. 2010), silver-palladium alloy (Studnicka et al. 2009), and silver-copper alloy (Sawosz et al. 2009) caused no abnormal development; only silver-copper alloy NPs caused limited signs of inflammation in the liver. An investigation performed by Hao et al. (2017) on chickens evaluated liver dysfunction at a molecular level (gene and protein expression) in offspring, after maternal oral exposure to zinc oxide NPs. Two groups were analyzed: one group fed with basal diet added with ZnSO4 (considered as the control group since ZnSO4 is a common diet additive for chickens) and one group fed with basal diet added with zinc oxide NPs. The given concentration of zinc was 200 mg/kg of feed for all the groups. It was found that zinc oxide NPs may be toxic on offspring liver development, primarily influencing lipid

synthesis, growth, and causing lesions or apoptosis. Thus, this NP should be taken into account as a possible agent of reproductive, embryonic, and developmental toxicity.

Nervous System Effects of NPs

Studies exploring embryo toxicology of NPs in mammals are less numerous than those for regarding fish and chickens (Taylor et al. 2012). However, considering titanium dioxide NPs, there are indications that point toward a nervous system toxicity after conception. Takeda et al. (2009) studied the effects of titanium dioxide NPs administered subcutaneously to pregnant mice. They observed that the titanium dioxide NPs were transferred to the offspring causing alterations in the genital apparatus and the cranial nerve systems. The presence of NPs in the offspring was confirmed in the damaged organs (i.e., brain and testis) via electron microscopy. Moreover, Shimizu et al. (2009) showed in mice that maternal exposure to anatase titanium dioxide NPs causes changes in the expression of genes associated with brain development, cell death, response to oxidative stress, and mitochondria in the brain during the perinatal period. Genes associated with inflammation and neurotransmitters are also affected, but in a later stage. Furthermore, the effects of the prenatal exposure to titanium dioxide NP on the central nervous system in mice have been investigated by measuring the levels of dopamine and its metabolites in various brain regions (Takahashi et al. 2010). It has been found that dopamine concentrations, as well as concentrations of its metabolites, were increased in the prefrontal cortex and neostriatum of the fetuses in the titanium oxide-treated group, thus demonstrating that this NP can affect the development of the central dopaminergic system in exposed fetuses.

The developmental and neurobehavioral effects of surface-coated nanosized titanium dioxide on mice fetuses, following maternal exposure by inhalation during pregnancy, were also investigated. When adults, the prenatally exposed fetuses displayed neurobehavioral alterations, tending to avoid the central zone of the open field, and exposed female offspring showed enhanced prepulse inhibition. Conversely, cognitive function was not affected. The effects of developmental exposure to titanium oxide NPs, after the oral administration in gestation, on synaptic plasticity in rats' hippocampal dentate gyrus (DG) area were also studied (Gao et al. 2011). Synaptic plasticity in this brain area is thought to be associated with determined complex functions of the central nervous system, such as learning and memory processes. Gao et al. (2011) also found that the exposed offspring showed a significant alteration of the nervous parameters of the hippocampal region, suggesting that the developmental brain is susceptible to titanium oxide NPs and that this NP may affect synaptic plasticity in this area.

Another NP, namely, silica NPs, has also been found to exert neuronal effects. Metabolic effects of silica NP exposure during neuronal differentiation in vitro were investigated. SH-SY5Y cells were exposed to silica NPs before and during differentiation; NP exposure impaired mitochondrial function depending on the time of exposure (before and during neuronal differentiation). Exposure during differentiation affected mitochondrial activity, and this may have consequences on neuronal cell differentiation (Ducray et al. 2017a), because mitochondria play a fundamental role during this process (Almeida and Vieira 2017). Specifically, neuronal differentiation results in a decreased cellular respiration rate since differentiated cells are characterized by lower glycolytic activity compared to undifferentiated cells. Moreover, it has been discovered that silica NPs were mainly taken up by microglial cells in the hippocampal cultures, but NPs were also found in differentiated SH-SY5Y cells. The uptake was time- and concentration-dependent. NP aggregates and single particles were found in the cytoplasm and in the endoplasmic reticulum, but not in other organelles. Although NP exposure did not impair cell viability, the neuronal differentiation markers indicated again a reduction in neuronal differentiation induction after the exposure (Ducray et al. 2017b). Based on these studies, NP exposure embryonically clearly has neurological effects on offspring, but how embryonic exposure to various NPs affect behavior in farm mammals will require further study.

3.4.3 Effects of NPs on Sperm Function

As mentioned earlier (Sect. 3.3.1), some NPs, such as titanium oxide, are spermatotoxic exerting a cytotoxic effect and causing high amount of DNA fragmentation on buffalo spermatozoa, impairing sperm functionality (Pawar and Kaul 2014). Other studies on sperm toxicity were conducted on NPs obtained from titanium (mice; Miura et al. 2017; rats; Morgan et al. 2017), nickel (marine invertebrates; Gallo et al. 2016), gold (mice; Zakhidov et al. 2010), polyvinyl alcohol-coated iron oxide (bovine; Makhluf et al. 2006), europium dioxide and europium hydroxide in conjunction with polyvinylpyrrolidone and polyvinyl alcohol (bovine; Makhluf et al. 2008), and zinc oxide and titanium dioxide (human; Gopalan et al. 2009). Polyvinyl alcohol-coated iron oxide did not show any detrimental effect on spermatozoa, while zinc oxide NPs and titanium oxide NPs caused sperm damage. Nickel NPs induced oxidative stress causing lipid peroxidation and DNA fragmentation and altered mitochondrial membrane potential and sperm morphology. Moreover, sperm exposure to nickel NPs impaired the fertilizing ability and caused developmental anomalies in the offspring. Very small gold NPs (2.5 nm diameter) caused failure of chromatin decondensation process, probably due to an interaction with

molecules of double-helix DNA. However, none of these studies investigated the mechanisms of action of the various NP sperm toxicity, and thus further investigations are needed.

Toxicity studies using silver, molybdenum trioxide, and alumina NPs were performed on mouse spermatogonial stem cells (Braydich-Stolle et al. 2005, 2010). A concentration-dependent cytotoxicity for all the NPs was found, with AgNP being the most toxic. In its case, the toxicity is probably due to interactions of NPs with a cell proliferation associated, intracellular kinase. Using mice's whole testicular cell cultures, Asare et al. (2012) reported decreased cell metabolic activity and cell viability after 24–48 h exposure to 100 μg/ml of 20 nm silver NPs. Whether these effects would be realized in farm animals will require further study.

3.4.4 Effects of NPs on Oocyte Function

Nano-toxicity in oocyte maturation has only been investigated in a few studies. In a research by Hou et al. (2009), rat preantral follicles were cultured in the presence of titanium dioxide NPs, and the effect on follicle development and oocyte maturation was observed. The follicle survival rate, the formation rate of antral follicles, and the release rate of cumulus-oocyte complexes decreased with increased titanium dioxide NP concentration in the culture medium (12.5, 25, and 50 μg/ml), whereas the same medium added with dioxide microparticle did not show detrimental effects on follicles, thus demonstrating that the toxicity is related to the nanodimensions.

Another combined in vivo and in vitro study (Hsieh et al. 2009) evaluated the effect of CdSe core quantum dots on mouse oocyte maturation, fertilization, and the subsequent embryo development (pre- and post-implantation). The CdSe core quantum dots caused a significant reduction of the oocyte maturation, fertilization, and in vitro embryo development rates, but this did not occur when the CdSe core quantum dots were ZnS-coated; thus, this surface modification was able to prevent the cytotoxic effect. Moreover, when oocytes were in vitro matured with a concentration of 500 nM CdSe core quantum dots to the maturation medium, the post-implantation resorption increased, while placental and fetal weights decreased. In addition to the action due to direct NP administration to animals, NP accumulation in the environment caused by the latest increase use of these products in many fields, must be considered and regarded as a possible future issue, which could have a great impact on veterinary species.

4 Anti-inflammatory Activity

Disease prevention potential of dietary supplements (i.e., polyphenols) has raised great attention in recent years, and many studies both in vitro and in vivo have been performed in animals and humans. In this context, a wide range of biological activities has been reported for polyphenols, ranging from anticancer and antioxidative effects to activities related to the prevention of chronic diseases.

There are challenges related to the use of polyphenols and in particular curcumin, a water-insoluble molecule extracted from the turmeric plant *Curcuma longa*, as a dietary supplement. These issues are primarily related to the scarce solubility of this molecule that impairs its oral bioavailability. Thus, different size of high-speed and high-pressure homogenated oil (medium-chain triacylglycerols)-in-water nanoemulsions were used to encapsulate curcumin to improve its anti-inflammatory activity, and the effects were evaluated using a mouse ear inflammation model. The results proved that, compared to 1% curcumin in 10% Tween 20 water solution, 1% curcumin emulsions had a greater anti-inflammatory effect showing a stronger inhibition on ear edema. Moreover, this effect was enhanced when the emulsion droplet size was smaller than 100 nm (Wang et al. 2008).

The oral bioavailability in mice of three different chitosan polymers used to produce curcumin nanocapsules has also been investigated (Marin et al. 2017). The chitosan polymers, characterized by high muco-adhesive capacity and safety (Wang et al. 2011b), differed in terms of molecular weight and degree of deacetylation (Marin et al. 2017).

Curcumin-loaded nanocapsules were given orally at a dose of 25 mg/kg. Interestingly, the bioavailability of curcumin nano-formulated was ninefold higher compared with those of the non-formulated curcumin (curcumin suspensions). Moreover, nanocapsules could cross the blood-brain barrier (BBB). Regarding the distribution of a curcumin nano-formulation, it has been observed that curcumin-loaded poly(lactic-co-glycolic acid) NPs, administered intravenously to rats, are distributed to the liver, heart, spleen, lung, kidney, and brain. Moreover, nano-formulation proved to significantly increase the AUC (area under concentration—time curve), the half-life, and the mean residence time of curcumin in all these organs, but not in the heart (Tsai et al. 2011).

5 Immunity

Feeding nano-zinc to livestock and poultry has produced encouraging immunity responses, since it has been observed that nano-zinc enhances the immunity of the animals.

5.1 Chickens

Supplementation of nano-zinc (at a dose of 0.06 ppm) is effective in improving immunity status of broilers (Sahoo et al. 2014). The effects of different types of zinc

supplementations on broiler chickens (15 ppm inorganic zinc, 15 ppm and 7.5 ppm organic zinc, and 0.3, 0.06, and 0.03 ppm nano-zinc) have been investigated. The cellular immune response was assessed by cutaneous basophilic hypersensitivity (CBH) test in using PHA-P (phytohaemagglutinin phosphate), while the humoral immune response was tested by the antibody production in response to sheep red blood corpuscles (SRBC) injection.

Significantly higher titer levels were observed in the groups fed with 15 ppm organic zinc and 0.06 ppm nano-zinc when compared to the group fed just with basal diet without zinc and to the group which had the inorganic zinc supplementation, while the CBH response was found higher in the group supplemented with 15 ppm of organic zinc. The antibody titer response to SRBC was significantly greater in the groups that received the organic zinc integration at a dose of 15 ppm and in those that received the nano-zinc integration at a dose of 0.06 ppm. The percent weight of bursa was significantly higher in the group that received the nano-zinc (dose of 0.06 ppm). In summary, dosages of 0.06 ppm nano-zinc added to the basal diet improved the immunity and health status of the broiler birds.

Another nanomineral tested in poultry regarding the immunity effect is copper. In several in vitro studies (Małaczewska 2014), copper NPs have been shown to have an immunotropic effect. In this matter, an in vivo study was performed by Ognik et al. (2018). They employed 308 male chickens raised until the age of 42 days and divided into groups that received basal diet with no copper integration and nano-copper supplementation at a dose of 0.5 mg/kg body weight/day and 1.5 mg/kg body weight/day. The correction of the deficient basal diet of chickens with a quantity of nano-copper exceeding the National Research Council (NRC) recommendation (National Research Council 1994) by 54% led to an increased antioxidant potential of the body and inhibited lipid peroxidation, and when the supplementation of nano-copper rose the total copper intake to a level exceeding the recommendation by 96%, signs of deterioration of the antioxidant system started to emerge with a decreased level of glutathione plus glutathione disulfide and increased levels in SOD, CAT, ceruloplasmin activity, and lipid hydroperoxide content. Conversely, an increase of just 7% over recommendations was followed by a deterioration in red blood cell parameters and stimulation of the immune system with an increase of IL-6, IgA, IgM, and IgY. These results, taken all together, indicate a simultaneous enhancement of the antioxidant system, and immune defense of chickens is achievable by supplementing their diet with nano-copper up to 12 mg/chicken in a period of 6 weeks.

Another NP evaluated in poultry in attempt to enhance the immunity was copper-loaded NPs. Diet supplementations were administered to broilers at dosages of 0, 50, 100, and 150 mg/kg of copper-loaded chitosan NPs, and the results were compared with those obtained feeding the animals with diets supplemented with chlortetracycline as positive control. Immune organ indexes (the thymus, spleen, and bursa of Fabricius) in broilers given dietary supplementation of 100 mg/kg of copper-loaded chitosan NPs were increased by 31.27, 22.64, and 19.61%, respectively (all statistically significant), compared to the control group. Moreover, the nano-copper administration increased the concentrations of immunoglobulins, complements, and lysozyme in serum, and, compared with the control group, the supplementation of 100 mg/kg significantly increased the serum concentrations of IgA, IgG, IgM, C3, C4, and lysozyme. Notably, the concentrations of IgA and C3 in broilers that received 100 mg/kg of copper-loaded chitosan NPs were significantly higher than those in the broilers receiving 50 mg/kg of chlortetracycline in the diet (Wang et al. 2011a).

5.2 Other Animals

The effects of the nanocomposite magnesium and silicon oxide (MgO-SiO2) on the immunity and tissue alterations of adult male rats exposed to aflatoxin B1 (AFB1) have been evaluated (Essa et al. 2017). Exposure to aflatoxin-contaminated feeds can cause the suppression of the cell-mediated immune responses, affect the function and count of T-lymphocytes, suppress the phagocytic activity, and impair the complement activity in many species such as poultry, pigs, and rats (Marin et al. 2002; Ottinger and Kaattari 2000; Cusumano et al. 1990). In this regard, the efficacy of the nanocomposite magnesium oxide and silicon oxide (MgO-SiO2) in reducing the toxic effects of AFB1 on the immunity and histological alterations has been tested in rats (Essa et al. 2017). The animals were divided into four groups: group 1 (control group; not fed AFB1 or the nanocomposite), group 2 was fed contaminated feed (200 ppb AFB1), group 3 was fed 200 ppb AFB1 and 0.5 g/kg nanocomposite of MgO-SiO2, and group 4 was fed only 0.5 g/kg nanocomposite of MgO-SiO2. The experiment lasted 8 weeks.

Rats fed MgO-SiO2 nanocomposite supplement exhibited significantly reduced AFB1 contents in the serum and liver. Conversely, the feces content was significantly increased indicating that MgO-SiO2 NPs affected the absorption of AFB1 causing an increased fecal excretion. The latter observation suggests that the dietary supplementation of MgO-SiO2 nanocomposite may offer a good protection against the toxic effects of AFB1 by preventing its absorption because of its absorbent capacity and the higher surface reactivity compared to traditional (non-nanosized) commercial products. Moreover, the cellular immune response, the total and differential leukocytic counts, the phagocytic activities of neutrophils and macrophages, the lymphocyte

transformation, and the lysozyme activity were severely reduced in group 2 fed with diet added with 200 ppb AFB1 compared to group 3 fed with the AFB1-contaminated fed but supplemented with the nanocomposite MgO-SiO2. These further suggest that supplementation with NPs of MgO-SiO2 can indirectly mitigate AFB1 effects by preventing AFB1 to act on the immune system.

In summary, the nanocomposite of MgO-SiO2 at a dose of 0.5 g/kg feed could be considered an effective adsorbing agent for AFB1, because of its ability to prevent the development of severe toxic effects on immunity and organs in rats. It should also be considered that it is an inexpensive and accessible feed additive that can be a useful tool, especially in hot, humid tropical and subtropical climates, for the prevention of aflatoxicosis in animals.

6 Risks and Challenges

Because of the great and rapid increase in interest in NPs both in human activities and veterinary and human medicine, regulatory ecotoxicity testing of these types of chemicals is becoming an issue of fundamental importance for our contemporary society, and great efforts have been made at the OECD to ensure that OECD test guidelines (TGs) fit the nanomaterials. In this context, the project MARINA (http://www.marina-fp7.eu/) has been born to develop and validate the Risk Management Methods for Nanomaterials. Eight OECD TGs were adapted in order to cover the two main variants of NPs, such as the ion-releasing and inert NPs. Thus, guidelines were developed to test a minimum of one ion-releasing NP (Ag) and two inert NPs (TiO2) (Hund-Rinke et al. 2016). Because standardized toxicity testing can underestimate the ecotoxicity, other factors such as exposure and resource availability may be needed to be considered to correctly estimate the effect of a toxicant (Stevenson et al. 2017).

7 Concluding Remarks and Future Directions

Nanotechnology has great potential for widespread applications in veterinary science including drug and vaccine delivery and diagnostic imaging. However, a balance between safety and effectiveness and risk and benefit will be mandatory including considerations of NP accumulation in the environment. Future in vitro and in vivo research studies will play a key role in developing the use of this technology in veterinary medicine as well as directing its correct and conscious use, which implies, in a one health perspective, the protection of animals, humans, and the environment.

Adsorption, distribution, mechanism of action, pathway of toxicity, and possible adverse effects on a species-specific basis need to be clarified for nanomaterials and NPs. Information regarding effects of NPs on farm animal metabolism and reproductive function are scarce and more need to be conducted. Also, studies using microarray technology will aid in discovering novel genes involved in the process of cellular functions (Türkez et al. 2017). Moreover, the real goal for veterinary nanotechnology is to have a positive impact in veterinary profession and to be useful for practitioners, as well as positively influencing animal healthcare.

References

Ahmed N, Haldar S, Pakhira MC et al (2005) Growth performances, nutrient utilization and carcass traits in broiler chickens fed with a normal and a low energy diet supplemented with inorganic chromium (as chromium chloride hexahydrate) and a combination of inorganic chromium and ascorbic acid. J Agric Sci 143:427–439

Akbari A, Wu J (2016) Cruciferin nanoparticles: preparation, characterization and their potential application in delivery of bioactive compounds. Food Hydrocoll 54:107–118

Almeida AS, Vieira HL (2017) Role of cell metabolism and mitochondrial function during adult neurogenesis. Neurochem Res 42(6):1787–1794

Anderson RA (1987) Chromium. In: Mertz W (ed) Trace elements in human and animal nutrition, 5th edn. Academic, San Diego, CA

Araujo L, Sheppard M, Lobenberg R et al (1999) Uptake of PMMA nanoparticles from the gastrointestinal tract after oral administration to rats: modification of the body distribution after suspension in surfactant solutions and in oil vehicles. Int J Pharm 176:209–224

Asare N, Instanes C, Sandberg WJ et al (2012) Cytotoxic and genotoxic effects of silver nanoparticles in testicular cells. Toxicology 291 (1–3):65–72

Asharani PV, Lian Wu Y, Gong Z et al (2008) Toxicity of silver nanoparticles in zebrafish models. Nanotechnology 19:255102

Austin CA, Hinkley GK, Mishra AR et al (2016) Distribution and accumulation of 10 nm silver nanoparticles in maternal tissues and visceral yolk sac of pregnant mice, and a potential effect on embryo growth. Nanotoxicology 10(6):654–661

Balasubramanian SK, Jittiwat J, Manikandan J et al (2010) Biodistribution of gold nanoparticles and gene expression changes in the liver and spleen after intravenous administration in rats. Biomaterials 31:2034–2042

Ban C, Park SJ, Lim S et al (2015) Improving flavonoid bioaccessibility using an edible oil-based lipid nanoparticle for oral delivery. J Agric Food Chem 63:5266–5272

Batley GE, McLaughlin MJ (2010) Fate of manufactured nanomaterials in the Australian environment. Department of the Environment, Water, Heritage and the Arts, New South Wales

Behne D, Höfer T, von Berswordt-Wallrabe R et al (1982) Selenium in the testis of the rat: studies on its regulation and its importance for the organism. J Nutr 112(9):1682–1687

Berube D, Cummings C, Cacciatore M et al (2011) Characteristics and classification of nanoparticles: expert Delphi survey. Nanotoxicology 5:236–243

Bhatia S (2016) Nanoparticles types, classification, characterization, fabrication methods and drug delivery applications. In: Bhatia S (ed) Natural polymer drug delivery systems. Springer, Cham

Braydich-Stolle L, Hussain S, Schlager JJ et al (2005) In vitro cytotoxicity of nanoparticles in mammalian germline stem cells. Toxicol Sci 88:412–419

Braydich-Stolle LK, Lucas B, Schrand A et al (2010) Silver nanoparticles disrupt GDNF/Fyn kinase signaling in spermatogonial stem cells. Toxicol Sci 116:577–589

Burda C, Chen X, Narayanan R et al (2005) Chemistry and properties of nanocrystals of different shapes. Chem Rev 105:1025–1102

Buzea C, Pacheco II, Robbie K (2007) Nanomaterials and nanoparticles: sources and toxicity. Biointerphases 2:17–71

Campbell JK, Mills CF (1979) The toxicity of zinc to pregnant sheep. Environ Res 20:1–13

Chakraborty C, Sharma AR, Sharma G et al (2016) Zebrafish: a complete animal model to enumerate the nanoparticle toxicity. J Nanobiotechnol 14(1):65

Challier JC, Panigel M, Meyer E (1973) Uptake of colloidal 198Au by fetal liver in rat, after direct intrafetal administration. Int J Nucl Med Biol 1:103–106

Chauke N, Siebrits FK (2012) Evaluation of silver nanoparticles as a possible coccidiostat in broiler production. S Afr J Anim Sci 42(5): 493–497

Chen J, Wang W, Wang Z (2011) Effect of nano-zinc oxide supplementation on rumen fermentation in vitro. Chin J Anim Nutr 8:023

Colagar AH, Marzony ET, Chaichi MJ (2009) Zinc levels in seminal plasma are associated with sperm quality in fertile and infertile men. Nutr Res 29(2):82–88

Colom J, Cano-Sarabia M, Otero J et al (2015) Liposome-encapsulated bacteriophages for enhanced oral phage therapy against Salmonella spp. Appl Environ Microbiol 81(14):4841–4849

Corbierre MK, Cameron NS, Sutton M et al (2005) Gold nanoparticle/polymer nanocomposites: dispersion of nanoparticles as a function of capping agent molecular weight and grafting density. Langmuir 21:6063–6072

Cromwell GL (2001) Antimicrobial and promicrobial agent. In: Lewis AJ, Southern LL (eds) Swine nutrition, 2nd edn. CRC, Boca Raton, FL. ISBN: 9780849306969

Croxford TP, McCormick NH, Kelleher SL (2011) Moderate zinc deficiency reduces testicular Zip6 and Zip10 abundance and impairs spermatogenesis in mice. J Nutr 141(3):359–365

Cusumano V, Costa GB, Seminara S (1990) Effect of aflatoxins on rat peritoneal macrophages. Appl Environ Microb 56:3482–3484

De Berardis B, Civitelli G, Condello M et al (2010) Exposure to ZnO nanoparticles induces oxidative stress and cytotoxicity in human colon carcinoma cells. Toxicol Appl Pharmacol 246:116–127

Ding Y, Bian XC, Yao W et al (2010) Surface potential-regulated transmembrane and cytotoxicity of chitosan/gold hybrid nanospheres. ACS Appl Mater Interfaces 2:1456–1465

Ducray AD, Felser A, Zielinski J et al (2017a) Effects of silica nanoparticle exposure on mitochondrial function during neuronal differentiation. J Nanobiotechnol 15(1):49

Ducray AD, Stojiljkovic A, Möller A et al (2017b) Uptake of silica nanoparticles in the brain and effects on neuronal differentiation using different in vitro models. Nanomedicine 13(3):1195–1204

Eguia AG, Fu CM, Lu FY et al (2009) Effects of nanocopper on copper availability and nutrients digestibility, growth performance and serum traits of piglets. Livest Sci 126:122–129

El Amin A (2006) Nanotech database compiles consumer items on the market. Available from: http://www.foodproductiondailyusa.com/news/ng.asp?id=66516

El-Sherbiny M, Cieslak A, Pers-Kamczyc E et al (2016) Short communication: a nanoemulsified form of oil blends positively affects the fatty acid proportion in ruminal batch cultures. J Dairy Sci 99(1): 399–407

Essa SS, El-Saied EM, El-Tawil OS et al (2017) Modulating effect of MgO-SiO(2) nanoparticles on immunological and histopathological alterations induced by aflatoxicosis in rats. Toxicon 140:94–104

Farré M, Gajda-Schrantz K, Kantiani L et al (2009) Ecotoxicity and analysis of nanomaterials in the aquatic environment. Anal Bioanal Chem 393:81–95

Fedulov AV, Leme A, Yang Z et al (2008) Pulmonary exposure to particles during pregnancy causes increased neonatal asthma susceptibility. Am J Respir Cell Mol Biol 38:57–67

Fondevila M, Herrer R, Casallas MC et al (2009) Silver nanoparticles as a potential antimicrobial additive for weaned pigs. Anim Feed Sci Technol 150:259–269

Gallo A, Boni R, Buttino I et al (2016) Spermiotoxicity of nickel nanoparticles in the marine invertebrate Ciona intestinalis (ascidians). Nanotoxicology 10(8):1096–1104

Gao X, Yin S, Tang M et al (2011) Effects of developmental exposure to TiO2 nano-particles on synaptic plasticity in hippocampal dentate gyrus area: an in vivo study in anesthetized rats. Biol Trace Elem Res 143:1616–1628

Ghosh P, Han G, De M et al (2008) Gold nanoparticles in delivery applications. Adv Drug Deliver Rev 60:1307–1315

Giri J, Usha K, Sunita T (1990) Evaluation of the selenium and chromium content of plants foods. Plant Foods Hum Nutr 40:49–59

Gopalan R, Osman I, de Matas M et al (2009) The effect of zinc oxide and titanium dioxide nanoparticles in the comet assay with UVA photoactivation of human sperm and lymphocytes. Environ Mol Mutagen 50:541–541

Haham M, Ish-Shalom S, Nodelman M et al (2012) Stability and bioavailability of vitamin D nanoencapsulated in casein micelles. Food Funct 3(7):737–744

Han XY, Wang X, Li S (2009) A simple route to prepare stable hydroxyapatite nanoparticles suspension. J Nanopart Res 11: 1235–1240

Han XY, Du WL, Fan CL et al (2010) Changes in composition a metabolism of caecal microbiota in rats fed diets supplemented with copper-loaded chitosan nanoparticles. J Anim Physiol Anim Nutr Berl 94:e138–e144

Hao Y, Liu J, Feng Y et al (2017) Molecular evidence of offspring liver dysfunction after maternal exposure to zinc oxide nanoparticles. Toxicol Appl Pharmacol 329:318–325

Heiden TC, Dengler E, Kao WJ et al (2007) Developmental toxicity of low generation PAMAM dendrimers in zebrafish. Toxicol Appl Pharmacol 225:70–79

Hill EK, Li J (2017) Current and future prospects for nanotechnology in animal production. J Anim Sci Biotechnol 8:26. https://doi.org/10.1186/s40104-017-0157-5

Holden P, Carr J, Honeyman M et al (2002) Minimizing the use of antibiotics in pig production. Iowa State University Extension, Iowa

Hosnedlova B, Kepinska M, Skalickova S et al (2018) Nano-selenium and its nanomedicine applications: a critical review. Int J Nanomed 13:2107–2128

Hou J, Wan XY, Wang F et al (2009) Effects of titanium dioxide nanoparticles on development and maturation of rat preantral follicle in vitro. Acad J Second Mil Med Univ 30(8):869–873

Hsieh MS, Shiao NH, Chan WH (2009) Cytotoxic effects of CdSe quantum dots on maturation of mouse oocytes, fertilization, and fetal development. Int J Mol Sci 10:2122–2135

Hund-Rinke K, Baun A, Cupi D et al (2016) Regulatory ecotoxicity testing of nanomaterials—proposed modifications of OECD test guidelines based on laboratory experience with silver and titanium dioxide nanoparticles. Nanotoxicology 10(10):1442–1447

Jain PK, El-Sayad IH, El-Sayed MH (2007) Au nanoparticles target cancer. Nano Today 2:18–29

Juniper DT, Phipps RH, Ramos-Morales E et al (2009) Effects of dietary supplementation with selenium enriched yeast or sodium selenite on selenium tissue distribution and meat quality in lambs. Anim Feed Sci Technol 149:228–239

Kim JS, Yoon TJ, Yu KN et al (2006) Toxicity and tissue distribution of magnetic nanoparticles in mice. Toxicol Sci 89:338–347

Kim YS, Kim JS, Cho HS et al (2008) Twenty-eight-day oral toxicity, genotoxicity, and gender-related tissue distribution of silver nanoparticles in Sprague-Dawley rats. Inhal Toxicol 20(6):575–583
Klaine SJ, Alvarez PJJ, Batley GE et al (2008) Nanomaterials in the environment: behavior, fate, bioavailability, and effects. Environ Toxicol Chem 27:1825–1851
Koo OM, Rubinstein I, Onyuksel H (2005) Role of nanotechnology in targeted drug delivery and imaging: a concise review. Nanomedicine 1(3):193–212
Li Z, Jiang H, Xu C, Gu L (2015) A review: using nanoparticles to enhance absorption and bioavailability of phenolic phytochemicals. Food Hydrocoll 43:153–164
Liu L, He Y, Xiao Z et al (2017) Effects of selenium nanoparticles on reproductive performance of male Sprague-Dawley rats at supranutritional and nonlethal levels. Biol Trace Elem Res 180(1):81–89
Makhluf SBD, Qasem R, Rubinstein S et al (2006) Loading magnetic nanoparticles into sperm cells does not affect their functionality. Langmuir 22:9480–9482
Makhluf SBD, Arnon R, Patra C et al (2008) Labeling of sperm cells via the spontaneous penetration of Eu3+ ions as nanoparticles complexed with PVA or PVP. J Phys Chem C 112(33):12801–12807
Małaczewska J (2014) Impact of noble metal nanoparticles on immune system of animals. Med Weter 70:204–208
Marin DE, Taranu I, Bunaciu PR et al (2002) Changes in performance, blood parameters, humoral and cellular immune response in weanling piglets exposed to low doses of aflatoxin. J Anim Sci 80:1250–1257
Marin E, Briceño MI, Torres A et al (2017) New curcumin-loaded chitosan nanocapsules: in vivo evaluation. Planta Med 83 (10):877–883
Massich MD, Giljohann DA, Schmucker AL et al (2010) Cellular response of polyvalent oligonucleotide-gold nanoparticle conjugates. ACS Nano 4:5641–5646
Maurer-Jones MA, Gunsolus IL, Murphy CJ et al (2013) Toxicity of engineered nanoparticles in the environment. Anal Chem 85: 3036–3049
McClements DJ, Xiao H (2012) Potential biological fate of ingested nanoemulsions: influence of particle characteristics. Food Funct 3:202–220
Mishra B, Patel BB, Tiwari S (2010) Colloidal nanocarriers: a review on formulation technology, types and applications toward targeted drug delivery. Nanomedicine 6(1):9–24
Miura N, Ohtani K, Hasegawa T et al (2017) High sensitivity of testicular function to titanium nanoparticles. J Toxicol Sci 42(3): 359–366
Morgan AM, Ibrahim MA, Noshy PA (2017) Reproductive toxicity provoked by titanium dioxide nanoparticles and the ameliorative role of Tiron in adult male rats. Biochem Biophys Res Commun 486(2):595–600
Müller RH, Lucks JS (1996) Arzneistofftraüger aus festen Lipidteilchen, Feste Lipidnanosphaüren (SLN). European Patent No. EP 0605497
National Research Council (1994) Nutrient requirements of poultry: ninth revised edition. The National Academies Press, Washington, DC. https://doi.org/10.17226/2114
Nowack B, Bucheli TD (2007) Occurrence, behavior and effects of nanoparticles in the environment. Environ Pollut 150:5–22
Ognik K, Sembratowicz I, Cholewińska E et al (2018) The effect of administration of copper nanoparticles to chickens in their drinking water on the immune and antioxidant status of the blood. Anim Sci J 89(3):579–588
Ostiguy C, Lapointe G, Ménard L et al (2006) Nanoparticles: actual knowledge about occupational health and safety risks and prevention measures. IRSST, Montréal, QC. ISBN: 2-89631-062-2
Ottinger CA, Kaattari SL (2000) Long-term immune dysfunction in rainbow trout (Oncorhynchus mykiss) exposed as embryos to aflatoxin B1. Fish Shellfish Immunopathol 10(1):101–106
Partha SS, Somu BNR, Somu BNR, Rajendran D et al (2016) Nano zinc, an alternative to conventional zinc as animal feed supplement: a review. Anim Nutr 2(3):134–141
Pawar K, Kaul G (2014) Toxicity of titanium oxide nanoparticles causes functionality and DNA damage in buffalo (Bubalus bubalis) sperm in vitro. Toxicol Ind Health 30(6):520–533
Pensel P, Paredes A, Albani CM et al (2018) Albendazole nanocrystals in experimental alveolar echinococcosis: enhanced chemoprophylactic and clinical efficacy in infected mice. Vet Parasitol 251:78–84
Pinar TS, Ismail S, Burcu GB et al (2018) Nanotechnology and nanopropolis in animal production and health: an overview. Ital J Anim Sci. https://doi.org/10.1080/1828051X.2018.1448726
Rock MJ, Kincaid RL, Carstens GE (2001) Effects of prenatal source and level of dietary selenium on passive immunity and thermometabolism of newborn lambs. Small Rumin Res 40(2):129–138
Sadauskas E, Wallin H, Stoltenberg M et al (2007) Kupffer cells are central in the removal of nanoparticles from the organism. Part Fibre Toxicol 4:10
Sahin K, Sahin N, Kucuk O (2002a) Effects of dietary chromium picolinate supplementation on serum and tissue mineral contents of laying Japanese quails. J Trace Elem Exp Med 15:163–169
Sahin K, Sahin N, Onderci M et al (2002b) Optimal dietary concentration of chromium for alleviating the effect of heat stress on growth, carcass qualities and some serum metabolites of broiler chickens. Biol Trace Elem Res 89:53–64
Sahoo A, Swain RK, Mishra SK (2014) Effect of inorganic, organic and nano zinc supplemented diets on bioavailability and immunity status of broilers. Int J Adv Res 2(11):828–837
Sawosz E, Grodzik M, Zielinska M et al (2009) Nanoparticles of silver do not affect growth, development and DNA oxidative damage in chicken embryos. Arch Geflugelkd 73:208–213
Schodek DL, Ferreira P, Ashby MF (2009) Nanomaterials: classes and fundamentals. In: Nanomaterials, nanotechnologies and design: an introduction for engineers and architects. Butterworth-Heinemann, Burlington. ISBN: 9780750681490
Sedic M, Senn JJ, Lynn A et al (2018) Safety evaluation of lipid nanoparticle-formulated modified mRNA in the Sprague-Dawley rat and cynomolgus monkey. Vet Pathol 55(2):341–354
Semmler-Behnke M, Fertsch S, Schmid G, et al (2007) Uptake of 1.4 nm versus 18 nm gold nanoparticles by secondary target organs is size dependent in control and pregnant rats after intratracheal or intravenous application. EuroNanoForum 102–104
Semo E, Kesselman E, Danino D et al (2006) Casein micelle as a natural nano-capsular vehicle for nutraceuticals. Food Hydrocoll 21:936–942
Shen XS, Wang GZ, Hong X et al (2009) Nanospheres of silver nanoparticles: agglomeration, surface morphology control and application as SERS substrates. Phys Chem Chem Phys 11:7450–7454
Shi LG, Yang RJ, Yue WB et al (2010) Effect of elemental nanoselenium on semen quality, glutathione peroxidase activity, and testis ultrastructure in male Boer goats. Anim Reprod Sci 118: 248–254
Shi L, Xun W, Yue W et al (2011a) Effect of elemental nano-selenium on feed digestibility, rumen fermentation and purine derivatives in sheep. Anim Feed Sci Technol 163:136–142
Shi LG, Xuna W, Yue W et al (2011b) Effect of sodium selenite, Se-yeast and nano-elemental selenium on growth performance, Se concentration and antioxidant status in growing male goats. Small Ruminant Res 96:49–52

Shimizu M, Tainaka H, Oba T et al (2009) Maternal exposure to nanoparticulate titanium dioxide during the prenatal period alters gene expression related to brain development in the mouse. Part Fibre Toxicol 6:20

Sikorska J, Szmidt M, Sawosz E et al (2010) Can silver nanoparticles affect the mineral content, structure and mechanical properties of chicken embryo bones? J Anim Feed Sci 2:286–291

Sirirat N, Lu JJ, Hung ATY et al (2013) Effect of different levels of nanoparticles chromium picolinate supplementation on performance, egg quality, mineral retention, and tissues minerals accumulation in layer chickens. J Agric Sci 5(2):150–159

Sri Sindhura K, Prasad TNVKV, Panner Selvam P et al (2014) Synthesis, characterization and evaluation of effect of phytogenic zinc nanoparticles on soil exo-enzymes. Appl Nanosci 4:819

Stevenson M, Katherine E, Krattenmaker EJ et al (2017) Standardized toxicity testing may underestimate ecotoxicity: environmentally relevant food rations increase the toxicity of silver nanoparticles to daphnia. Environ Toxicol Chem 36(11):3008–3018

Studnicka A, Sawosz E, Grodzik M et al (2009) Influence of nanoparticles of silver/palladium alloy on chicken embryos' development. In: Kaleta T (ed) Annals of Warsaw University of Life Sciences – SGGW, Animal Science. Warsaw University of Life Science Press, Warsaw, pp 237–242

Swain PS, Rajendran D, Rao SBN, Dominic G (2015) Preparation and effects of nano mineral particle feeding in livestock: a review. Vet World 8(7):888–891

Takahashi S, Matsuoka O (1981) Cross placental-transfer of Au-198-colloid in near term rats. J Radiat Res 22:242–249

Takahashi Y, Mizuo K, Shinkai Y et al (2010) Prenatal exposure to titanium dioxide nanoparticles increases dopamine levels in the prefrontal cortex and neostriatum of mice. J Toxicol Sci 35:749–756

Takeda K, Suzuki KI, Ishihara A et al (2009) Nanoparticles transferred from pregnant mice to their offspring can damage the genital and cranial nerve systems. J Health Sci 55:95–102

Taylor U, Barchanski A, Kues W et al (2012) Impact of metal nanoparticles on germ cell viability and functionality. Reprod Domest Anim 47(4):359–368

Thornton PK (2010) Livestock production: recent trends, future prospects. Philos Trans R Soc Lond B Biol Sci 365(1554):2853–2867

Tsai YM, Chien CF, Lin LC et al (2011) Curcumin and its nanoformulation: the kinetics of tissue distribution and blood-brain barrier penetration. Int J Pharm 416(1):331–338

Türkez H, Arslan ME, Sönmez E et al (2017) Toxicogenomic responses of human alveolar epithelial cells to tungsten boride nanoparticles. Chem Biol Interact 273:257–265

Underwood C, van Eps AW (2012) Nanomedicine and veterinary science: the reality and the practicality. Vet J 193(1):12–23

Valdes C, Bustos G, Martinez JL et al (2018) Antinociceptive antibiotics-loaded into solid lipid nanoparticles of prolonged release: measuring pharmacological efficiency and time span on chronic monoarthritis rats. PLoS One 13(4):e0187473

Vandamme TF, Anton N (2010) Low-energy nanoemulsification to design veterinary controlled drug delivery devices. Int J Nanomed 21(5):867–873

Vyas SP, Khar RK (2002) Targeted and controlled drug delivery: novel carrier systems, 1st edn. CBS, New Delhi. ISBN: 978-8123907994

Wang MQ, Xu ZR (2004) Effect of chromium nanoparticle on growth performance, carcass characteristics, pork quality and tissue chromium in finishing pigs. Asian Aust J Anim Sci 17(8): 1118–1122

Wang H, Zhang J, Yu H (2007) Elemental selenium at nano size possesses lower toxicity without compromising the fundamental effect on selenoenzymes: comparison with selenomethionine in mice. Free Radic Biol Med 42:1524–1533

Wang X, Jiang Y, Wang YW et al (2008) Enhancing anti-inflammation activity of curcumin through O/W nanoemulsions. Food Chem 108(2):419–424

Wang C, Wang MQ, Ye SS et al (2011a) Effects of copper-loaded chitosan nanoparticles on growth and immunity in broilers. Poult Sci 90(10):2223–2228

Wang JJ, Zeng ZW, Xiao RZ, Xie T, Zhou GL, Zhan XR et al (2011b) Recent advances of chitosan nanoparticles as drug carriers. Int J Nanomed 6:765–774

Wegener HC (2003) Antibiotics in animal feed and their role in resistance development. Curr Opin Microbiol 6:439–445

Wu ASH, Oldfield JE, Shull LR et al (1979) Specific effect of selenium deficiency on rat sperm. Biol Reprod 20:793–798

Xi G, Xu ZR, Wu SH et al (2001) Effect of chromium picolinate on growth performance, carcass, characteristics, serum metabolites and metabolism of lipid in pigs. Asian-Aust J Anim Sci 14(2):258–262. https://doi.org/10.5713/ajas.2001.258

Yamashita K, Yoshioka Y, Higashisaka K, Mimura K, Morishita Y, Nozaki M et al (2011) Silica and titanium dioxide nanoparticles cause pregnancy complications in mice. Nat Nanotechnol 6:321–328

Yildiz A, Parlat S, Yazgan O (2004) The effects of organic chromium supplementation on production traits and some serum parameters of laying hens. Rev Med Vet 12:642–646

Zakhidov ST, Marshak TL, Malolina EA, Kulibin AY, Zelenina IA, Pavluchenkova SM et al (2010) Gold nanoparticles disturb nuclear chromatin decondensation in mouse sperm in vitro. Biol Membr 4:349–353

Zha LY, Wang MQ, Xu ZR, Gu LY (2007) Efficacy of chromium(III) supplementation on growth, body composition, serum parameters, and tissue chromium in rats. Biol Trace Elem Res 119:42–50. https://doi.org/10.1007/s12011-007-0042-8

Zha LY, Xu ZR, Wang MQ, Gu LY (2008) Chromium nanoparticle exhibits higher absorption efficiency than chromium picolinate and chromium chloride in caco-2 cell monolayers. J Anim Physiol Anim Nutr 92:131–140. https://doi.org/10.1111/j.1439-0396.2007.00718.x

Zha LY, Zeng JW, Chu XW, Mao LM, Luo HJ (2009) Efficacy of trivalent chromium on growth performance, carcass characteristics and tissue chromium in heat-stressed broiler chicks. J Sci Food Agric 89:1782–1786. https://doi.org/10.1002/jsfa.3656

Zhang JS, Wang XF, Xu TW (2008) Elemental selenium at nano size (Nano-Se) as a potential chemopreventive agent with reduced risk of selenium toxicity: comparison with Se-methylselenocysteine in mice. Toxicol Sci 101:22–31. https://doi.org/10.1093/toxsci/kfm221

Zielinska AK, Sawosz E, Grodzik M, Chwalibog A, Kamaszewski M (2009) Influence of nanoparticles of gold on chicken embryos' development. In: Kaleta T (ed) Annals of Warsaw University of Life Sciences – SGGW, Animal Science. Warsaw University of Life Science Press, Warsaw, pp 249–253

Veterinary Nutraceuticals Stability Testing

Dan DuBourdieu

Abstract

Nutraceuticals have gained increasing importance in veterinarian use over the past 30 years. However, the stability of many of these nutraceuticals is a challenge to manufacturers to maintain over a 2-year shelf life. Factors that affect nutraceutical stability include environmental conditions, chemical instability, matrix interactions, packaging, and other issues. Certain nutraceuticals, such as glucosamine sulfate, have very short stability in finished products, whereas others, such as chondroitin sulfate, are very stable. Testing nutraceuticals involves monitoring them under real-time and accelerated conditions to determine how much they will degrade. The strategies that companies use to increase the stability of nutraceuticals involve quality by design, encapsulation methods, and advances in packaging techniques.

Keywords

Veterinary nutraceuticals · Nutraceutical stability · Nutraceutical testing

1 Introduction

Nutraceuticals have gained increasing prominence for veterinary use over the past 30 years and have turned into a billion-dollar industry. This trend reflects a shift in the mind-set of manufacturers, veterinarians, and animal owners. Rather than relying solely on medicinal or drug products for the prevention and treatment of diseases, nutritional alternatives for both disease management and health promotion are readily available in conventional pet foods as well as individual supplements. This trend comes from a highly regulated environment of drugs where manufacturing is tightly controlled and where the stability of active ingredients is completely known in finished products. However, the nutraceutical manufacturing environment is currently not as controlled as the drug manufacturing environment. Expiration dates are not required on nutraceutical labels from a regulatory standpoint in the USA. This has led to justifiable concerns about the stability of shelf life of nutraceuticals used in both the human and veterinary arenas and the concern whether the nutraceutical is still effective. The primary reason for stability testing is the concern for the well-being of the animal suffering from the disease for which the product is designed. A second important concern is to protect the reputation of the manufacturer by assuring that the product will retain fitness for use with respect to all functionally relevant attributes for as long as they are on the market. As such, manufacturers, veterinarians, and pet owners wish to know whether the nutraceuticals that are being used are stable and will still be efficacious, even 2 years or longer after the date of manufacture.

Stability testing of nutraceutical products is a complex set of procedures involving considerable cost, time, and scientific expertise in order to build in quality, efficacy, and safety in a nutraceutical formulation. The most important steps during the developmental stages of a nutraceutical ingredient include the actual ingredient analysis and stability studies that are required to determine and assure the identity, potency, and purity of ingredients, as well as those of the formulated products (Bajaj et al. 2012). Stability/shelf life for a drug or nutraceutical can be defined as the capacity of that substance or product in a specific container-closure system to remain within established specifications to maintain its identity, strength, quality, and purity throughout the retest or expiration dating periods. Stability testing thus evaluates the effect of environmental factors on the quality of the nutraceutical substance or a formulated product which is utilized for prediction of its shelf life, to determine proper storage conditions, and for suggested labeling instructions. Moreover, the data generated during stability testing is an important requirement for the manufacturer to have to properly use.

D. DuBourdieu (✉)
Vets Plus Inc., Menomonie, WI, USA
e-mail: dand@vets-plus.com

R. C. Gupta et al. (eds.), *Nutraceuticals in Veterinary Medicine*, https://doi.org/10.1007/978-3-030-04624-8_55

In the USA, approximately 90% of veterinarians sell some type of novel ingredient such as nutraceuticals. Close to 30% of pet owners have used or considered the use of novel ingredients such as nutraceuticals and herbs/botanicals in their animals. Consumers often look for expiration dates on products, but US federal regulations do not currently require expiration dating for nutritional supplements. Manufacturers would like at least a 2-year stability on their nutraceutical products if possible, meaning that the listed "active" ingredients on the label will still be able to meet the label claim if retested at 2 years post manufacturing. The best a consumer can do is examine the label for date of manufacturing and hope that the product will be stable for at least 2 years, following proper storage. However, manufacturers, veterinarians, and consumers should be aware that not all nutraceuticals may meet this label claim.

2 Factors That Affect Stability of Nutraceuticals

There are a number of issues that can affect the concentration and stability of nutraceuticals in their raw and finished formats. These include environmental conditions, chemical instability, matrix interactions, packaging, and other intangible issues. A nutraceutical product may undergo change in appearance, consistency, content uniformity, clarity (solution), moisture content, particle size and shape, pH, and package integrity, thereby affecting its stability. Such physical changes may be because of impact, vibration, abrasion, and temperature fluctuations such as freezing, thawing or shearing, etc. Chemical reactions like solvolysis, oxidation, reduction, racemization, etc. that occur in the nutraceutical products may lead to the formation of degradation products, loss of potency of active nutraceutical ingredient, and loss of excipient activity like antimicrobial preservative action and antioxidant activity. Stability of a nutraceutical product can also be affected because of microbiological changes like growth of microorganisms in non-sterile products and changes in preservative efficacy. For details, see Fig. 1.

One source of problems for nutraceutical stability is the starting raw material itself, such as actives from plants. Plants

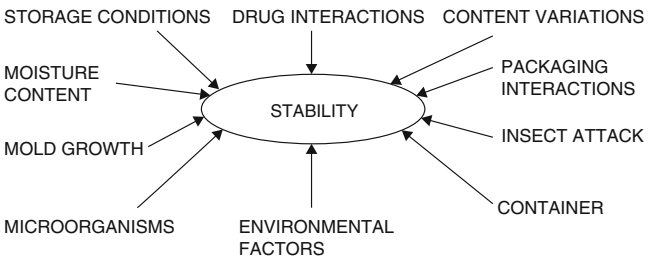


Fig. 1 Factors affecting nutraceutical stability

containing nutraceutical actives are collected or harvested either from cultivated sources or wild sources at certain times of the year and then are typically dried. The plant material then goes through a garbling step to remove extraneous matter such as dirt or non-desired plant parts or other adulterants. This should be done before it is baled or packaged. These raw materials can then be processed further to partially or more fully purify a particular active compound (i.e., the specific nutraceutical) of interest. For many botanical and herbal nutraceuticals, environmental conditions such as rainfall, altitude, temperature, soil, and storage conditions as well as different harvesting procedures; time and method of collection; manufacturing processes such as selecting, drying, purifying, and extracting; and genetic variability can create substantial variability in the product quality and stability and in the concentration of plant chemicals within different products. Nutraceuticals derived from animals or other sources parallel the same basic issues as the collection of plant-based nutraceuticals.

Phyto-formulations often suffer degradation during storage via oxidation, hydrolysis, crystallization, emulsion breakdown, enzymatic deterioration, and chemical reactions with the additives and excipients. Each ingredient, whether therapeutically active or inactive, in a dosage form can affect stability. Temperature and moisture are the two major factors that affect quality and stability of most herbal products. A chemical reaction increases by 2–3 times for every 10 °C rise in temperature. Moisture absorbed on to the surface of a solid nutraceutical often increases the rate of decomposition if it is susceptible to hydrolysis. The presence of enzymes in the product also increases the rate of chemical degradation. Light is also a prominent factor affecting phyto-formulations by generation of free radicals. The environmental factors of oxygen and carbon dioxide can affect stability. Similarly, factors such as particle size, pH, the properties of water and other solvents employed, the nature of the container, and the presence of other chemicals resulting from contamination or from the intentional mixing of different products can influence stability. Nutraceuticals derived from animal sources are also affected by these chemical and physical reactions. Table 1 demonstrates the susceptibility of those vitamins typically found in various nutraceutical preparations to several external factors.

Many herbal formulations are complex mixtures of different components obtained during an extraction process. This is not surprising since the efficacy of many plant-based nutraceuticals is not based on a single pure compound such as drug might be, but rather the efficacy is from groups of compounds in the plant. Each component has variable shelf life, activity, concentration, and consistency. This creates a problem during storage condition determination as it is not easy to determine the stability of the final herbal preparation based on the activity and stability profile of a single

Table 1 External factors that affect vitamin stability

Vitamin	Temperature	Oxygen	Humidity	Light	Acid pH	Alkaline pH
A	xx	xx	x	xx	x	0
D3	x	xx	x	x	x	0
E	x	0	x	x	x	x
K3	x	x	xx	x	xx	0
B1	x	x	xx	x	0	xx
B2	0	0	x	x	0	0
B6	xx	0	x	x	x	0
B12	x	x	x	0	0	0
Calcium pantothenate	x	0	x	0	0	0
Nicotinic acid	0	0	0	0	0	0
Biotin	0	0	x	x	xx	0
Folic acid	xx	0	x	xx	0	0
C	0	xx	xx	0	0	0

0, stable; x, sensitive; xx, very sensitive
Source: Gadient (1986)

component. However, most manufacturers will base any stability label claims from a single compound due to the complicated and costly nature of the analytical work required to get at least some sort of stability measurement.

Moisture content above a critical value and mold growth in natural products can cause an interaction of the active components with the packaging material. Natural medicines and nutraceuticals often suffer physical instability due to the presence of impurities and reaction with the container. Conditions like the growth of microorganisms and insect feeding affect the secondary metabolites and chemical composition of plants. Volatile active components of natural medicines and nutraceuticals have a problem of volatility and decreasing activity following long-term storage. Interactions of active components with other ingredients of formulations, such as additives, cause alteration in the novel drug activity. Herbal formulations have many active constituents, such as alkaloids, glycosides, tannins, flavonoids, etc., and each component will have different stability conditions. Therefore, actual stability conditions for the herbal formulation are different than for its individual components.

Packaging of the final product also has great bearing on the stability of the nutraceutical. Whether the packaging does not allow moisture, light, or air to reach the nutraceutical and whether the packaging has any kind of ability to moderate temperature will greatly affect the overall stability of the final product.

3 Categories of Nutraceuticals and Their Stability

There are many types of nutraceuticals. Each of these has their own inherent stability, and manufacturers have to be aware that some are going to be more stable than others for the particular matrix that the nutraceutical is delivered in. The top categories of supplements used in veterinary situations include joint protectants, antioxidants, fatty acids with anti-inflammatory ability, and probiotics.

The joint protectants that are commonly used include glucosamine, chondroitin sulfate, and methylsulfonylmethane. Some of these ingredients are not very stable. For example, glucosamine is not a stable compound in its free-base form. It is known to degrade quickly in aqueous solutions (Hrynets et al. 2015). There are numerous more stable forms of glucosamine available to consumers. These different forms include glucosamine sulfate, glucosamine hydrochloride, and *N*-acetyl-glucosamine. These different chemicals have some similarities. However, they may not have the same effects when taken as a dietary supplement, nor the same stability. From the results presented in Table 2, it is clear that glucosamine sulfate is a particularly unstable substance since it is readily oxidized and strongly

Table 2 Glucosamine sulfate stability at different temperatures and humidity

	25 °C/30% RH	15 °C/30% RH	25 °C/45% RH
Day	% Value	% Value	% Value
0	99.6	99.7	100.5
6			99.8
7	99.1	99.3	–
12		–	90.3
15	98.3	98.4	–
24		–	74.5
30	99.5	99.5	–
36		–	29.6
60	87.8	98.1	–
120	75.2	97.2	–
360	40.4	84.1	–

Table adapted from Senin (1987)

hygroscopic. The conditions under which it is kept are clearly very important (particularly the ambient relative humidity) and must be rigorously controlled. Only at temperatures less than 15 °C, with a relative humidity not greater than 30%, can a stability of about 4–5 months be obtained, while at 25 °C, under the same conditions of relative humidity, signs of degradation are shown even after about 60 days. Further, if the conditions of temperature and humidity are such as to be considered normal (25 °C and 60% relative humidity), the first signs of degradation appear even after 4 h, and the glucosamine sulfate is completely decomposed after just 36 h (Senin 1987). It is known that glucosamine salts destruct in solutions to 5-hydroxymethylfurfural (Shu 1998; Kompantsev 2012).

On the other hand, glucosamine hydrochloride is more stable than the sulfate form at an acid pH, and degradation begins when the temperature reaches 190 °C. Consequently, it is more stable under conditions of pasteurization and at an acid pH. Its stability in foods has been studied (EFSA 2009). For example, in lemonade and 100 % juice held at 100 °C for 5 min, there is a 100% recovery of glucosamine. Similarly, there is 100% recovery in isotonic drinks at pH 3.0, juice at pH 3.0, and "fitness water" at pH 2.9, all held at room temperature for 9, 24, and 17 months, respectively. However, the stability of glucosamine HCl in finished products of solid matrices such as tablets will still be dependent on the amount of moisture, pH, and temperature that the finished product has been subjected to. It is expected that degradation will have occurred since the date of manufacture in most products to a degree. While the amount may still meet label claims after 2 years, it will depend on the particular product. Unfortunately, there is no simple way to tell from the label, unless testing occurred on the finished product at 2 years past the manufacturing date and that information is stated somewhere. Fortunately, manufacturing groups will continue to research ways to further stabilize glucosamine in finished products.

The stability of chondroitin sulfate (CS) used in joint products has a much better track record for stability than glucosamine. CS raw material held at room temperature has little degradation over a year (Fig. 2). CS has been studied under acidic, neutral, and basic conditions at 30 and 60 °C, and CS is remarkably stable under neutral conditions at low temperature. However, it degrades at 60 °C, producing low-molecular-mass fragments and desulfated products (Volpi et al. 1999).

Methylsulfonylmethane (MSM) is an organosulfur compound having a sulfonyl functional group that is often used in joint products. It occurs naturally in some plants and is used in disease related to chronic pain, inflammation, and arthritis. MSM appears to be fairly stable due to its ability to withstand high temperatures (Trivedi et al. 2015). The shelf life of MSM can be 5 years in pure preparations according to manufacturers.

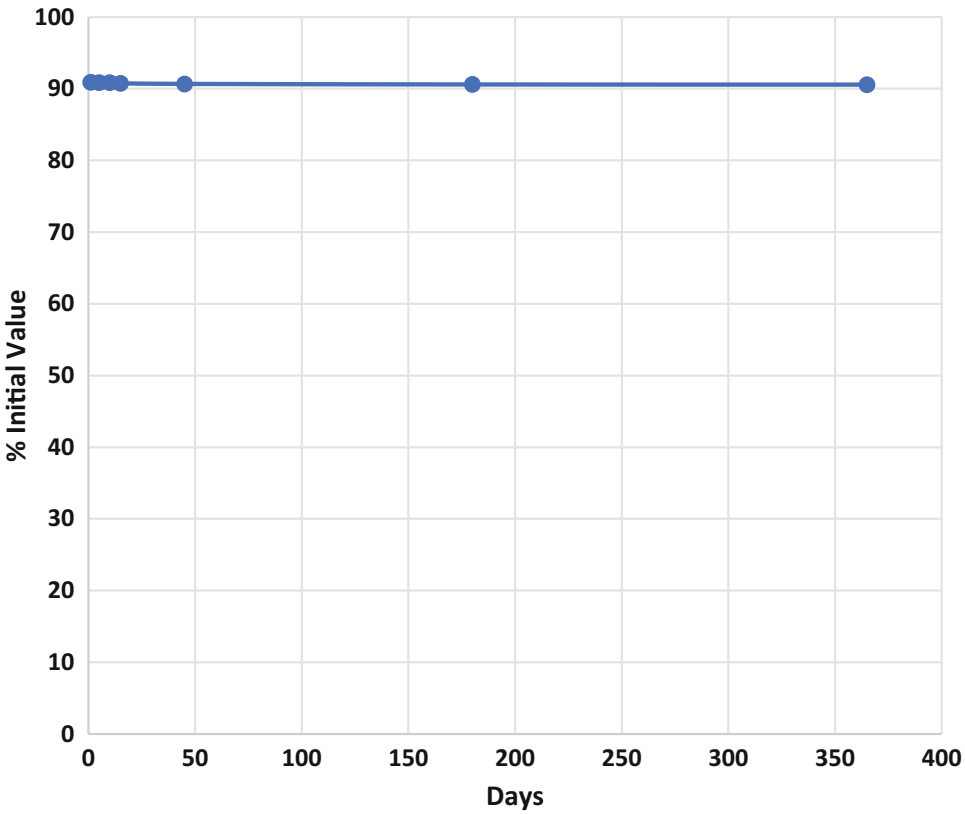


Fig. 2 Stability of chondroitin sulfate at room temperature

Antioxidants refer to a heterogeneous group of compounds that prevent free radical damage to cell membranes, proteins, and DNA. They are beneficial in inflammatory diseases, aging, and certain cancers. Common antioxidants used as supplements in food or direct oral intake include vitamins E and C (ascorbic acid), various bioflavonoids found in plants, and carotenoids such as astaxanthin found in marine life. Many of these antioxidants have poor long-term stability and therefore a shortened shelf life of the finished products. Analytical work has shown (Oyetade et al. 2012) that packaging materials, exposure to air, storage temperature conditions, and matrix type can significantly affect the stability of vitamins that have been used as antioxidants. A significant negative correlation exists between ascorbic acid decline, which depends on the storage temperatures, and matrix type as seen in Table 3 (table adapted from Oyetade et al. 2012). Ascorbic acid content was more stable when stored under refrigeration.

The stability of other individual vitamins in premixes and finished feeds varies according to a number of factors (Table 4).

Minerals are more resistant to manufacturing processes than vitamins. Minerals are naturally occurring fundamental substances that maintain proper bone health and the nervous and the muscular system and have the ability to assimilate nutrients. However, they do undergo changes when exposed to heat, air, or light. Minerals such as copper, iron, and zinc are also affected by moisture and may react with other matrix components such as proteins and carbohydrates. Various forms of iron are used in fortification. Among the most popular are ferrous sulfate and elemental iron powders because of their relatively high bioavailability. Other potential iron sources include ferric orthophosphate, sodium iron phosphate, ferrous fumarate, and iron-EDTA. The stability of different forms of iron depends on various factors, including the nature of the final nutraceutical product that it is added to, particle size, and exposure to heat and air (Table 4).

Proteins, such as enzymes, possess greater biochemical and structural complexity compared to most single active ingredients used in nutraceutical products. Thus, the formulation and delivery of proteins and enzymes into stable, well-characterized, and efficacious nutraceutical products represent significant challenges to the formulator. Tablets are a suitable dosage form for application of these materials as they provide ease of administration, metering accuracy, robustness, good stability, and efficient production. However, simple compression of a bulk material, either powder or granular, to a robust tablet is dependent on a great number of influences, mainly compression force, particle deformation, and formation of adhesive forces. Therefore, the physical and chemical properties of proteins and enzymes can be influenced by formulation and technological factors, such as excipients, temperature, storage conditions, compression, or shear forces (Manning et al. 1989).

There has been a surge in clinical evidence regarding the therapeutic benefits of probiotic bacteria including *Lactobacillus*, *Bifidobacteria*, and *Enterococcus* species to gut health and a growing commercial interest in food and/or nutraceutical applications of these bacteria (Park et al. 2016). One beneficial effect of probiotics on human and animal gut flora is the restoration of impaired intestinal barrier function. Regular intake of probiotic strains stimulates the growth of preferred microorganisms, crowds out potentially detrimental strains, and reinforces the natural defense mechanisms. However, the formulation of probiotic bacteria along with other ingredients, or even alone, in a stable dosage form is hampered by their extremely poor viability during the preparation process and/or storage period. The majority of microorganisms generally exhibit extreme liability to oxygen, temperatures, low pH, and additives (Kailasapathy and

Table 3 Changes in ascorbic acid contents in matrices stored at different temperatures

Tablets	Temperature				
Holding time (minutes)	4 °C	35 °C	55 °C	75 °C	95 °C
0	84.5	84.5	84.5	84.5	84.5
60	84.5	77.5	72.9	69.9	61.6
120	84.5	75.8	63.3	59.3	48.9
180	84.5	69.8	60	48.6	36.8
240	84.5	62.9	52.3	38.6	30.9
Grape juice					
0	58.2	58.2	58.2	58.2	58.2
60	46.0	42.5	40.5	31.9	29.3
120	46.0	40.3	39.8	30.2	26.9
180	46.0	37.8	37.8	28.9	24.3
240	45.8	37	33.9	26.3	19.3

Table 4 Percent retention of vitamins in pelleted feed stored 6 months at room temperature

Vitamin	Vitamins only	Vitamins mixed with minerals and choline chloride
A	89–95	70–100
D3	90–100	80–100
E	90–100	90–100
K3	50–70	30–50
Thiamine	85–100	70–80
Riboflavin	90–100	90–100
B6	70–90	60–80
B12	60–90	50–80
Pantothenic acid	90–100	80–100
Niacin	90–100	90–100
Folic acid	70–100	50–70
Biotin	90–100	70–90

Source: Gadient (1986)

Chin 2000). Moreover, the viability of living bacteria in gastric fluid is rather poor. Few of them exhibit resistance to adverse environmental conditions to colonize the mucosal membrane of the small and/or large intestine. To help overcome these stability problems, enteric coating techniques have been explored in order to protect living bacteria from the unfavorable interaction within the dosage form and adverse gastrointestinal environments (pH, enzymes, bile salts, etc.) and to deliver bacteria to the intestine of animals. For example, *L. acidophilus* and *Enterococcus faecalis* were coated (encapsulated) and imbedded in tablets and compared with corresponding bare bacteria, the uncoated bacteria, in tablets under ambient storage conditions (25 °C/60% relative humidity) for 6 months (Fig. 3). The particular coating procedures clearly helped maintain stability and viability of the bacteria (Park et al. 2016).

Omega-3 long-chain polyunsaturated fatty acids including EPA and DHA are substances with a range of important structural and regulatory functions in animal physiology. Deficiencies in their dietary intake and tissue levels are related to chronic disease susceptibility. Omega-3 fatty acids have been shown to be beneficial in dogs with atopic dermatitis, pruritis, lymphoma, arthritis, and chronic valvular disease. The National Research Council has recognized omega-3 fatty acids as an essential nutrient for dogs and cats. As such, a substantial number of finished products are produced with EPA-/DHA-containing oils derived from marine sources, notably from anchovy and cod liver. Other sources, such as purposefully grown microalgae, are of growing importance. Preparing solid formulations, such as powder and tablets, that contain omega-3, can be challenging as the necessary production processes expose the unsaturated omega-3 fatty acids to high temperatures, light, and mechanical stress in the presence of air. When added to laboratory rodent formulations, these fatty acids are subject to rapid and/or extensive oxidation and other chemical changes by exposure to air, light, or heat during processing of pellets or when stored for various lengths of time (Lytle et al. 1992). Under ambient and real-life conditions, the absolute oxidative stability of EPA-/DHA-containing oils cannot be achieved, but it can be slowed down considerably by limiting the exposure to air and by reducing the rates of peroxidation and additional reactions secondary to primary oxidation, for example, by maintaining sufficiently high concentrations of reduced antioxidants and by limiting exposure to predisposing factors such as heat and light (Bannenberg et al. 2017). The use of encapsulation technology for fatty acids can help delay degradation, as well as enhance stability.

4 Stability Testing Methods

Stability testing is a routine procedure performed on nutraceutical substances and products and is employed at various stages of product development (Mehta 2009). In the early stages, accelerated stability testing (at relatively high temperatures and/or humidity) is used to determine the type of degradation products which may be found after long-term storage. Testing under less rigorous conditions (i.e., those recommended for long-term shelf storage) at slightly elevated temperatures is used to determine a product's shelf life and expiration date. The major aim of nutraceutical stability testing is to provide reasonable assurance that the products will remain at an acceptable level of fitness/quality throughout the period during which they are available for supply to the animal and will be fit for their consumption until the patient uses the last unit of the product.

Real-time stability testing is the best way of establishing accurate values. In real-time stability tests, a product is stored at recommended conditions and tested/monitored for the period of time in the packaging that the nutraceutical product is intended to be marketed in. However, real-time testing is very time consuming and can take years to complete. Real-time stability testing is normally performed for a longer duration of the test period to allow significant product degradation under recommended storage conditions. The long-term test storage conditions usually are 25 ± 2 °C/60 ± 5% relative humidity (RH) in stability chambers. These are

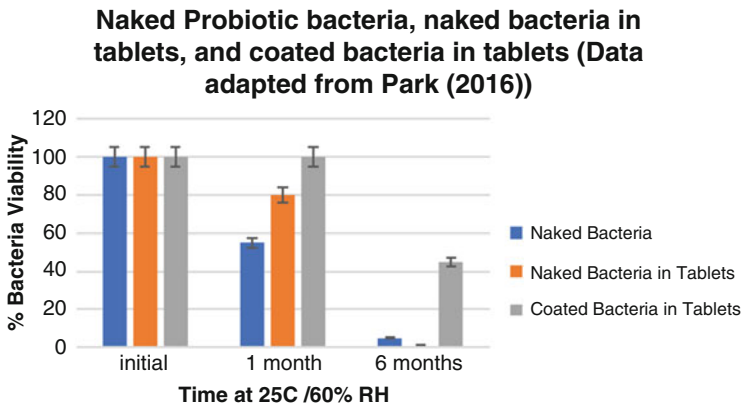


Fig. 3 Coated probiotic bacteria stability in tablets

specialized environmental chambers that can simulate storage conditions and enable evaluation of product stability based on real-time, accelerated, and long-term protocols. Samples also should be kept refrigerated long term at 5 °C and frozen long term at –20 °C to see what happens to the product when cooled or frozen and then thawed.

It is always recommended to follow the procedures given in official compendia if possible for any particular nutraceutical, as results obtained using the official tests in general find better acceptance. If alternate methods are used, they are required to be duly validated. However, assay of the nutraceutical should be carried out using a stability-indicating method, established by carrying out stress tests on the nutraceutical under forced decomposition conditions. This method should be validated for specificity, accuracy, precision, and linearity, in the range within which the nutraceutical is expected to fall during stability studies. For the assay of degradation products, the validated method should also include the limits of detection/quantification. The methods reported in literature should be used after confirming reproducibility and carrying out minimal validation of linearity, range, etc. All analytical methods are required to be validated before initiating the stability studies. Similarly, the acceptance criteria for analytical results, as well as that for the presence of degradation products, should also be fixed before doing the assays. These acceptance criteria should also include individual and total upper limits for degradation products.

The test period depends upon the stability of the product. This period should be long enough to indicate clearly that no measurable degradation occurs and must distinguish degradation from inter-assay variation. During analysis, data is collected at an appropriate frequency such that a trend analysis is able to distinguish instability from day-to-day ambiguity. Frequency of testing should be such that it is sufficient to establish the stability profile of the new nutraceutical substance. For products with a proposed shelf life of at least 24 months, the testing frequency at long-term storage conditions should be every 3 months over the first year, every 6 months over the second year, and annually thereafter through the proposed shelf life expiration date, if such a date is going to be put on the label. Stability studies at developmental stages are generally carried out on a single batch, while studies intended for registration of new product or unstable established product are done on the first three production batches. In general, the selection of batches should constitute a random sample from the population of pilot or production batches. The reliability of data interpretation can be increased by including a single batch of reference material for which stability characteristics have already been established. Stability of the reference material also includes the stability of reagents, as well as consistency of the performance of the instrument to be used throughout the period of stability testing. However, system performance and control for drift and discontinuity resulting from changes in both reagents and instrumentation must be monitored.

Since manufacturers typically are not going to wait 2–3 years before launching a nutraceutical product, an accelerated stability testing program is used concurrently with real-time studies. In accelerated stability testing, a product is stressed at several high (warmer than ambient) temperatures, and the amount of heat input required to cause product failure is determined (Fig. 4). Accelerated conditions of 40 ± 2 °C/75 ± 5% RH is commonly used as well as minimum of three time points, including the initial and end points [0, 3, and 6 months is recommended (Magari 2003)].

Accelerated stability testing is done to subject the product to a condition that accelerates degradation. This information is then projected to predict shelf life or used to compare the relative stability of alternative formulations by using the Arrhenius equation. Arrhenius equation gives the dependence of the rate constant of a chemical reaction on the absolute temperature, a pre-exponential factor, and other constants of the reaction:

$$k = A\mathrm{e}^{-E_a/(RT)}$$

where k is the rate constant, T is the absolute temperature (in kelvins), and A is the pre-exponential factor, a constant for each chemical reaction. According to collision theory, A is the frequency of collisions in the correct orientation, E_a is the activation energy for the reaction (in the same units as

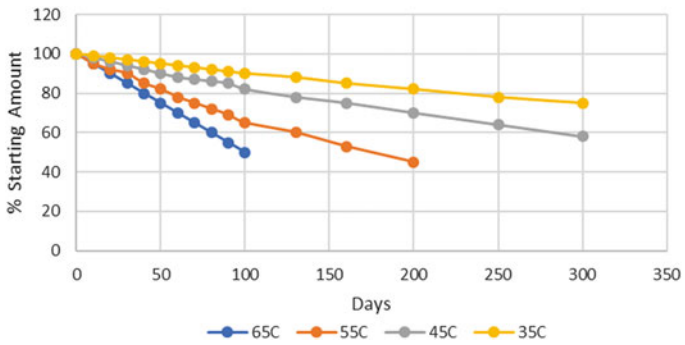


Fig. 4 Example of accelerated stability testing at different temperatures for determining degradation of an active compound

$R \times T$), and R is the universal gas constant. This equation describes the relationship between storage temperatures and degradation rate. Using Arrhenius equation, projection of stability (an estimate) from the degradation rates observed at high temperatures for some degradation processes can be determined. When the activation energy is known, the degradation rate at low temperatures may be estimated from those observed at "stress" temperatures at any particular time. Real-time results can be graphed and compared to the estimated degradation curve generated from accelerated stability testing data and the Arrhenius calculations (Fig. 5).

The results produced from the Arrhenius equation usually provide an early indication of the product shelf life, thus shortening the development schedule. In addition to temperature, stress conditions applied during accelerated stability testing are moisture, light, agitation, gravity, pH, and packaging. In accelerated stability testing, the samples are subjected to stress, refrigeration after stressing, and then simultaneously assay. Because the duration of the analysis is short, the likelihood of instability in the measurement system is reduced in comparison to real-time stability testing. Further, in accelerated stability testing, comparison of the unstressed product with stressed material is made within the same assay, and the stressed sample recovery is expressed as percent of unstressed sample recovery. For statistical reasons, the treatment in accelerated stability projections is recommended to be conducted at four different stress temperatures. However, for thermolabile and proteinaceous components, relatively accurate stability projections are obtained when denaturing stress temperatures are avoided. While estimated degradation from Arrhenius calculations can give some idea about what may occur in real life, accelerated data should not be solely relied upon by manufacturers for what truly happens under real-life conditions. Real-time data has to occur simultaneously with accelerated studies to fully determine what occurs for the stability of any nutraceutical.

5 Regulatory Guideline Aspects Related to Nutraceutical Stability

The Dietary Supplement Health and Education Act of 1994 is a statute of US federal legislation which defines and regulates dietary supplements and how nutraceuticals are at least partially regulated. Under the act, supplements are effectively regulated by the FDA for good manufacturing practices (GMP). The act has been criticized because supplement manufacturers are not required to demonstrate supplements' safety before marketing them. To help nutritional supplement companies make sure they have the necessary data to support expiration dating on product labels, NSF International, the developer of US national standard for dietary supplements, helped create a voluntary Stability Testing Guideline (NSF 2011). This guideline outlines the science-based criteria necessary to support expiration dating in order to comply with the current GMP for nutraceutical supplements. The guideline suggests that nutritional supplement companies identify the physical, chemical, and microbiological characteristics of their product under long-term storage. It specifies that companies understand the impact of manufacturing, packaging, labeling, distribution, and holding/warehouse processes may have on a product's stability. Factors involved in stability testing include dietary ingredient strength, chemical fingerprints, microbial growth, preservative content, moisture content, pH, viscosity, and oxidation, among other parameters, such as the product's container-closure system.

To assure that optimally stable molecules and products are manufactured, distributed, and given to patients, regulatory authorities in several countries have made provisions in the drug regulations for the submission of stability data by the manufacturer. These same basic drug principles hold true for nutraceuticals. Basic purpose of these regulatory guidelines was to bring uniformity to testing from manufacturer to manufacturer. These guidelines include basic issues related

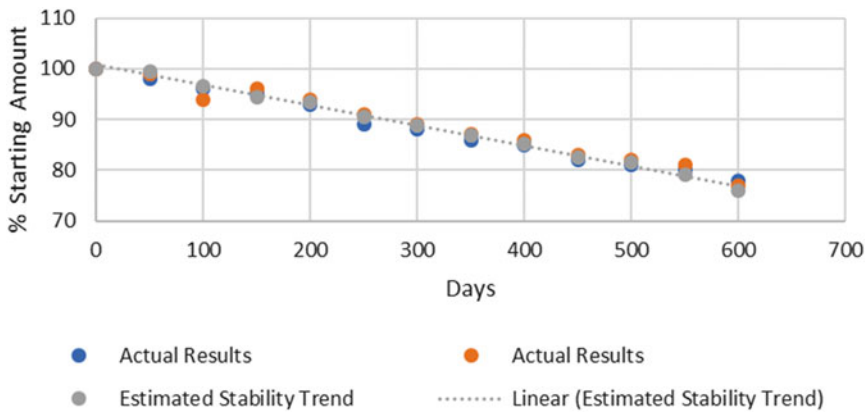


Fig. 5 Example of real-time stability compared to estimated stability

to stability, data requirements for the application dossier, and steps for their execution. Such guidelines were initially issued in the 1980s. These were later made uniform by the International Conference on Harmonization (ICH 2003) in order to overcome the bottleneck to market and register products in other countries. The ICH was a consortium formed with input from both regulatory agencies and industry from the European commission, Japan, and the USA.

6 Concluding Remarks and Future Directions

Stability testing is a key procedural component in the pharmaceutical development program for a new drug, and the same principles are beginning to be applied to the nutraceutical industry. Stability tests are carried out so that recommended storage conditions and shelf life can be included on the label to ensure that the drug or nutraceutical is safe and effective throughout its shelf life. Over a period of time and with increasing experience and attention, the regulatory requirements have been made increasingly stringent to achieve the above goal in all possible conditions to which the product might be subjected during its shelf life. While expiration dates are currently not required on nutraceuticals in the USA as of 2018, the trend is for nutraceuticals to become more and more regulated and tightly controlled for manufacturing, as is currently carried out in the drug industry. One approach to this is quality by design (QbD). QbD is a systematic approach to pharmaceutical product development that begins with predefined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk management. The same approach is starting to be used by the nutraceutical industry. It means designing and developing formulations and manufacturing processes to ensure a predefined quality. Thus, QbD requires an understanding of how formulation and process variables influence product quality (Lionberger et al. 2008). QbD is a scientific, risk-based, holistic, and proactive approach to pharmaceutical development that will work for the nutraceutical industry. It is a deliberate design effort from product conception through commercialization.

One way the nutraceutical industry is currently dealing with stability issues is to simply add more of the nutraceutical to any particular formulation than would be stated on the label at time of manufacturing. Taking into account that decay of the nutraceutical will occur, it is hoped that the overage of the particular active nutraceutical will be enough so that when decay does occur, there will be enough left to meet any label claims. While this approach may be simple in theory, it may be costly to do or impractical in certain formulations or simply undesirable for other reasons like palatability. Another strategy is to use chemical salt forms of the nutraceutical that make the molecule inherently more stable. Encapsulation strategies of nutraceuticals is an area where much research effort is undergoing (Thakur et al. 2011; Ruiz and Campos 2017). Development of encapsulation systems based on lipophilic nutraceuticals' binding ability with some globular proteins is an area that holds promise. Newer packaging strategies to limit moisture, light, and air are another way to help maintain nutraceutical stability. Apart from simply keeping nutraceutical products refrigerated to maintain stability, packaging that helps prevent temperature from affecting the active ingredients will be a step forward using various temperature-controlled packaging solutions. Structurally, nutraceutical packages may be more experimental and unique than pharmaceutical containers in order to intrigue the novelty-oriented baby-boomer generation. Regardless of the strategies used, the goal of manufacturers will still be to achieve the longest shelf life for the active agent and give assurance to the veterinarian and the animal owner that the animal is receiving an effective nutraceutical.

References

Bajaj S, Singla D, Sakhuja N (2012) Stability testing of pharmaceutical products. J Appl Pharm Sci 02(3):129–138

Bannenberg G, Craig Mallon C, Edwards H et al (2017) Omega-3 long-chain polyunsaturated fatty acid content and oxidation state of fish oil supplements in New Zealand. Sci Rep 7:1488

EFSA (2009) Opinion of the safety of glucosamine hydrochloride from *Aspergillus niger* as food ingredient. EFSA J 1099:1–19

FDA for Good Manufacturing Practices under 21 CFR Part 111. http://www.accessdata.fda.gov/scripts/cdrh/cfcfr/cfrsearch.cfm?cfrpart=111

Gadient M (1986) Effect of pelleting on nutritional quality of feed. In: Proceedings of the 1986 Maryland nutrition conference feed manufacturers, College Park, MD

Hrynets Y, Ndagijimana M, Betti M (2015) Studies on the formation of Maillard and caramelization products from glucosamine incubated at 37 °C. J Agric Food Chem 63(27):6249–6261

ICH (2003) International conference on harmonization. Stability testing of new drug substances and products Q1A(R2)

Kailasapathy K, Chin J (2000) Survival and therapeutic potential of probiotic organisms with reference to *Lactobacillus acidophilus* and *Bifidobacterium* spp. Immunol Cell Biol 78:80–88

Kompantsev DV (2012) Stability of glucosamine dosage forms. Russ J Gen Chem 82(3):579–585

Lionberger RA, Lee SL, Lee ML et al (2008) Quality by design: concepts for ANDAs. AAPS J 10(2):268–276

Lytle JS, Lytle TF, Newmark HL et al (1992) Stability of a commercially prepared fish oil (omega-3 fatty acid) laboratory rodent diet. Nutr Cancer 17(2):187–194

Magari RT (2003) Assessing shelf life using real-time and accelerated stability tests. BioPharm Int 16(11):36–48

Manning MC, Patel K, Borchardt RT (1989) Stability of protein pharmaceuticals. Pharm Res 6(11):903–918

Mehta J (2009) Practical challenges of stability testing of nutraceutical formulations. In: Huynh-Ba K (ed) Pharmaceutical stability testing to support global markets. Springer, New York, pp 85–91. 20 Oct 2009, www.iosrjournals.org

NSF (2011) www.nsfstability.org
Oyetade OA, Oyeleke GO, Adegoke BM et al (2012) Stability studies on ascorbic acid (vitamin C) from different sources. IOSR J Appl Chem 2(4):20–24
Park HK, Lee GH, Jun J et al (2016) Multiple-unit tablet of probiotic bacteria for improved storage stability, acid tolerability, and *in vivo* intestinal protective effect. Drug Des Dev Ther 10:1355–1364
Ruiz JCR, Campos PS (2017) New polymers for encapsulation of nutraceutical compounds. Wiley, Chichester
Senin (1987) Stable compounds of glucosamine sulfate. US Patent 4,642,340
Shu CK (1998) Degradation products formed from glucosamine in water. J Agric Food Chem 46(3):1129–1131
Thakur L, Ghodasra U, Patel N et al (2011) Novel approaches for stability improvement in natural medicines. Pharmacogn Rev 5 (9):48–54
Trivedi MK, Branton A, Trivedi D, Nayak G et al (2015) Evaluation of physical, thermal and spectral parameters of biofield energy treated methylsulfonylmethane. J Mol Pharm Org Process Res 3:129
Volpi N, Mucci A, Schenetti L (1999) Stability studies of chondroitin sulfate. Carbohydr Res 315(3–4):345–349

Part VII

Regulatory Aspects and Country-Specific Requirements for Nutraceuticals

Basic Regulatory Guidelines for Veterinary Nutraceuticals

Dan DuBourdieu, Anita Sinha, and Rajiv Lall

Abstract

Close to 30% of pet owners use nutraceuticals on their animals. Veterinary nutraceuticals face regulations from regulatory authorities including the Food and Drug Administration, the American Federation of Feed Control Officials, states, and countries. These regulations can be confusing as to how nutraceuticals for veterinary use can be marketed legally. Trade organizations such as the National Animal Supplement Council recommend guidelines to manufacturers in coordination with the regulatory agencies, at both the state and federal level to help establish a system of regulatory oversight. This approach helps clarify the regulatory environment for veterinary nutraceutical manufacturer to continue manufacturing and marketing their products.

Keywords

Veterinary nutraceuticals · Regulations

1 Introduction

Close to 30% of pet owners have used nutraceuticals of one type or another for their animals, while in the USA, approximately 90% of veterinarians recommend some type of nutraceutical ingredient (Boothe 2017). As such, the global pet food nutraceutical market is estimated to reach a value of $8.25 billion by 2023 (Mordor Intelligence 2018). Clearly this is a large market. Therefore, how nutraceuticals are regulated is of obvious interest to manufacturers and veterinarians and to the animal owners that use them. The American Veterinary Medical Association recognizes the importance of these medicaments through its guidelines regarding complementary or alternative medicine, which includes veterinary nutraceutical therapy (AVMA 2018). While the nutraceuticals for human use have some regulatory framework that surround them, the use of nutraceuticals for veterinary use is somewhat more murky and confusing from a regulatory standpoint. Veterinary nutraceuticals' manufacturing and use are regulated by a number of entities including governmental agencies, trade groups, and indirectly by customer requirements. How these various entities work together to regulate veterinary nutraceuticals is what manufacturing companies must have an understanding of in order to market their nutraceutical products for veterinary use.

2 DSHEA

Dr Stephen DeFelice coined the term "nutraceutical" from "nutrition" and "pharmaceutical" in 1989 (Kalra 2003). The term nutraceutical is commonly used in marketing pieces but has no real regulatory definition. Attempts to put some sort of regulatory aspects surrounding nutraceuticals started in the early 1990s. In 1994, the Dietary Supplement Health Education Act (DSHEA) was passed by the US Congress and signed into law by President Clinton in October. This legislation created a specific category of products, dietary supplements, as a subset of food under the Federal Food, Drug, and Cosmetic Act that allowed the marketing of dietary supplements for human use. Unfortunately, when the issue was debated in Congress, animals were not considered, and language was not included in the legislation to allow similar products for animals to be regulated as "dietary supplements" as provided for people. DSHEA established supplements intended for human consumption as a new class of food for purposes of federal regulation. But importantly, DSHEA does not allow dietary supplement manufacturers to make unapproved health claims, such as cure, prevent, mitigate, treat, or diagnose a disease. That is where problems occur in marketing veterinary nutraceuticals since manufacturers want

D. DuBourdieu (✉) · A. Sinha · R. Lall
Vets Plus Inc., Menomonie, WI, USA
e-mail: dand@vets-plus.com

R. C. Gupta et al. (eds.), *Nutraceuticals in Veterinary Medicine*, https://doi.org/10.1007/978-3-030-04624-8_56

to make these unapproved health claims with nutraceuticals but are not allowed to and get into trouble when they do make these claims.

DSHEA defines a dietary supplement as a product intended to supplement the diet which contains at least one or more of the following ingredients: a vitamin, a mineral, an herb or other botanical, an amino acid, a dietary substance for use to supplement the diet by increasing total dietary intake, or a concentrate, metabolite, constituent, extract, or combination of any of the previously mentioned ingredients. The main effect of DSHEA was to remove certain dietary ingredients from regulation as food additives, which requires premarket approval. Quite a few animal supplement products are being sold as a result of DSHEA, and these products generally contain similar ingredients to those in human dietary supplements. Examination of the *Congressional Record*, when the bill was finalized, shows Congress neither specifically included nor excluded the application to animals. Although it may have been reasonable to at least include animals which are not intended for human consumption, such as dogs, cats, and horses, the topic of animals was overlooked, probably for the principle reason that the animal supplement industry did not really exist to any significant degree in 1994. In addition, the primary purpose of the legislation was to address the increasing consumer demand for dietary supplements for people. However, the Food and Drug Administration (FDA) published a notice in the *Federal Register* in 1996 explaining why the FDA believes that DSHEA does not apply to animals (Schultz 1996). The FDA believed many of the types of products marketed for animals contained ingredients that may be unsafe food additives or unapproved new animal drugs, making the products unsafe for the animals. The Center for Veterinary Medicine (CVM) was concerned about these products because the CVM did not have scientific data to show that they are safe or even contain the ingredients listed on the label. Thus, many substances that are permitted in human dietary supplements may not be legally sold in animal supplements. In announcing that DSHEA does not apply to animal products, CVM reasoned that many substances that qualify as dietary supplements for human consumption, such as botanicals, have a history of use in humans that can be used to establish reasonably safe levels. However, the same is not true for many of the same ingredients in animals as evidential support concerning the use of such substances is incomplete or unavailable. Animals may react very differently to substances than humans, and even small doses can cause adverse effects. Moreover, each animal species requires different nutrients, absorbs and metabolizes nutrients differently, and can exhibit different toxic reactions to food and its components. Because CVM has determined that DSHEA does not apply to animal supplements, many claims that are permitted for human dietary supplements are not permitted for animal nutritional supplements. The courts have interpreted "food" as a substance that provides nutrition, taste, or aroma. Thus, claims on animal food and supplement products that establish the intended use of the product to affect the structure or function of the body of animals in a manner other than via nutrition, aroma, or taste cause the product to be a drug.

3 FDA/CVM

The primary agency responsible for the regulation of animal food and drugs is the Food and Drug Administration's Center for Veterinary Medicine. FDA/CVM works closely with the states through regulatory associations like the Association of American Feed Control Officials (AAFCO) to ensure both federal and state laws are followed and companies remain in compliance. The CVM generally takes the position that animals on balanced rations, such as companion animals (i.e., dogs, cats, and horses), do not require extra nutritional supplementation beyond their typical feed. This is because CVM believes that dog and cat foods are rich in nutrients and that most animals either receive adequate amounts of vitamins, minerals, protein (essential amino acids), fat (fatty acids), and carbohydrates from their diet or are able to synthesize them from a ration balanced to observe National Research Council's nutrient requirements (FDA 2015a). There is no requirement that animal foods have premarket approval by CVM. The Federal Food, Drug, and Cosmetic Act does require that animal foods, like human foods, be pure and wholesome, contain no harmful or deleterious substances, and be truthfully labeled. When a substance, including one considered food, is intended to be used for the treatment or prevention of disease or for a "nonfood" structure/function effect, FDA considers it a drug. Drugs are an article intended for use in the diagnosis, cure, mitigation, treatment, or prevention of disease or an article intended to affect the structure or function of the body other than food. In the drug definition, the courts have interpreted "food" as something that provides nutrition, taste, or aroma. If a food affects the structure or function of the body, it does so by these properties (e.g., a food may provide nutrients such as calcium to support proper bone structure). However, if a substance affects the structure or function of the body apart from its nutritive value, such as improvement in joint function, it may be considered a drug. Structure/function effects extending beyond the "food" umbrella also include claims for improved or increased production and performance and alteration or improvement in function. A new animal drug must be shown to be safe and effective for its intended use by adequate data from controlled scientific studies as part of a New Animal Drug Application (NADA). If a product on the market is not approved, it may be deemed an adulterated drug and subject to regulatory action.

Most animal health supplements were considered "drugs of low regulatory priority" by the FDA, but a subtle shift has

occurred, and the FDA/CVM now views these supplements as "un-approved drugs for which enforcement discretion may be exercised" based on their content, labeling, intended use, and overall conduct of the company. If the product is intended as a source of minerals and vitamins for a nonhuman food chain animal (dogs, cats, and horses), it is classified as a nutritional or feed supplement and regulated by the FDA and individual state feed control officers through the AAFCO Model Bill. Most states follow the recommendations of the AAFCO. Nevertheless, CVM generally does not object to the over-the-counter marketing of dietary supplements in tablet, capsule, powder, or liquid form for companion animals similar to dietary supplements marketed to humans. However, CVM takes the position that such products should provide meaningful amounts of each of the nutrients they are represented to contain and that the nutrients should be of known value for the intended or target animal. The FDA uses the Nutritional Requirements of Dogs and Cats (National Research Council 2006) to arrive at a level of supplementation that represents the best information presently available for these species. FDA typically accepts as adequate those products providing a meaningful level of nutrition when compared with the NRC recommendations.

Generally, the CVM will not object to the marketing of nutritional supplements for oral administration to companion animals provided they conform to the following restrictions: there is a known need for each nutrient ingredient represented to be in the product for each animal for which the product is intended; the label represents the product for use only in supplementation of, and not as a substitute for, good daily rations; the product provides a meaningful but not excessive amount of each of the nutrients it is represented to contain; the product is neither overpotent nor under-potent nor otherwise formulated so as to pose a hazard to the health of the target animal; the labeling should bear no disease prevention or therapeutic representations, including growth promotion; and the labeling should not be otherwise false or misleading.

The FDA does send warning letters to manufactures in certain cases. But typically, the rationale is not because the supplement is not effective but rather because of the language used in describing the supplement. When the language is acceptable to the FDA, then the product can be sold again. For example, a supplement maker was accused by the FDA of selling a product that the FDA called an unapproved kidney disease drug for pets but was then allowed to continue selling the same product as a supplement instead with a new product name and different marketing (JAVMA 2015).

4 AFFCO

In general, the FDA requires that animal feed be pure and wholesome, contain no harmful or deleterious substances, and be truthfully labeled. Currently, only those substances that are (1) listed as generally recognized as safe (GRAS) in FDA regulations at 21 C.F.R. Part 582, (2) listed as an approved food additive in FDA regulations at 21 C.F.R. Part 573, or (3) listed as a defined ingredient in the Official Publication of the Association of American Feed Control Officials (the AAFCO book) are permitted in animal feed, including animal/pet supplements that are being sold as feed. AAFCO is a voluntary membership association of local, state, and federal agencies charged by law to regulate the sale and distribution of animal feeds and animal drug remedies. Although AAFCO has no regulatory authority, most states have adapted AAFCO guidelines when regulating animal/pet food. AAFCO provides a forum for the membership and industry representation to achieve three stated main goals: safeguarding the health of animals and humans, ensuring consumer protection, and providing a level playing field of orderly commerce for the animal feed industry (AFFCO 2018). These goals are achieved by developing and implementing uniform and equitable laws, regulations, standards, definitions, and enforcement policies for regulating the manufacture, labeling, distribution, and sale of animal feeds—resulting in safe, effective, and useful feeds by promoting uniformity among member agencies.

The CVM categorizes ingredients in animal supplements as one of two types: (1) a nutritional ingredient (intended to provide nutrition) or (2) a non-nutritive ingredient (does not provide nutrition). The AAFCO Official Publication (OP) contains nutrient profiles for cats and dogs that list the ingredients considered to be essential nutritive ingredients. According to the CVM, a claim that an animal supplement affects the structure or function of the body based on its nutritive value (a nutritional ingredient) is considered a food claim, and the product will be categorized as a food. For example, calcium is listed as an essential nutritive ingredient in the AAFCO OP for both dogs and cats. Thus, a claim that a supplement that contains dried milk provides calcium to support proper bone structure would be a permitted food claim, and the supplement would be categorized as a food. Conversely, a claim that an animal supplement affects the structure or function of the body apart from its nutritive value (a non-nutritive ingredient) is considered a drug claim, and the product will be categorized as a drug. Moreover, claims for (1) improved or increased production and performance, (2) alteration or improvement in function, or (3) treatment or prevention of disease are also considered drug claims, regardless of whether the ingredient is nutritive or non-nutritive. FDA has explicitly stated that pet supplements should not "bear such vague therapeutic suggestions as promotion of 'health,' 'stamina,' 'strength,' or that they are of any special value for breeding purposes or for show or racing purposes or for working animals, or that by virtue of their formulation [i.e., 'chelated,' 'timed release,' 'natural'] they are superior to the ordinary vitamin-mineral preparations of commerce" (FDA 2015a). Likewise, direct or implied representations

for the product as a tonic, conditioner, or toner are considered drug claims. Because of all this, the labeling of various nutraceuticals for animals typically will say something about "maintains normal conditions or supports something" for whatever the nutraceutical is really intended for, as a way of getting around drug claims.

On a case-by-case basis, CVM has permitted some exceptions and agreed to exercise regulatory discretion for certain references to "nutritional support" for specific organs or body functions. For instance, CVM has explained that it would not object to a claim that vitamin E serves as an antioxidant in the body of animals. AAFCO has obviously considered that nutraceuticals will be used by manufacturers and veterinarians and indirectly lists them in a sort of tacit way in the AFFCO book. AFFCO categorizes ingredients that are approved for use in different ways. Various ingredients that might be considered to be nutraceuticals can fall into these various categories. For example, various botanical or plant-based nutraceuticals can be found listed under the category of "Spices and other natural seasonings and flavorings." Others can be found listed under the category of "Essential oils, oleoresins (solvent-free) and natural extractives (including distillates)." Still other nutraceuticals can be found listed in other categories.

5 Claims for Probiotics

FDA has issued a special Compliance Policy Guide (CPG) related to products that are purported to contain live (viable) microorganisms (bacteria and/or yeast). FDA refers to such products as "direct-fed microbial products (probiotics)" (FDA 2015b). FDA's policy is as follows: a direct-fed microbial product with label/promotional claims for disease cure, mitigation, treatment, or prevention is a new animal drug and is adulterated under Section 501(a)(5) unless it is the subject of a NADA. If the claims are in promotional material that cannot be documented as labeling, the product is misbranded under Section 502(f)(1). A direct-fed microbial product with label/promotional claims for affecting the structure or function of the body is a new animal drug because the claims are not derived from its food properties (i.e., the product does not act only as a source of nutrition in the animal's body) and is adulterated under Section 501(a)(5) unless it is the subject of an approved NADA. Such claims are usually improved animal productivity claims. If the claims are in promotional material that cannot be documented as labeling, the product is misbranded under Section 502(f)(1). Ordinarily, a direct-fed microbial product that is not labeled/promoted with any therapeutic or structure/function claims but that contains one or more microorganisms not listed in the AAFCO Official Publication is a food additive and is adulterated under Section 402(a)(2)(C), unless it is the subject of a food additive regulation.

A direct-fed microbial product listed by the AAFCO Official Publication and labeled with the AAFCO-approved label statement for live microorganism content, and not labeled or promoted with any therapeutic or structure/function claims, will be regulated as a food as defined in Section 201(f)(3) and usually will not require FDA regulatory attention. It is anticipated that the states will monitor these products. However, if FDA has safety concerns about these products, it will treat them as not generally recognized as safe and will regulate them as food additives subject to FDA enforcement attention. AAFCO has adopted a standard statement for viable microorganism labeling. The AAFCO statement is, "Contains a source of live (viable) naturally occurring microorganisms," followed by a listing of each of the microorganisms and the content guarantee, as colony-forming units per gram. The guarantee expression is specified in Regulation 4(g) of the Uniform State Feed Bill in the AAFCO Official Publication. Additionally, AAFCO specifies that there should be a verifiable method to test the veracity of the microorganism-guaranteed content specified in the labeling.

A product containing microorganisms listed by the AAFCO Official Publication but not purported to contain live microorganisms and with no label/promotional representations other than as a source of designated nutrients will be regulated as a food as defined in Section 201(f)(3). Although claims as nutrient sources could be misleading, depending on directions for use, this type of product usually will not require FDA regulatory attention. It is anticipated that the states will monitor these products. However, if FDA has safety concern about these products, it will treat them as not generally recognized as safe and will regulate them as food additives subject to FDA enforcement attention.

6 Trade Organizations Related to Veterinary Nutraceuticals

The interest in veterinary nutraceuticals in the early 1990s resulted in the formation of several trade organizations. The North American Veterinary Nutraceutical Council (now defunct) was formed in 1996 by interested persons in industry, practice, and academia. It defined a veterinary nutraceutical as "a [non-drug] substance which is produced in a purified or extracted form and administered orally to a patient to provide agents required for normal body structure and function and administered with the intent of improving the health and well-being of animals." The commonality among novel ingredient products is that legally they are neither food, food additives, nor drugs as recognized by the FDA. As such, they undergo no premarket approval process, and neither safety, efficacy, nor manufacturing is assured. At least two groups have formed in North America with an interest in veterinary nutraceuticals: the Nutraceutical Alliance (2018)

based in Canada and the National Animal Supplement Council (NASC 2018), based in the USA. Founded in 2013, the Nutraceutical Alliance provides an interface between product development, product testing, animal and literature research, product registration, and the regulatory environment.

NASC is a nonprofit trade organization founded in 2001 that is comprised of companies committed to providing health supplements and nutritional supplements of the highest quality for companion animals, primarily dogs, cats, and horses. The NASC has taken an assertive approach in trying to implement voluntary actions among nutraceutical manufacturers that will cause regulators to respond to their products in a positive fashion. NASC is trying to make sure that veterinary nutraceuticals that are on the market are safe to use on animals. Novel ingredients often are used without doctor supervision. Up to 70% of humans do not report herbal use to physicians, in part because of their failure to recognize the products as drugs. Likewise, pet owners often do not cite nutraceutical or herbal use when queried regarding drug therapy for their pet. The availability through medically recognized and trusted sources often leads the consumer to assume both their accuracy in labeling, efficacy, and safety. Yet, the lack of regulations and guidelines should lead to the "buyer beware." NASC created a quality seal as a way for consumers to know that when they buy a product, they buy from a reputable manufacturer. Only NASC member companies operating under the stringent guidelines of NASC for manufacturing, labeling and adverse event reporting, and demonstrating responsible participation are permitted to use the NASC Quality Seal.

The FDA/CVM would consider marketing claims that if an ingredient used in an animal affects the structure or function of the body, then the active ingredient is generally considered a drug. This has led to confusion when it comes to marketing nutraceuticals. For example, glucosamine is not listed as an essential nutritive ingredient in the AAFCO Official Publication for either dogs or cats. Thus, a claim that a pet supplement helps to improve joint function based on glucosamine in the product would be a drug claim, and the supplement would be considered a drug. With the above FDA/CVM thinking in mind, many unapproved ingredients are currently marketed for use in animal supplements, including burdock, echinacea, ginseng, horsetail, and nettles. Currently, none of these ingredients are permitted for use in pet supplements with approval from the FDA/CVM or AFFCO. Obviously, the veterinary nutraceutical industry has a problem regarding marketing claims about whether glucosamine or any other nutraceutical is a drug or not.

The increased consumer demand for animal health supplements creates a significant issue for the industry in that many ingredients, even common ones found in human dietary supplements such as glucosamine, chondroitin, methylsulfonylmethane (MSM), etc., are not approved for use in animal food/feed. Surveys conducted by NASC indicate that there are over 400 ingredients currently marketed by NASC members that are unapproved for use in animal feed products. However, trade organizations have taken steps to be able to market glucosamine and these other nutraceuticals legally in the USA and other countries. In response to the "unapproved" status of these ingredients, NASC submitted ingredient definition applications in 2002 and 2003 petitioning FDA/CVM to allow the use of glucosamine and MSM in animal feed. These ingredients were chosen because nutritional requirements for them have not been established in any animals, including "healthy animals." Additionally, due to the marketing of human products, consumers recognize the use of these and other ingredients for purposes other than nutritional, i.e., providing benefits to support the structure and/or function of the body, or their use with specific medical conditions such as osteoarthritis.

7 Legally Marketing an Animal Nutraceutical in the USA

Nutraceutical products such as dietary supplements for humans that marketed for animals have only two possible legal categories under US law. They are either an animal food/feed or they are a drug. Dietary supplements, such as vitamins and minerals, fall into the category of animal feeds. Dietary supplements for animals such as vitamin and mineral products have been marketed for many years. Most of these products include ingredients that are approved food additives, generally GRAS substances, or ingredients listed in AFFCO. But for many nutraceuticals, these determinations leave the industry with only one other option under current law: marketing nutraceutical products as animal drugs consistent with their intended use. Legally marketing nutraceutical products as animal drugs requires the submission of a New Animal Drug Application to the FDA/CVM that demonstrates criteria for safety and efficacy have been satisfied. Submission of NADA for animal health supplements is problematic because of costs associated with development and with patent protection of natural substances. The courts have held that natural substances cannot be protected via intellectual property such as patents (Harrison 2014). The isolation and purification of an active ingredient from a plant may not be enough to allow patentability. Since many nutraceutical agents come from plants, this causes problems for manufacturers. Patents allow a company to recover the considerable investment in product development with an appropriate selling price of the product. The consumer has the ability to purchase human dietary supplements that may be similar in formulation and a less

expensive alternative for their companion animals than a patented animal product that has been through the NADA process.

In sum, in order to ensure that a pet supplement is legally marketed as an animal feed, each of the ingredients in the pet supplement should be listed in one of the three following methods: (1) FDA regulations at 21 C.F.R. Part 573 as an approved food additive, (2) FDA regulations at 21 C.F.R. Part 582 as a GRAS substance, or (3) the ingredient definitions in the AAFCO book (although the status of this category of ingredients is subject to change). In addition, any claims that the product affects the structure or function of the body of animals should be based on an essential nutritive ingredient, according to the AAFCO nutrient profiles.

If the supplement is marketed as a feed, it is recommend that labeling the product is in accordance with FDA requirements and the AAFCO feed labeling guide. However, not all states have adopted the AAFCO model regulations, and some states have adopted the model regulations with modifications. Accordingly, it is possible that some states require changes to the label depending on the product. Many states require that animal feed products, including supplements that are sold as feed, be registered with the state before being sold in that state.

Another option is to market the product with ingredients that are not included on the three listed methods above, with the knowledge that the product will technically be considered an unapproved new drug. Generally, FDA considers these products to be "un-approved drugs for which enforcement discretion may be exercised." Animal supplements are low on FDA's priority list, and the agency has generally not been active with enforcement actions in this area. Instead, enforcement is typically handled on the state level, which makes it a state-by-state issue. Some states are much more permissive when it comes to ingredients that are not included on one of the three lists discussed previously. However, there is a chance that one or more states will object to the sale of the product, and those states could ultimately suspend or prohibit the sale of the product in the state. Ultimately, it is a business decision for the company to make depending on how much risk it is willing to take.

If a company intends to market the product as a non-feed or health product, it is recommend evaluating whether the product can be marketed as a non-feed supplement under the NASC guidelines. If so, the NASC has published templates for labels for non-feed products. However, it is important to note that some states struggle with the NASC template and will likely require the nutraceutical product to comply with the feed regulations in that state. Manufacturers that market nutraceuticals that go the route of a non-feed supplement may avoid the requirements to register with many states as an animal feed and may avoid the annual fees associated with the same; however, these companies need to register as animal remedies in those states with remedy laws.

8 Regulatory Rules in Different Countries for Veterinary Nutraceuticals

In the USA, the FDA/CVM, AFFCO, and NASC all have some say in how veterinary nutraceuticals are regulated and marketed to a greater or lesser extent depending on the organization. While the FDA is authorized to act against any unsafe product on the market, in the European Union, the European Food Safety Authority (EFSA) does not have the same mandate (Santini et al. 2018). EFSA is a European agency funded by the European Union that operates independently of the European legislative and executive institutions (Commission, Council, Parliament) and EU member states. It was set up in 2002 following a series of food crises in the late 1990s to be a source of scientific advice and communication on risks associated with the food chain. The agency was legally established by the EU under the General Food Law—Regulation 178/2002. EFSA must authorize/approve in detail any health claim before it is considered at a national or European level prior to being put on the market following a specific request from member states, European Parliament, or stakeholders. Following the EFSA opinion, each European member country can decide independently to set specific approval regulations and/or authorization. In the USA, however, manufacturers and other stakeholders do not have to register their products with the FDA because there is no need to obtain FDA approval and/or authorization before producing or selling food supplements or nutraceuticals. Surveillance activity is relegated to governmental agencies, and manufacturers are responsible for ensuring that information reported on the product label is true and not misleading. In Canada, nutraceuticals are regulated more like a drug than as a food category (L'abbé et al. 2008).

Other countries have specific legislation; for example, Indian legislation does not ascribe any specific legal status to nutraceuticals. The government of India established the Food Safety and Standards Act (FSSA) in 2006 to introduce a legislation system. FSSA does not separate functional foods, nutraceuticals, and dietary supplements; instead, each is indicated as food for a special dietary application. It considers products with beneficial health claims to be similar to food without any statements about nutraceuticals with clinical trial results. In 2015, India notified the World Trade Organization of a draft regulation for nutraceuticals and foods for special diets and medical purposes. The draft regulation defined these categories based on ingredients, labeling, additives, contaminants, and health and nutritional claims. The draft regulation also determined the criteria for the manufacturing

and sale of these categories of foods and recommended doses or consumption levels. The new regulation, namely, Food Safety and Standards for Food for Health Supplements, Nutraceuticals, Food for Special Dietary Use, Food for Special Medical Purposes, Functional Food and Normal Food Regulations 2016, is based on and framed from Section 22 of the FSSA (FSSA 2018).

Japan, by contrast, was among the first countries to face the issue of regulating food supplements and foodstuff by issuing the Food for Specified Health Uses (FOSHU) based on a voluntary request from stakeholders for approval. This legislation, originally set in 1991, evolved into the 2003 Health Promotion Law (Yamada et al. 2008). Possible approval is available for food with beneficial health activities even if these activities are not substantiated with scientific evidence if the product meets the level of FOSHU requirements (safety, nutritionally appropriate ingredient content, etc.). Even food without an assessed and defined mechanism of effectiveness for its function, namely, qualified FOSHU and standardized FOSHU, can be considered. Any reduction of disease risk claim is allowed if the reduction of disease risk is clinically and nutritionally established in one ingredient.

In general, many countries, such as Australia or China, regulate nutraceuticals simply as a category of food, and the national regulations valid for food apply (Tapsell 2008, Yang 2008). A simple registration-based approach has been adopted by some countries, such as Colombia, Brazil, and Argentina. A notification-based approach addressed to the local competent authority is valid in Mexico and Chile. Nevertheless, other countries, such as Brazil, China, and Taiwan, have stricter requirements, and prior to registration, a complete animal or human clinical study is required. Based on this information, it is foreseeable that a safety assessment and complete clinical study may be necessary before any nutraceutical or any ingredient is put on the market. Moreover, a health claim should be authorized and attributed only after a complete clinical study is proposed to the appropriate authority for approval with the aim of substantiating its safety and efficacy with respect to the claimed beneficial health effect based on an understanding of the mechanism of action and the absence of undesired side effects (Fig. 1).

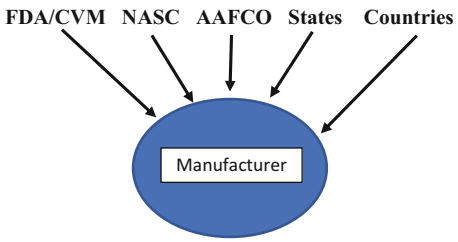


Fig. 1 Sources of nutraceutical regulations manufacturers face

9 The Future of Veterinary Nutraceutical Regulations

It is unclear how long the FDA/CVM will continue to recognize the definitions in the AAFCO Official Publication. In a memorandum of understanding entered into in 2007 and renewed in March 2015, FDA and AAFCO agreed to collaborate in the development and approval of animal feed ingredient definitions. However, also in March 2015, FDA announced its intention to bring the AAFCO ingredient definition and standards completely under the FDA GRAS and food additive framework. Specifically, the agency intends to (re)evaluate all feed ingredients that appear in the AAFCO book but are not listed in FDA regulations as GRAS or approved as food additives. FDA intends to determine whether there is sufficient scientific evidence to affirm the ingredient as GRAS or approve it as a food additive. When there is not sufficient evidence available for FDA to make a GRAS or food additive determination, the agency intends to require companies to submit food additive petitions in order to continue using an ingredient in animal feed products. Thus, while an ingredient might currently be permitted due to its listing in the AAFCO book, companies should understand that the permissibility of the ingredient is subject to change in the future (Harrison and Jackson 2016).

Because the present regulatory aspect of veterinary nutraceuticals is both complex and confusing to most veterinarians and manufacturers, a restructuring of the entire regulatory framework of dietary supplements would be helpful. A version of DSHEA for nutraceuticals intended for animals that models itself after NASC guidelines could be started. NASC already has an adverse reporting system in place that could be adopted to ensure veterinary nutraceutical safety. National competent authorities could ask manufacturers to provide data on the safety and mechanism of action supporting any claims contained on the labels of their products, especially when the term nutraceutical is used. Relevant information and data could be related to the complex of substances forming a nutraceutical and not refer to a single substance. What these new regulations will look like will depend on who wins the battle between what manufacturer want and the regulatory agencies want. A restructuring will not be easy but hopefully will result in a more clarity for veterinary nutraceutical regulations.

References

AFFCO (2018) www.affco.org
AVMA (2018) https://www.avma.org/About/Governance/Documents/2014W_2013W_Resolution3_Attch2.pdf
Boothe DM (2017) The use of nutraceuticals in veterinary medicine. https://www.isvma.org/wp-content/uploads/2017/10/Nutraceuticals_Dietary_Supplements.pdf

FDA (2015a) Compliance Policy Guide Section 690.100, Nut. https://www.fda.gov/ICECI/ComplianceManuals/CompliancePolicyGuidanceManual/ucm074708.htm
FDA (2015b) Compliance Policy Guide 689.100. www.fda.gov/ICECI/ComplianceManuals/CompliancePolicyGuidanceManual/ucm074707.htm
FSSA (2018) www.fssai.gov.in/
Harrison C (2014) Patenting natural products just got harder. Nat Biotechnol 32(5):403–404
Harrison T, Jackson M (2016) Pet nutrition: a legal rundown. Nutraceutical World, 04.01.16
JAVMA (15 Oct 2015) https://www.avma.org/News/JAVMANews/Pages/151015n.aspx
Kalra EK (2003) Nutraceutical—definition and introduction. AAPS PharmSci 5(3):Article 25
L'abbé MR, Dumais L, Chao E et al (2008) Health claims on foods in Canada. J Nutr 138:1221–1227
Mordor Intelligence (2018) Global pet food nutraceutical market—segmented by pet type, function, ingredient, and geography—growth, trends and forecast (2018–2023). https://www.mordorintelligence.com
National Research Council (2006) Nutrient requirements of dogs and cats. National Academies Press, Washington, DC
National Animal Supplement Council (2018) www.nasc.cc
Nutraceutical Alliance (2018) www.nutraceuticalalliance.com
Santini A, Cammarata SM, Capone G et al (2018) Nutraceuticals: opening the debate for a regulatory framework. Br J Clin Pharmacol 84(4):659–672
Schultz WB (1996) Federal Register/Vol. 61, No. 78/Monday, April 22, 1996
Tapsell LC (2008) Evidence for health claims: a perspective from the Australia–New Zealand region. J Nutr 138:1206–1209
Yang Y (2008) Scientific substantiation of functional food health claims in China. J Nutr 138:1199–1205
Yamada K, Sato-Mito N, Nagata J et al (2008) Health claim evidence requirements in Japan. J Nutr 138:1192–1198

Regulatory Aspects of Veterinary Nutraceuticals in the USA and Canada

Daljit Vudathala

Abstract

The Food and Drug Administration (FDA) in the US provides clarification, guidance, and regulatory oversight to protect human and animal health. The Center for Veterinary Medicine (CVM), a branch of FDA, ensures safety of veterinary medical products and animal feed including pet food. Nutraceuticals are neither food nor drugs and present a regulatory challenge. Although most nutraceuticals are generally regarded as safe, there is a growing concern about their safety, quality, and efficacy. To safeguard human health, the FDA has not provided any special provision to natural supplements intended to be included in animal feed. The FDA believes that there is insufficient information on the safe use of natural products in food animals to ensure human health from potential toxic residues. However, the use of oral natural supplements is not prohibited in pets if a product is harmless and not labeled to treat or cure an ailment. In Canada, veterinary nutraceuticals are defined as "natural supplements" and are regulated by Health Canada. Guidance includes information on permitted and excluded products, manufacturing, labeling, and adverse event reporting system. All products are required to obtain a notification number (NN) prior to sale in Canada. The objective of Canadian regulations is to facilitate the approval of safe products while keeping harmful supplements off the market to protect human and animal health.

Keywords

Veterinary nutraceuticals · Regulatory guidelines in the USA and Canada

D. Vudathala (✉)
PADLS New Bolton Center Toxicology Laboratory, Department of Pathobiology, School of Veterinary Medicine, University of Pennsylvania, Kennett Square, PA, USA
e-mail: vudathal@vet.upenn.edu

1 Introduction

Public health and safety is a core government function. Regulations and guidelines ensure consumer safety by keeping harmful food and health-care products from distribution to the consumer while enabling flow of safe products into the market. Manufacturers, distributors, and stakeholders are all expected to follow the regulations of a country where the product is going to be marketed. Regulations may require manufacturers and distributors to disclose factual information to prevent giving out inaccurate or misleading information. Accurate information of a food and health-care product empowers people to make informed choices and reduces the incidence of adverse health events.

Nutraceuticals, also known as dietary supplements or "functional foods," are part of a growing segment of the health-care industry (Childs 2000). A nutraceutical product is used to improve overall well-being, slow the aging process, prevent chronic diseases, and support bodily functions. A large number of products are currently sold in the market as alternatives to traditional medicine with the intent to prevent and treat various ailments (McKeever 2017). A common public perception is that pharmaceuticals are chemicals, synthesized in a laboratory, and therefore harmful. On the contrary, natural products are from the bounty of nature and therefore perceived to be safe. Focus on disease prevention and rising drug costs are some of the other driving forces for the increased use of nutraceuticals. A healthy diet and dietary supplements for overall well-being and prevention of chronic diseases to improve quality of life are sought by many consumers not only for themselves but also for their pets (Bauer 2001). Natural supplements to improve overall health and productivity of farm animals are routinely used as feed additives.

Products classified as nutraceuticals seem to reside in a gray area between food and pharmaceutical (Crandell and Duren 2007; Boothe 1997). Food is defined as a substance that provides nutrition, taste, or aroma. By comparison, a

R. C. Gupta et al. (eds.), *Nutraceuticals in Veterinary Medicine*, https://doi.org/10.1007/978-3-030-04624-8_57

drug is intended for diagnosis, cure, mitigation, treatment, or prevention of a disease in man or animals. Unlike human food, animal feed not only meets the nutritional requirements of a species but may also serve as a delivery mechanism of pharmaceuticals. A medicated feed can therefore be classified both as a food and a drug (US Food and Drug Adminstration 1998). Regulatory agencies make great strides to ensure the safety of humans and animals by monitoring the food supply and regulating the drug products.

2 FDA Perspective

Different branches of the Food and Drug Administration (FDA) in the US, are responsible for protecting the public health by ensuring the safety, efficacy, and security of human and veterinary drugs, biological products, and medical devices. Under the umbrella of the FDA, the Center for Veterinary Medicine (CVM) regulates products intended for use in animals (US FDA 1998). CVM ensures that animal feed, pet food, and treats are safe, are made under sanitary conditions, and are appropriately labeled. A drug is approved and then monitored for safety and efficacy throughout the life cycle of the product. Animal drugs may be administered directly or incorporated in feed.

In 1994, the FDA enacted the Dietary Supplement Health and Education Act (DSHEA) to enhance consumer safety without imposing unnecessary barriers that could slow the flow of safe products to consumers (National Institute of Health 1994). A framework of guidelines for dietary supplements (DS) was put in place to replace regulatory policy used to handle any concerns. Prior to the DSHEA, any reported adverse effects of a marketed product were handled on a case-by-case basis.

DSHEA defines a DS as a product (other than tobacco) intended to supplement the diet with one or more of the following ingredients:

(a) A vitamin
(b) A mineral
(c) An herb or a botanical
(d) An amino acid
(e) A dietary supplement used by man to supplement the diet by increasing the total dietary intake
(f) A concentrate, a metabolite, a constituent, an extract, or a combination of ingredients described in (a), (b), (c), (d), or (e)

Although dietary supplements are generally considered safe, the FDA takes action against products that are unsafe to protect consumers. According to the FDA, ingredients sold in the USA before October 15, 1994, are considered safe based on their historic use and don't need further safety evaluation. For a new dietary ingredient sold after October 15, 1994, the manufacturer must notify the FDA and provide reasonable evidence that it is safe for human consumption. Labeling requirements of a DS outlined to provide truthful information to the consumer are:

- The product must be labeled as a dietary supplement.
- Labeled not to treat, diagnose, prevent, or cure diseases.
- "Supplemental facts" replaces the "nutritional facts."
- The name of each ingredient is listed in descending order by weight or total quantities of all ingredients in a proprietary blend per serving.
- Herbs must list the part of the plant from which they originated (leaf, flower, root, etc.).
- Botanicals are listed by their common name.

The FDA received inquiries as to whether the DSHEA of 1994 was applicable to products intended for use in animals. In response, the CVM published an assessment in the Federal Register on April 22, 1996 (US FDA 1996), after examining the language, intent, and legislative history of the DSHEA. The definition of dietary supplement in the DSHEA doesn't specify if it includes products intended for use in animals other than humans. In their assessment, the FDA stated that on reviewing the complete DSHEA, it can be concluded that the Act was written for human and not animal products.

3 Inapplicability of DSHEA to Veterinary Products

The CVM concluded that the DSHEA was written without fully considering human food safety and animal health. Lack of information and safety concerns about potential residues in meat, milk, and eggs from animals given natural products was a major CVM concern (US FDA 1996). The FDA has concluded that dietary supplements intended for food-producing animals can't be given special treatment provided by the DSHEA to better protect public health. Furthermore, many products that are currently used to affect animal performance could potentially be covered as dietary supplements under the DSHEA. These products have been marketed with FDA approval after extensive scientific studies by the manufacturer to demonstrate safety and efficacy in animals and humans. The FDA believes that allowing a new product to be marketed under the provision of the DSHEA not only raises food safety concerns but provides an unfair advantage over existing products that have been approved after demonstration of safety and may act as a deterrent to develop innovative products in the future.

Finally, animal health is another concern addressed in the assessment. There is limited information on the safe use of dietary supplements in animals as compared to humans. Herbs and other botanical products have a history of use in humans to make a reasonable safety assessment for their

human use. However, the same ingredients lack sufficient safety data for use in animals. Furthermore, animal species differ in their nutritional requirements and react differently to various feed components. Absorption, metabolism, and transmission of an ingredient or its metabolites into meat, milk, or eggs can also vary in different animal species.

The assessment has concluded that to safeguard the human food supply and to protect animal health, the DSHEA of 1994 is not applicable to products for animal use. Under current regulations, natural supplements to be included in animal food are treated like any other feed additive.

4 Feed Additives

All additives in animal feed, including dietary supplements, require FDA approval after determining that the product will not leave harmful residues in meat, milk, and eggs (21 U.S.C. 348(b)(2) and (c)(5), and 21 CFR part 570) (US FDA 2018a). Approval of a feed additive intended for an animal species involves establishing a withdrawal period for the product in a finishing animal and tolerance levels in edible food. If a compound or any of its metabolites are carcinogenic, the act imposes additional requirements on its approval (21 U.S.C. 348(C) (3)(A) and 21 CFR part 500, subpart E). The use of a compound that can induce cancer in humans is prohibited for use in food-producing animals unless no residues of the carcinogenic compound can be demonstrated under conditions of use (US FDA 2012). A product may be eligible for "generally regarded as safe" (GRAS) status based on the view of qualified experts drawn from available and accepted data collected using scientific principles or through a common use in food prior to January 1, 1958 (US FDA 2018b).

Animal feed is regulated by the CVM in cooperation with state regulatory officials and partners who provide subject matter expertise in animal science and nutrition. The *Association of American Feed Control Officials* (AAFCO), a nonprofit organization with no regulatory authority, provides a forum for state and federal regulatory officials to come together and create model guidance to ensure that the regulation of animal feeds is as uniform as possible from state to state. Regulations include the establishment of uniform feed ingredient definitions and proper labeling to assure the safe use of feeds. AAFCO guidelines safeguard the health of animals and ensure consumer protection from harmful veterinary additives.

Labeling requirements for animal feed provide sufficient information to keep animals healthy and safe. A typical label includes:

- Brand name, if any
- Product name
- Purpose statement
- Guaranteed analysis
- List of ingredients
- Direction for use
- Warning or caution statements
- Name and address of manufacturer
- Quality statement

The purpose, list of ingredients, directions for use, and warning statements are all helpful in prevention of overdosing of feed additives to target animals or inadvertent exposure to nontarget species. Natural supplements to be incorporated in animal feed must meet CVM and AAFCO requirements.

5 Nutraceuticals for Pets

The CVM believes that animals on balanced rations receive an adequate amount of nutrients from natural ingredients or supplements that are added during feed manufacturing. The amounts of vitamins, minerals, protein, fat, and carbohydrates in a commercial pet food are adequate and meet the National Research Council nutrient requirements (US FDA 1995). The CVM has no objection to the marketing of oral nutritional supplements for companion animals, provided the product marketer meets the following restrictions:

1. Nutrient ingredient represented to be in the product is beneficial for each animal species for which the product is intended.
2. The product is labeled for use as a supplement and not as a substitute for good daily rations.
3. The product provides useful but not excessive amount of each of the nutrients it is represented to contain.
4. The label should not claim to cure or prevent a disease, including growth promotion.
5. The label should not make any false or misleading claims.
6. The potency and formulation of the product should not pose a health hazard to the target animal.

Non-compliance results in appropriate regulatory action against products that violate the policy with a "Warning" letter as the initial action to achieve compliance. The FDA holds the authority to take additional regulatory action against adulterated products that pose a health hazard (US FDA 1995). According to the FDA, the above policy is for oral products and excludes all injectable nutritional

supplements. Injectables are considered drugs and any issues are dealt with on a case-by-case basis.

6 Safety Concerns

Although natural products are considered safe, there is a growing concern about their safety in pets. The safety of a drug or an additive is generally assessed pre- and post-market approval. Prior to the sale of a product, studies are undertaken to identify potential adverse effects and then through surveillance studies to monitor for unexpected or previously identified adverse events. Currently, natural supplements are available for pets without pre-market approval. A product with safety data established in humans may not be safe in animals. Furthermore, a product that is safe in one species may not be safe in another. Current guidelines lack a robust post-market surveillance system to gather any adverse events.

Concerned with the safety of natural supplements, the FDA reached out to the National Research Council (NRC) to comment on safety in general and specifically the safety of three products: lutein, evening primrose oil, and garlic in pets. A committee of experts was formed to provide a safety assessment report and has published their findings (NRC 2009). The committee identified three main concerns that challenge the safety assessment of supplements:

1. Limited safety studies are conducted.
2. Lack of standardization among active ingredients.
3. Insufficient adverse event reporting system.

To strengthen the safety evidence of a supplement, the committee created a pyramid consisting of ten data supporting factors in increasing progression, starting with expert opinion as the base and meta-analysis as the apex. The list of parameters is as follows:

1. Expert opinion
2. In vitro and ex vivo research
3. Pathophysiologic rationale
4. Research in other species
5. Historical use/exposure
6. Case series/signals
7. Models of disease
8. Randomized controlled studies in target species
9. Epidemiologic studies
10. Meta-analysis

Information gathered by these various scientific means would be helpful in a safety evaluation. Safety study design needs to consider dosage and contamination in the supplement. Although the use of other species is generally helpful in assessing the safety of supplements, this provides no safety guarantee in the intended species. For example, a garlic supplement is considered safe for humans but may cause hemolytic anemia in dogs, cats, and horses (Cope 2005; Kovalkovičova et al. 2009; NRC 2009). A suitable safety study model of a supplement that naturally occurs in the diet of a surrogate and target animal that have a similar metabolic and natural dietary pattern would be preferred.

FDA regulations for veterinary DS are insufficient to fully address their safety, quality, and efficacy. Products are available for pets without any regulatory approval, and post-market surveillance is insufficient to properly monitor adverse events (Boothe 1998). Updated regulations are greatly needed to ensure safety and efficacy of products.

7 Regulations in Canada

Nutraceuticals intended for animals in Canada are defined as veterinary health products (VHPs) and are regulated by Health Canada. VHPs are used to maintain or promote the health and welfare of companion and food-producing animals. However, they are not for use to treat, prevent, or cure disease. In Canada, guidance and recommendation for veterinary products come from the Veterinary Drugs Directorate (VDD) under the direction of Health Canada. An Expert Advisory Committee on Veterinary Natural Health Products (EAC-VNHP or EAC) started meeting with VDD in 2008 to discuss the issues, goals, and priorities with regard to veterinary natural health products (Health Canada 2011). The committee outlined the following objectives to:

- Protect the well-being of animals from harmful products.
- Establish safety standards for products that are intended for use in food animals to protect human food supply, health, and the environment.
- Simplify regulations to facilitate approval of products that are presumed safe while keeping harmful products out of the market.

In addition, the EAC wanted to establish quality control standards for the manufacture of products and efficient post-market surveillance to allow Health Canada to take swift action against a non-compliant or a harmful product. The EAC also wanted to create standards for efficacy claims that would be helpful to consumers in making informed decisions.

A temporary Interim Notification Pilot Program (INPP) was launched in 2012 for low-risk veterinary health products, followed by a notification program that came in effect on November 13, 2017 (Health Canada 2012, 2017). The notification program was initiated with the objective to provide a flexible and risk appropriate regulatory framework for veterinary health products (VHPs) that have a history of safe use. A compliance and enforcement approach is based on the potential risk with authority to take appropriate action against

non-compliance and to stop the sale of an unsafe product. A notification number (NN) must be obtained as part of the program within the timeframe outlined below:

- At least 30 days before selling a VHP for the first time in Canada
- At least 30 days before making a change to a VHP that is already notified in Canada
- Before importing a VHP into Canada

Health Canada has provided guidance to comply with the notification program consisting of information on:

- Included and excluded products
- Label and claim requirements
- Manufacturing guidelines
- Reporting of adverse reactions
- Compliance and enforcement

8 Included and Excluded Products

VHPs are considered low-risk drugs that may contain ingredients, such as:

- Vitamins
- Minerals
- Traditional medicines

Oral, topical, otic, and dental/periodontal products, depending upon the ingredients, are included in this category. Traditional, homeopathic, and nonmedical substances that may be used to promote or maintain health are allowed, with a required warning on the product label (Health Canada 2017). Permitted product details are available at https://health-products.canada.ca/vhp-psa/en/about/3, and a current list of permitted substances is maintained and can be obtained at https://health-products.canada.ca/vhp-psa/en/substance-list.

A number of products, depending upon the purpose, route of administration, types, and classification, are excluded from the category. A product intended to cure, treat, or diagnose an animal is excluded. Any product that needs to be injected, inhaled, or delivered via intramammary or intrauterine route is not permitted under the program. Ophthalmic, transdermal patches and implants are also not included under the VHP provision.

Product types excluded are:

- Medicated feed and pet food
- Prescription drugs and controlled substances
- Dewormers and insect repellants
- Antimicrobial and hormones except botanicals with antimicrobial and mild hormonal activity
- Teat dips
- Specified risk materials (SRMs) of ruminants with potential risk of bovine spongiform encephalopathy (BSE) causing prions and including tissues, such as brain, skull, spinal cord, tonsil, eyes, etc.
- Combination products of homeopathic and traditional Chinese medicine with other types of ingredients such as vitamins or botanicals which are not in the same healing model

A complete list and the details of the currently excluded products are available at the Health Canada website https://health-products.canada.ca/vhp-psa/en/about/4. A current list of notified products is being maintained and can be obtained at https://health-products.canada.ca/vhp-psa/en/product-list.

9 Labeling Guidance

Health Canada guidelines require that labels and claims must not be false or misleading to create an inaccurate impression regarding the benefit or safety of a VHP. A product can claim to help a bodily function but not to cure a disease. For example, a product can have a claim "Maintains balance of healthy microflora" but not "Helps combat digestive problems." Homeopathic products consisting of a single ingredient can be labeled "Homeopathic," "Homeopathic Medicine," "Homeopathic Remedy," or "Homeopathic Preparation." A homeopathic product consisting of multiple ingredients can have a general health claim based on data in *Homeopathic Materia Medica*. A traditional Chinese medicine product can have the claim of traditional use but not for treatment or prevention, if the product has been used for a minimum of 50 consecutive years and its preparation and use have not changed. An example of an acceptable claim will be "In Traditional Chinese Medicine . . . used to replenish Qi."

Specific claims are permitted under the following conditions:

- An ingredient is absent in a product, and the ingredient is a safety concern and could potentially be present in the product.
- An ingredient can be listed on a claim made if its presence in sufficient quantity would have the expected effect.
- Absence of side effects is permitted if the claim is based on scientific evidence, and the incidences of side effects don't exceed that from placebo.

In addition, the manufacturer is responsible for naming the product such that the name will not cause confusion due to sound-alike or look-alike names. Promotional terms such as safe, nontoxic, nonpoisonous, or non-allergenic are considered misleading and prohibited. However, the word "safe" can

be used in providing directions such as "for safe use, do not exceed three tablets daily." Words like "natural alternative to. . ." or "alternative to. . ." are considered misleading and not permitted as such statements can potentially delay proper treatment of an animal, when needed (Health Canada 2012).

Details of the permissible statements that can be used for general and specific claims, including reference to homeopathic and Chinese VHP, can be obtained at https://health-products.canada.ca/vhp-psa/en/about/7.

Label recommendations provided in the guidance are:

- Brand name
- Dosage form
- Notification number (NN)
- Name and address of the manufacturer or distributor
- Lot number
- Expiration date or shelf life
- Adequate directions for use
- Quantity of each active (medicinal) ingredient per dosage unit
- Net amount (net contents)
- List of excipient ingredients (i.e., non-medicinal ingredients)
- Contact information "For questions or to report a side effect"

Label requirements are written to prevent an inaccurate or misleading impression about the nature, usefulness, safety, recommended dosage, quantity, and composition of a product. Contact information readily available on the label would be helpful in reporting adverse effects to the manufacturer or to a Canadian representative of an imported product. Details and additional information about VHP label requirements can be obtained at https://health-products.canada.ca/vhp-psa/en/about/6.

10 Manufacturing Requirements

Manufacturing of VHP is required to follow Part 3—Good Manufacturing Practices (GMPs) described in the Natural Health Products Regulations (SOR/2003-196). GMP guidelines include the specification, premises, equipment, personnel, sanitation program and operation, as well as the quality assurance program of the manufacturer (Health Canada 2012).

11 Reporting of Adverse Effects

A manufacturer or a Canadian representative, if the product is imported, is required to monitor and maintain a record of adverse reactions of a marketed product. All serious adverse reactions must be reported within 15 days of receiving event information. Adverse reaction reports are used by Health Canada to issue advisories or warnings and to change safety information of a product when required. Health Canada has the authority to remove any unsafe product from the market (Health Canada 2012).

12 Compliance and Enforcement

Health Canada approaches a regulatory non-compliance based on the risk posed to the general public and has authority to take appropriate action to address complaints, where non-compliance is found (Health Canada). Applicable actions are outlined in the Compliance and Enforcement Policy (POL_001) of Health Canada and are available at http://www.hc-sc.gc.ca/dhp-mps/compli-conform/gmp-bpf/pol/pol_1_tc-tm-eng.php.

Any changes to the notified VHP that need to be made following Health Canada review and approval, and major changes may result in a new NN for the product. Major changes include route of administration, dosage, duration of use, active ingredients, recommended use or purpose, and intended species.

Clear and precise Health Canada guidelines cover all major topics to ensure the safety of DS in animals and to protect the human food supply. Objectives, lists and details of included and excluded products, labeling requirements, manufacturing guidelines, and an adverse event reporting system are clearly outlined on the Health Canada website, easily accessible to manufacturers, distributors, consumers, and the general public.

13 Concluding Remarks and Future Directions

Nutraceuticals' legal definition and the products included are somewhat dissimilar in the USA and Canada. Nutraceuticals are called "dietary supplements" in the USA and "veterinary health products" in Canada. In the USA only oral products are considered dietary supplements, whereas in Canada topical and otic products are also permitted, depending upon the ingredients. However, in both Canada and the USA, nutraceuticals are not intended to cure, treat, or prevent a disease in animals.

Regulatory variations between the USA and Canada would hinder trade and marketing of nutraceutical products. Current FDA regulations are inadequate to fully address the safety, efficacy, and quality of natural supplements. Although no special provision is being given to supplements intended to be incorporated in feed in order to protect human health, products are being sold for pets without any pre-market approval. Safety is the biggest concern. Guidelines to assess the safety of a supplement in different species and appropriate dosage are needed. Effective regulations to ensure that

manufacturing meets quality standards of products that are free of contaminants are required. In addition, a comprehensive surveillance system to report adverse events to the manufacturer is required to fully assess the safety of the marketed products. On the contrary, Health Canada has outlined extensive guidelines for natural products to ensure animal safety without creating an unnecessary barrier for the sale of safe products. Prior to the sale, the manufacturer or the distributor is required to register and provide evidence to support the claim that the product is safe and effective. Guidelines have covered types of products that are included or excluded, manufacturing standards and labeling requirements to ensure safety and efficacy. A comprehensive system to record adverse events has been established with risk-based actions to deal with non-compliance to protect the general public.

Although many challenges exist in addressing safety, efficacy, and quality of nutraceuticals, comprehensive guidelines are necessary to ensure a product's safety and usefulness. Appropriate regulations are essential to ensure a product's safety and efficacy at proper dosage. The challenges with nutraceuticals can be resolved by scientists and regulators working together both nationally and internationally to provide a harmonized approach to get safe and effective products that are alternative to traditional medicine to the market, to meet the growing demand.

References

Bauer JE (2001) Evaluation of nutraceuticals, dietary supplements, and functional food ingredients for companion animals. J Am Vet Med Assoc 218(11):1755–1760

Boothe DM (1997) Nutraceuticals in veterinary medicine. I. Definitions and regulations. The Compendium on continuing education for the practicing veterinarian (USA)

Boothe DM (1998) Nutraceuticals in veterinary medicine. Part II Safety and efficacy. Compend Contin Educ Pract Vet 20(1):15

Childs NM (2000) Nutraceutical industry trends. J Nutra Funct Med Foods 2(1):73–85

Cope RB (2005) Allium species poisoning in dogs and cats. Vet Med 100(8):562

Crandell K, Duren S (2007) Nutraceuticals: what are they and do they work? J Biotechnol 34(3):29–36

Health Canada (2011) Expert advisory committee on veterinary natural health products. Available at: https://www.canada.ca/en/health-canada/services/drugs-health-products/public-involvement-consultations/veterinary-drugs/advisory-committees/expert-advisory-committee.html

Health Canada (2012) Interim notification pilot program. Available at: https://www.canada.ca/en/health-canada/services/drugs-health-products/veterinary-drugs/other-issues/interim-notification-pilot-program-low-risk-veterinary-health-products.html

Health Canada (2017) About the VHP notification program. Available at: https://health-products.canada.ca/vhp-psa/en/about/1

Kovalkovičová N, Šutiaková I, Pistl J (2009) Some food toxic for pets. Interdiscip Toxicol 2(3):169–176

McKeever KH (2017) Nutraceuticals: a goldmine but for whom? Comp Exerc Physiol 13(3):121–126

National Research Council (2009) Safety of dietary supplements for horses, dogs, and cats. National Academies Press, Washington, DC

National Instititute of Health (1994) Office of dietary supplements-Dietary supplement health and education act of 1994. Available at: https://ods.od.nih.gov/About/DSHEA_Wording.aspx

US FDA (1995) Compliance policy guides—CPG Sec. 690.100 nutritional supplements for companion animals. Available at: https://www.fda.gov/ICECI/ComplianceManuals/CompliancePolicyGuidanceManual/ucm074708.htm

US FDA (1996) Food and drug administration [Docket No. 95N–0308] inapplicability of the dietary supplement health and education act to animal products. Available at: https://www.gpo.gov/fdsys/pkg/FR-1996-04-22/pdf/96-9780.pdf

US FDA (1998) Center for veterinary medicine regulating animal foods with drug claims. Guide 1240.3605. Available at: https://www.fda.gov/downloads/AnimalVeterinary/GuidanceComplianceEnforcement/PoliciesProceduresManual/UCM046883.pdf

US FDA (2012) Code of Federal Regulations Title 21, 500.82. Available at: https://www.gpo.gov/fdsys/pkg/CFR-2018-title21-vol6/pdf/CFR-2018-title21-vol6-sec500-82.pdf

US FDA (2018a) Animal and veterinary product regulation. Available at: https://www.fda.gov/AnimalVeterinary/Products/AnimalFoodFeeds/ucm050223.htm

US FDA (2018b) CVM animal food GRAS notice description. Available at: https://www.fda.gov/AnimalVeterinary/Products/AnimalFoodFeeds/GenerallyRecognizedasSafeGRASNotifications/ucm192224.htm

Regulatory Guidelines for Nutraceuticals in the European Union

Doriana Eurosia Angela Tedesco and Petra Cagnardi

Abstract

Nutraceuticals include a wide range of substances that can be used as medicinal products, feed material, or feed additives. This makes a substantial difference in the regulatory aspect of the marketing authorization in the European Union (EU) because for obtaining the appropriate marketing authorization, different procedures have to be followed. Since specific regulations do not apply to nutraceuticals, when they are used as feed additives for animal nutrition, they shall comply with Regulation No 1831/2003 on additives for use in animal nutrition. While nutraceuticals are administered as feed ingredients, they must comply with Commission Regulation (EU) No 68/2013. If nutraceuticals are administered with medical claims or if they exert a pharmacological effect, their use must comply with Directive 2001/82/EC. The EU legislation on this topic is very detailed and complex. Nevertheless, it allows the obtaining of marketing authorization with a wide safety margin, precautionary for animal health, human health, and the environment. The Scientific Committees and panels of the European Food Safety Authority (EFSA) are responsible for producing opinions that are used by the European Commission to adopt legislation related to animal nutrition. For veterinary medicinal products, the responsibility for marketing authorization is both granted by competent national authorities of the Member States or by the European Medicine Agency (EMA). This chapter describes legislation that is relevant to the marketing authorization of nutraceuticals for animals in the EU, elucidating the different categories of use, i.e., feed materials, feed additives, and veterinary medicinal products.

Keywords

Nutraceuticals · European guidelines · Feed materials · Feed additives · Veterinary medicinal products

1 Introduction

The use of nutraceuticals in animals has seen a substantial increase in the last few years, and several issues have arisen related to the correct identification for their use and how to obtain the appropriate authorization. In the category of nutraceuticals, there are a wide range of substances that can be used as medicinal products, feed material, or feed additives. The substantial differences in the regulatory aspects for marketing authorization in the European Union (EU) are strictly correlated with the lack of specific regulations on nutraceuticals and from the difficulties in categorizing them for a specific use. If nutraceuticals are administered with medical claims or if they exert a pharmacological effect, their use must to comply with the body of European Union legislation in the pharmaceutical sector for medicinal products for veterinary use according to Commission Directive 2001/82/EC (EC 2001).

Nutraceuticals can be administered as a feed or feed additive when they do not have a medical claim or a pharmacological effect. If nutraceuticals are administered as feed ingredients, they must comply with Commission Regulation (EU) No 68/2013 (EU 2013). When used as a feed additive for animal nutrition, nutraceuticals as herbs or their extracts, probiotics and prebiotics, shall comply with Regulation (EC) No 1831/2003 on additives for use in animal nutrition (EC 2003a). Some nutraceuticals are beneficial as their inclusion in the diet favorably affects animal welfare, the maintenance of animal health and of a healthy digestive system. In

D. E. A. Tedesco (✉)
Department of Environmental Science and Policy, Università degli Studi di Milano, Milano, Italy
e-mail: doriana.tedesco@unimi.it

P. Cagnardi
Department of Health, Animal Science and Food Safety, Università degli studi di Milano, Milano, Italy
e-mail: petra.cagnardi@unimi.it

R. C. Gupta et al. (eds.), *Nutraceuticals in Veterinary Medicine*, https://doi.org/10.1007/978-3-030-04624-8_58

any case, if nutraceuticals are administered as veterinary medicinal products, feed ingredient, or feed additive, only products with appropriate marketing authorization can be used.

The complexity of the marketing authorization of veterinary medicines, especially in food-producing animals, greatly limits the presence of some nutraceuticals (herbs) among the currently allowed molecules listed in Table 1 of the Commission Regulation (EU) No 37/2010 (EU 2010). In the list of allowed molecules, homeopathic compounds are included. In this case, the high dilution prevents the possible risks correlated with the dose administered. For food-producing animals, the risk of residues in edible tissues requires the definition of maximum residual limits (MRL) for all compounds administered according to Regulation (EC) No 470/2009 (EC 2009a).

In the same way, the EU legislation for inclusion of nutraceuticals in feed additives is very complex and limits new applications for marketing authorization.

2 Guidelines for the Distinction Between Feed Materials, Feed Additives, and Veterinary Medicinal Products

The distinction between feed materials, feed additives, and other products such as veterinary drugs has implications for placing them on the market, depending on the relevant applicable legislation. In order to provide an appropriate level of legal certainty, the European Commission has recently adopted Recommendation No 2011/25/EU (EU 2011) to provide non-binding guidelines for the distinction between feed materials, feed additives, and veterinary medicinal products. The aims of these guidelines are (a) to avoid inconsistencies in the treatment of such products, (b) to facilitate the work of national competent authorities, and (c) to help interested economic operators to act in a framework that provides an appropriate level of legal certainty. Guidelines are based on the provisions laid down in the legislative framework governing the different kinds of products concerned and their particular definitions.

2.1 Feed Legislation

According to the food law legislation reported in Regulation (EC) No 178/2002 (EC 2002), the following definitions can be found. Feed is any substance or product, including additives, whether processed, partially processed, or unprocessed, intended to be used for oral feeding to animals. Regulation (EC) No 767/2009 states in detail that "feed may take the form of feed materials, compound feed, feed additives, premixtures or medicated feeding stuffs" (EC 2009b).

Feed materials are products of vegetable or animal origin whose principal purpose is to meet animals' nutritional needs, in their natural state, fresh, or preserved, and products derived from the industrial processing thereof, as well as organic or inorganic substances, that may or may not contain feed additives, which are intended for use in oral animal feeding. Feed materials are primarily used to meet animals' needs for energy, nutrients, minerals, or dietary fibers. They are usually not chemically well-defined except for basic nutritional constituents. Effects which can be justified by scientific assessment and which are exclusive to feed additives or veterinary drugs should be excluded from the objective uses of feed materials (EC 2009b).

Feed additives are substances, microorganisms, or preparations, other than feed material (a product cannot be a feed material and a feed additive at the same time), which are intentionally added to feed or water in order to perform one or more specific function. According to Regulation (EC) No 1831/2003, the feed additive shall (a) favorably affect the characteristics of feed; (b) favorably affect the characteristics of animal products; (c) favorably affect the color of ornamental fish and birds; (d) satisfy the nutritional needs of animals; (e) favorably affect the environmental consequences of animal production; (f) favorably affect animal production, performance, or welfare, particularly by affecting the gastrointestinal (GI) flora or digestibility of feed stuffs; or (g) have a coccidiostatic or histomonostatic effect. Coccidiostats and histomonostats are substances intended to kill or inhibit protozoa and are the only veterinary medicinal products used as feed additives (EC 2003a).

Feed additives are defined by their functions and are intentionally added for those specific functions. However, these functions are not exclusive to feed additives. Thus, a feed material can also exert an additive function (e.g., as a thickener), but this should not be the only intended use (EU 2011).

Veterinary medicinal products (VMPs) are any substance or combination of substances presented as having properties for treating or preventing disease in animals or any substance or combination of substances that may be used in, or administered to, animals with a view either to restore, correct, or modify physiological functions by exerting a pharmacological, immunological, or metabolic action or to make a medical diagnosis. Substance is intended to represent any matter of the following origin: human (i.e., blood and blood products), animal (i.e., microorganisms, whole animals, parts of organs, animal secretions, toxins, extracts, blood products), vegetable (i.e., microorganisms, plants, parts of plants, vegetable secretions, extracts), and chemical (i.e., elements, naturally occurring chemical

materials and chemical products obtained by chemical change or synthesis) (EC 2001).

To confirm the substantial differences between feed materials and feed additive and VMPs, the labeling or the presentation of feed materials shall not claim that it will prevent, treat, or cure a disease, except for coccidiostats and histomonostats (EC 2009b).

In the same recommendation (EU 2011), the following definitions are reported:

Feed intended for particular nutritional purposes, regulated by Commission Directive 2008/38/EC, means feed which can satisfy a particular nutritional purpose by virtue of its composition or method of manufacture, which clearly distinguishes it from ordinary feed. A list of the intended uses of animal feed stuffs for particular nutritional purposes was established. This list indicates the precise use, i.e., the particular nutritional purpose (EC 2008a). Feed intended for particular nutritional purposes does not include VMPs or medicated feeding stuffs within the guidelines of Directive 90/167/EEC (EC 2009b; ECC 1990). Particular nutritional purposes (i.e., support of liver function in the case of chronic liver insufficiency, reduction of urate stones formation, or reduction of the risk of milk fever) can be achieved by feed. Feed intended for particular nutritional purposes meets the specific nutritional needs of animals whose process of assimilation, absorption, or metabolism is, or could be, temporarily or irreversibly impaired and who can therefore benefit from the ingestion of feed appropriate to their condition (EC 2009b).

Medicated feeding stuffs are not VMPs but, according to Regulation (EC) No 767/2009, a form of feed containing medicated premixes subject to a prescription by a veterinarian (EC 2009b).

3 Nutraceuticals as Feed Material

If nutraceuticals are administered as functional feed, they need to be included in the feed ingredients. The feed materials permitted in animal nutrition are reported in the Community Catalog of Feed Materials (EU 2013). This Catalog has been created as a tool to improve the labeling of feed materials and compound feed, according to Article 24 of Regulation (EC) No 767/2009 (EC 2009b).

All feed materials reported in the Catalog comply with restrictions on the use of feed materials in accordance with the relevant legislation of the Union. Feed business operators using a feed material entered in the Catalog shall ensure that it complies with the safety and marketing requirements set forth in Article 4 of Regulation (EC) No 767/2009 (EC 2009b), for example, the botanical purity of a feed material not less than 95%, the chemical impurity level resulting from manufacturing process and from processing aids, lacking or with a fixed, maximum content according to Regulation (EC) No 183/2005 (EC 2005). Particular attention shall be paid to compliance with Regulation (EC) No 1829/2003 for feed materials that are, or are produced from, genetically modified organisms or result from a fermentation process involving genetically modified microorganisms (EC 2003b).

The feed materials, which have a natural presence of microorganisms, may be placed on the market as the intended use of the feed materials is not linked to a function exerted by microorganism(s) according to Annex I of Regulation (EC) No 1831/2003 (EC 2003a). The presence of microorganisms, as well as any function resulting thereof, shall not be claimed on the feed materials and the compound feed containing them.

In part C of Commission Regulation (EU) No 68/2013, the list of feed materials reports the name of feed materials, the description, and the compulsory declarations (EU 2013).

The use of this Catalog (EU 2013) by the feed business operators shall be voluntary. However, the name of a feed material listed in the Catalog may be used only for a feed material complying with the requirements of the entry concerned. The person who, for the first time, places a feed material on the market that is not listed in the Catalog shall immediately notify the representatives of the European feed business sectors. The representatives of the European feed business sectors shall publish a register of such notifications on the Internet and update the register on a regular basis (EC 2009b). The Catalog was last updated with Regulation (EU) 2017/1017 (EU 2017).

Among feed materials, *Medicago sativa* is one of the most important crops used as fodder for livestock, and it is known as a source of natural bioactive compounds with potential health-promoting effects (Rafińska et al. 2017). Oats (*Avena sativa*) and barley (*Hordeum vulgare*) are two cereal grains that are good sources of β-glucan, a water-soluble fiber fraction that has plasma lipid- and glycemic-lowering effects. Therefore, the use of oats and barley as functional ingredients in pet foods may be beneficial in the control or prevention of obesity, diabetes mellitus (DM), and dyslipidemia (de Godoy et al. 2013). Fenugreek presents a unique and rare nutraceutical opportunity for providing multiple health benefits (Garg 2016). Many other different feed materials with nutraceutical properties can be found in the Catalog of Feed Material (EU 2013). In Table 1 some examples are reported.

4 Nutraceuticals as Feed Additives

The conditions for the use of feed additives are established by Regulation (EC) 1831/2003. Only those additives approved under the procedure provided for in this Regulation can be placed on the market, used, and processed in animal feeding under conditions set out in the authorization (EC 2003a).

Table 1 Examples of feed materials from Commission Regulation (EU) No 68/2013 on the Catalog of Feed Materials

Number	Name	Description	Compulsory declarations
1.1.1	Barley	Grains of *Hordeum vulgare* L. It may be rumen protected	
1.4.1	Oats	Grains of *Avena sativa* L. and other cultivars of oats	
2.8.1	Linseed	Seeds of linseed *Linum usitatissimum* L. (minimum botanical purity 93 %) as whole, flattened, or ground linseed. It may be rumen protected	
2.18.11	Soya (beans)	Soya beans (*Glycine max* L. Merr.)	Urease activity if > 0.4 mg N/g × min
3.5.1	Fenugreek seed	Seed of fenugreek (*Trigonella foenum-graecum*)	
4.1.14	Fructo-oligosaccharides	Product obtained from sugar beet through an enzymatic process	Moisture if > 28 %
4.3.1	Carrots	Root of the yellow or red carrot *Daucus carota* L.	
4.4.1	Chicory roots	Roots of *Cichorium intybus* L.	
4.4.9	Inulin (the name shall be supplemented by the plant species)	Inulin is a fructan extracted from roots of *Cichorium intybus* L., *Inula helenium*, or *Helianthus tuberosus*; raw inulin may contain up to 1 % sulfate and 0.5 % sulfite	
4.5.1	Garlic, dried	White to yellow powder of pure, ground garlic, *Allium sativum* L.	
5.1.1	Acorn	Whole fruits of the pedunculate oak *Quercus robur* L., the sessile oak *Quercus petraea* (Matt.) Liebl., the cork oak of *Quercus suber* L., or other species of oak	
5.3.1	Anise seed	Seeds of *Pimpinella anisum*	
5.12.1	Broken chestnuts	Product of the production of chestnut flour, consisting mainly of particles of endosperm, with fine fragments of envelopes and a few remnants of chestnut (*Castanea* spp.)	Crude protein Crude fiber
5.25.1	Grape pips	Pips from *Vitis* L. separated from grape pulp, from which the oil has not been removed	Crude fat Crude fiber
5.28.1	Perilla seed	Seeds of *Perilla frutescens* L. and its milling products	
5.31.1	Plantago seed	Seeds of *Plantago* (L.) spp.	
5.34.1	Thistle seed	Seeds of *Carduus marianus* L.	
5.36.1	Yarrow seed	Seeds of *Achillea millefolium* L.	
6.10.1	Lucerne; [alfalfa]	*Medicago sativa* L. and *Medicago* var. Martyn plants or parts thereof	Ash insoluble, in HCl if > 3.5 % of dry matter
7.1.1	Algae (the name shall be supplemented by the plant or algae species)	Algae, live or processed, including fresh, chilled, or frozen algae. May contain up to 0.1 % antifoaming agents	Crude protein Crude fat Crude ash
7.9.1	Liquorice root	Root of *Glycyrrhiza* L.	
7.10.1	Mint	Product obtained from drying aerial parts of the plants *Mentha spicata*, *Mentha piperita*, or *Mentha viridis* (L.), regardless of their presentation	

Nutraceuticals can be used as functional feed additives. These feed additives can adequately stimulate the local defensive responses and favorably influence resident GI microflora for optimizing growth and intestinal health status. Additives protect animals against physiopathological disturbances but are also able to improve nutrient digestion and absorption (Domeneghini et al. 2006). Plants/herbs and their extracts are being used increasingly not only as sensory additives but also for other purposes, notably better growth or feed conversion, improved meat quality, and for prophylaxis. Their use is further increased as replacement additives for the antibiotic growth promoters which were prohibited at the end of 2005 (Franz et al. 2005). Among other nutraceutical substances included in the feed additives categories are probiotics, prebiotics, vitamins, antioxidants, and some amino acids.

In order to protect human health, animal health, and the environment, feed additives should undergo a safety assessment through a community procedure before being placed on the market, used, or processed within the community. Any person seeking an authorization for a feed additive or for a new use of a feed additive shall submit an application. Regulation (EC) No 1831/2003 establishes the rules governing the community authorization of additives for use in animal nutrition. Moreover, Regulation (EC) No 429/2008 (EC 2008b) provides detailed rules for the implementation of Regulation (EC) No 1831/2003 concerning the preparation and presentation of applications and the assessment and authorization of feed additives.

The applicant should specify the intended effect of the additive and make a proposal for the classification of the

Table 2 Categories of feed additives (excluding coccidiostatic or histomonostatic) and examples of the most functional groups (EC 2003a)

Categories of feed additives	Examples of functional groups
Technological additives: Any substance added to feed for a technological purpose	• Antioxidants: substances prolonging the storage life of feed stuffs and feed materials by protecting them against deterioration caused by oxidation • Stabilizers: substances which make it possible to maintain the physicochemical state of feed stuffs • Binders: substances which increase the tendency of particles of feed stuffs to adhere • Anticaking agents: substances that reduce the tendency of individual particles of a feed stuff to adhere • Acidity regulators: substances which adjust the pH of feed stuffs • Denaturants: substances which, when used for the manufacture of processed feed stuffs, allow the identification of the origin of a specific food or feed materials • Substances for reduction of the contamination of feed by mycotoxins: substances that can suppress or reduce the absorption, promote the excretion of mycotoxins, or modify their mode of action • Hygiene condition enhancers: substances or, when applicable, microorganisms which favorably affect the hygienic characteristics of feed by reducing a specific microbiological contamination
Sensory additives Any substance, the addition of which to feed improves or changes the organoleptic properties of the feed or the visual characteristics of the food derived from animals	• Colorants – Substances that add or restore color to feed stuffs – Substances which, when fed to animals, add colors to food of animal origin – Substances which favorably affect the color of ornamental fish or birds • Flavoring compounds: substances which increase feed smell or palatability
Nutritional additives	• Vitamins, provitamins, and well-defined chemical substances having similar effect • Compounds of trace elements • Amino acids, their salts, and analogs
Zootechnical additives Any additive used to favorably affect the performance of animals in good health or used to favorably affect the environment	• Digestibility enhancers: substances which, when fed to animals, increase the digestibility of the diet through action on target feed materials • Gut flora stabilizers: microorganisms or other chemically defined substances, which, when fed to animals, have a positive effect on the gut flora • Substances which favorably affect the environment

additive in one or more categories and functional groups according to its major effects (Table 2.).

Applications for authorization of feed additives should take into account different documentation requirements for food-producing and other animals. At the time of application for authorization, the applicant should send the following details and documents directly to the European Food Safety Authority (EFSA) and the inclusion into the Register of Feed Additives (European Union 2018):

(a) Name and address
(b) Identification of the feed additive, a proposal for its classification by category, and functional group and its specifications, including purity criteria
(c) A description of the method of production, manufacturing, and intended uses of the feed additive, a description of the method of analysis of the additive according to its intended use, and, where appropriate, the method of analysis for the determination of the level of residues of the feed additive, or its metabolites, in food
(d) A copy of the studies which have been carried out and any other material which is available to demonstrate that the feed additive fulfills safety criteria and has favorably effects
(e) Proposed conditions for placing the feed additive on the market, including labelling requirements and, specific conditions for use and handling, level of use in complementary feed stuffs and animal species, and categories for which the feed additive is intended
(f) A written statement that three samples of the feed additive have been sent by the applicant directly to the community reference laboratory
(g) For additives which do not belong to either technological or sensory category and for additives consisting of, containing or produced from GMOs, a proposal for post-market monitoring

Table 3 Examples of feed additives from Annex I: List of additives from the Register of Feed Additives (European Union 2018)

Category	Functional Group	Subclassification	Additive
2 (Sensory additives)	b (Flavoring compounds)	Natural products—botanically defined	*Astragalus membranaceus* L. = *A. pycnocladus* Boiss.et Haussk. ex Boiss.: *Astragalus* tincture
			Crataegus oxyacantha L.p.p. et auct.: Hawthorne tincture CoE 156
			Curcuma longa L.: turmeric extract CAS 8024-37-1 FEMA 3086 CoE 163 EINECS 283-882-1/turmeric oleoresin CAS 84775-52-0 FEMA 3087 CoE 163 EINECS 283-882-1/turmeric oil CAS 8024-37-1 FEMA 3085 CoE 163 EINECS 283-882-1/turmeric tincture CoE 163
			Echinacea purpurea (L.) Moench.: *Echinacea* absolute/*Echinacea* extract [cats and dogs]
			Eleutherococcus senticosus Rupr. et Maxim. = *Acanthopanax s.* Harms: taiga root extract/taiga root tincture
			Panax ginseng C. A. Mey.: Ginseng extract CoE 318 [cat and dog]
			Salix alba L.: white willow extract/white willow tincture
			Silybum marianum (L.) Gaertn. = *Carduus marianus* L.: Milk thistle extract CoE 551/milk thistle tincture CoE 551
			Zingiber officinale Rosc.: ginger oleoresin CAS 84696-15-1 FEMA 2523 CoE 489 EINECS 283-634-2/ginger oil CAS 8007-08-7 FEMA 2522 CoE 489 EINECS 283-634-2/ginger tincture CoE 489
4 (Zootechnical additives)	b (Gut flora stabilizers)	Gut flora stabilizers	*Saccharomyces cerevisiae*
			Enterococcus faecium
			Bacillus subtilis

(h) A summary containing the information provided in the previous points
(i) For additives falling within the scope of community legislation relating to the marketing of products consisting of, containing, or produced from GMOs, details of any authorization granted in accordance with applicable legislation

For certain additives, safety criteria (d) for the target animals can be presumed without the need for specific information. For all other additives, safety for the target animals can be assessed by extensive literature searches for studies on target animals, toxicity data (either existing or new) from repeated dose studies in laboratory animals, or tolerance studies in target animals.

In order to satisfy the assessment of additives in animal nutrition, and the rules concerning the procedure for the authorization, the European Food Safety Authority (EFSA) has published the following guidelines:

- Guidance on the assessment of the safety of feed additives for the target species (EFSA 2017a).
- Guidance on the assessment of the safety of feed additives for the consumer (EFSA 2017b)
- Guidance on the identity, characterization, and conditions of use of feed additives (EFSA 2017c)
- Guidance on the characterization of microorganisms used as feed additives or as production organisms (EFSA 2018a)
- Guidance on the assessment of the efficacy of feed additives (EFSA 2018b).

The authorized additive entered in the Register of Feed Additives shall be kept up to date (European Union 2018). Authorizations under Regulation (EC) No 1831/2003 shall be renewable for 10-year periods. An application for renewal shall be sent to the Commission at least 1 year before the expiration of the authorization (EC 2003a).

In the Register of Feed Additives, there are many examples of authorized additives with nutraceutical properties. Some of these additives represent a real alternative to in-feed antibiotics and include organic acids, prebiotics, and probiotics. Herbs and herbal products/botanicals represent a large array of nutraceuticals (Franz et al. 2005). In the category of sensory additives, a functional group of flavoring compounds (156 natural products—botanically defined) are included. Some examples of natural products—botanically defined—and gut flora stabilizers are listed in Table 3.

5 Nutraceutical Properties Claimed on Animal Feed Labels

In addition to the mandatory labeling requirements, the labeling of feed materials and compound feeds may also include voluntary labeling particulars. The labeling and presentation of feed may draw particular attention to the presence or the absence of a substance to a specific nutritional characteristic or to a specific function. The nutraceutical property can be highlighted in the voluntary labeling of feed for animals as described in Regulation (EC) 767/2009 (EC 2009a).

At the request of the competent authority, scientific substantiation of the claim is required. This allows the accuracy of the information given on the label to be verified, including the exact percentages by weight of feed materials used. Claims concerning optimization of nutrition and support or protection of physiological conditions are permitted, unless they contain a claim such as "it will prevent, treat or cure a disease" (EC 2009b). Claims considered medicinal, such as dosage, cures, treats, remedies, prevents, relieves, heals, or repairs, should be avoided. Conversely, claims not considered as medicinal can be used, such as use, administration, application, soothes, preparation, maintains, apply, cleanses, and health/healthy (FEDIAF 2011).

The Codes of Good Labelling Practice have been created as a tool to improve the labeling of feed materials and compound feeds in order to provide industry guidance to operators for the management of claims. The codes were developed for pet food (FEDIAF 2011) and for food-producing animals (COPA-COGECA/FEFAC 2016). Some claims can be particularly useful to provide information related directly to the nutraceutical. There is scientific evidence that particular feeds can make a significant contribution toward the promotion of health (e.g., support the immune function) and reduction of disease risk (e.g., decrease the risk of developing joint disease) (FEDIAF 2011).

A functional claim describes the effect of a feed material, nutrient, component, or additive on growth, development, or normal functions of the body. The claim may concern optimization of the nutrition and support or protection of the physiological conditions (Art. 13.2 EC 2009b). These effects go beyond meeting basic nutritional needs of the animals. The code of practice on voluntary labeling is applicable to compound feeds and feed materials used for food-producing and non-food-producing animals. Table 4 lists the authorized claims that can be used on the label when the scope is to underline some characteristics related to the nutritional, compositional, and functional claims.

In animal feed stuffs intended for particular nutritional purposes (EC 2008a), functional claims should be clearly separated from particular nutritional purposes that indicate the precise use. However, this does not prevent particular nutritional products from making additional functional claims. Besides the statutory statements on the precise use (i.e., brand name dietetic pet food for cats for reduction of struvite stone recurrence, the functional claim with fish oil for a shiny coat) may be used (FEDIAF 2011).

6 Nutraceuticals as Veterinary Medicinal Products (VMPs)

According to the definition previously reported in Directive 2001/82/EC (EC 2001), nutraceutical products having a beneficial effect on animal health require a marketing authorization if medicinal claims are made or if they contain certain ingredients that exert a pharmacological effect on the target animal. Thus, they have to follow legislation for medicinal products for veterinary use (EudraLex Volume 5 2015b).

Legislation for the marketing authorization of VMPs in the EU is rather complex and must address differences correlated to legislation of the Member States and also to the veterinary species. This includes food-producing animals and companion animals. Any VMPs to be legally placed on the market in the European Union or in any Member State must be the subject of a marketing authorization granted by the competent national authorities of the State or by the European Medicine Agency (EMA). The EMA provides guidance and support to companies researching and developing veterinary medicines. This includes scientific and regulatory information on how to design and run clinical trials, compliance standards, how to establish maximum residue limits for medicines and biocides, support to innovation, and incentives for companies developing medicines for minor use/minor species (MUMS)/limited markets (EudraLex Volume 5 2015b).

The body of European Union legislation in the pharmaceutical sector of veterinary products is compiled under "The rules governing medicinal products in the European Union," EudraLex Volume 5—EU pharmaceutical legislation for medicinal products for veterinary use—which is updated periodically to take into account new legislation (EudraLex Volume 5 2015b). The basic legislation is supported by a series of guidelines that are also published in volumes 4–9 (EudraBook V1 May 2015/EudraLex V30 January 2015). All regulations, directives, and guidelines are summarized in Table 5.

6.1 Marketing Authorization Procedure of VMP in the EU

With regard to the marketing authorization procedure of VMP, the general principles of assessing the quality, safety,

Table 4 Categories of authorized claims and examples of claims (COPA-COGECA/FEFAC 2016; FEDIAF 2011)

Categories of authorized claims	Typology of claims	Definition	Examples
Nutritional and compositional claims Refers to the presence or a high or low inclusion level of a particular component	Component claims (major components)	Refer to the presence of a particular feed material which can be accompanied by a qualifier	– "Brand name" with carrots and rice – High in [substance] (e.g., energy)
	Component claims (minor components)	Refer to the presence of a particular minor component	– "Brand name" with parsley – Low in [substance] (e.g., fiber)
	Nutrient and additive claims Presence/absence of a substance	Reference to the presence or a specific level of a nutrient or additive, including fatty acids, vitamins, etc., with no further connection to health effects	– "Brand name" contains vitamin E – Enriched with omega-3 fatty acids – Contains/brings/source of/provides/concentrated in/rich in [substance]
Functional claims	Nutrient function claims	Links the presence of a nutrient/combination of nutrients contained in a product to the physiological role in growth, development, and normal functions of the body	– Contains calcium for strong healthy bones and teeth – Vitamin E helps to protect the fat in body tissues from oxidation
	Enhanced function claims Support physiological functions of the animal or enable return to normal physiological status	Describes the specific beneficial effect of nutrients or other substances, alone or in combination, on physiological functions or biological activities beyond their established role in growth, development, and normal functions of the body. No reference should be made to particular diseases or pathological states	– Contains antioxidants to support the immune system – Contains chicory to improve growth of beneficial bacteria in the gut – Contributes to good liver function – Preserves udder integrity – Supports starting growth – Facilitates digestive transit – Fosters feed/drinking water intake/digestion/appetence – Maintains bowel flora balance
	Health maintenance and decreased disease risk claims	Relates to the consumption of a nutrient or other substances, alone or in combination, that help reduce the risk of disease development or maintain physiological functions or health	– "Brand name" contains omega-3 fatty acids to maintain healthy joints

and efficacy of a product are at the core of its benefit-risk balance. The European Union currently maintains four different registration procedures (Table 6) and multiple regulatory authorities for the registration of VMPs. Historically, each EU Member State maintained its own **National Procedure** (NP), which required national assessments and resulted in licenses valid only in one country. The **Centralized Procedure** (CP) must be submitted to the EMA and will enable it to issue a marketing authorization valid in all Member States, Iceland, Norway, and Liechtenstein. The **Mutual Recognition Procedure** (MRP) allows obtaining authorizations for a product in two or more Member States. If an authorization has already been issued in one EU State, the holder may apply to one or more Member States to issue identical authorizations based on mutual recognition of the reference authorization. This procedure intends to harmonize between Member States the individual assessment of the same data set, which may result in different label claims. The

Table 5 European law on veterinary medicinal products

Name	Description
Regulation	
EudraLex—volume 5	EU pharmaceutical legislation for medicinal products for veterinary use
Guidelines	
EudraLex—volume 4	Guidelines for good manufacturing practices for medicinal products for human and veterinary use
EudraLex—volume 6	Notice to applicants and regulatory guidelines for medicinal products for veterinary use. Volume 6A, Procedures for marketing authorization; volume 6B, Presentation and content of the dossier; volume 6C, Regulatory Guidelines
EudraLex—volume 7	Scientific guidelines for medicinal products for veterinary use
EudraLex—volume 8	Maximum residue limits. Notice to applicants and guideline—Veterinary medicinal products—establishment of maximum residue limits (MRLs) for residues of veterinary medicinal products in foodstuffs of animal origin
EudraLex—volume 9	Guidelines for pharmacovigilance for medicinal products for human and veterinary use. Volume 9B—Pharmacovigilance for Medicinal Products for Veterinary Use
Directives	
Directive 2001/82/EC	Community code relating to veterinary medicinal products
Regulation (EC) No 470/2009 of the European Parliament and of the Council of 6 May 2009	Community procedures for the establishment of residue limits of pharmacologically active substances in foodstuffs of animal origin
Commission Regulation (EU) No 37/2010 of 22 December 2009	Pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin. Table 1, Allowed substances; and Table 2, Prohibited substances

Table 6 Marketing authorization procedures available in EU for VMP (EC 2001)

Type	Assessment	License validity
National Procedure (NP)	National competent authorities	Only in one country
Centralized Procedure (CP)	European Medicines Agency (EMA)	All Member States, Iceland, Norway, and Liechtenstein
Mutual Recognition Procedure (MCP)	Authorization issued in the EU, the holder applies to one or more other Member States to issue identical authorizations based on mutual recognition of the "reference" authorization	Two or more Member States
Decentralized Procedure (DCP)	No marketing authorization issued in any country. The applicant requests one country to become the Reference Member State (RMS) in the procedure and asks recognition by national authorities of a first assessment performed by one Member State	Several Member States

Decentralized Procedure (DCP) has many similarities with the MRP and can be used to obtain marketing authorization in several Member States in parallel, if the applicant does not have marketing authorization for the medicinal product in any country. The Decentralized Procedure is based on recognition by national authorities of a first assessment performed by one Member State. The applicant requests one country to become the Reference Member State (RMS) in the procedure, while submitting an application for marketing authorization in all countries simultaneously (EC 2001).

6.2 VMPs and Use in Food-Producing or Companion Animals

To increase the complexity of VMP there are the animal species characteristics. The use of VMP in companion animals is performed under the veterinary responsibility with veterinary drugs authorized for specific diseases in the specific species. The "off-label use," intended as the use of a VMP not in accordance with the summary of the product characteristics, including the misuse and serious abuse of the

product, is allowed in these animals, so as the use of drugs for human use, unless no specific veterinary product is available or following a cascade system as reported in EMA/CVMP/AWP/237294/2017 (EC 2001; EMA 2017).

When animals are reared to produce food intended for human consumption, i.e., meat, offal, milk, eggs, and honey, the focus is on the potential for pharmacologically active residues in the tissues or products of treated animals at the time of slaughter or collection, which may subsequently be ingested by the consumer.

A VMP may not be the subject of a marketing authorization for administering to food-producing species unless the pharmacologically active substances which it contains appear in Table 1 of Commission Regulation 37/2010 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin (EC 2001; EU 2010). This limits the possibility of therapeutic treatments in food-producing animals to those substances that have been thoroughly evaluated to be safe for animals and also for human consumer. Thus, special attention needs to be paid to products intended for use in food-producing animals, as it is often, but not always, necessary to make an application for the assessment of human food safety and MRLs (EudraLex Volume 8 2015e). In the EU, all MRL applications must be submitted to the EMA, but marketing authorization applications can be made to either the EMA, i.e., in case of Centralized Procedure, or to the national authorities, i.e., National Procedure, as reported above (EC 2001).

The general principle at the base of an MRL definition for parent compounds and/or metabolites is to use data from studies in laboratory animals to establish an acceptable daily intake (ADI) of the drug residues without risks for consumers' health. The setting of the ADI is very precautionary for human health and includes multiple safety factors and worst-case assumptions. Once the ADI has been established, the MRLs of pharmacologically active substances are set for each edible tissue (meat, offal, milk, honey, egg) so that the consumer could ingest food of animal origin every day without exceeding the ADI and consequently posing any risks for his health (EC 2009a).

In food-producing animals it is not possible to administer pharmacologically active substances that are not listed in Table 1 (EU) No 37/2010 (EU 2010). Nevertheless, when particular treatments are necessary with substances not on the list, animals have to be excluded from the production of food destined for human consumption (EC 2001, 2009a).

Considering the premises, nutraceuticals also administered in food-producing species must be listed in Table 1 of allowed substances in Commission Regulation (EU) No 37/2010 (EU 2010). The natural products botanically defined included in the list are approximately 115, varying from *Absinthium extract* to *Viscum album* and for none of these an MRL is required (EU 2010). An excerpt of the list is reported in Table 7.

6.2.1 Homeopathic Veterinary Medicinal Products

Natural products botanically defined may also be used as "homeopathic veterinary medicinal products." A simplified registration scheme has been implemented for homeopathic remedies, which are placed on the market without medical claims and where there is sufficient dilution to guarantee safety of the product.

Only homeopathic veterinary medicinal products, which satisfy all of the following conditions, may be subject to a special, simplified registration procedure:

- They are administered by a route described in the European Pharmacopoeia or, in the absence thereof, by the pharmacopoeias currently used officially in Member States
- No specific therapeutic indication appears on the labeling of the veterinary medicinal product or in any information relating thereto
- There is a sufficient degree of dilution to guarantee the safety of the medicinal product. In particular, the medicinal product shall not contain more than one part per 10,000 of the mother tincture (EC 2001)

Homeopathic medicinal products may be administered to companion animals under the responsibility of a veterinarian. In food-producing animals, the administration of homeopathic veterinary medicines is permitted if the active constituents appear in Table 1 of Commission Regulation (EU) No 37/2010 (EU 2010).

7 Concluding Remarks and Future Directions

The noun "nutraceutical" is the union of the words "nutrition" and "pharmaceutical," and nutraceutical substances can be considered as both feed and drug. In any case, they are used to promote health promotion. The complexity for obtaining a marketing authorization for nutraceuticals is related to the definition of their intention to use, i.e., feed material or feed additive or VMPs. Once the intention is defined, the EU regulatory guidelines are very detailed although complex. They are freely and easily accessible on institutional websites (e.g., EFSA, EMA, EUR-Lex, etc.) by the applicant. So, regular updates of the authorized or allowed products are available.

Table 7 Extract of some natural compounds listed as pharmacologically active substances published in Table 1 of Commission Regulation (EU) No 37/2010 (EU 2010)

Pharmacologically active substance	Marker residue	Animal Species	MRL	Target tissues	Other provisions (according to Article 14(7) of Regulation (EC) No 470/2009)	Therapeutic classification
Adonis vernalis	NA	All food-producing species	No MRL required	NA	For use in homeopathic veterinary medicinal products prepared according to homeopathic pharmacopoeias at concentrations in the products not exceeding one part per hundred only	No entry
Aloe vera gel and whole leaf extract of *Aloe vera*	NA	All food-producing species	No MRL required	NA	For topical use only	No entry
Aloes, Barbados, and Capae, their standardized dry extract and preparations thereof	NA	All food-producing species	No MRL required	NA	No entry	No entry
Echinacea	NA	All food-producing species	No MRL required	NA	For use in homeopathic veterinary medicinal products prepared according to homeopathic pharmacopoeias at concentrations corresponding to the mother tincture and dilutions thereof only. For topical use only. For use in homeopathic veterinary medicinal products prepared according to homeopathic pharmacopoeias at concentrations corresponding in the products not exceeding one part per ten only	No entry
Ginseng, standardized extracts and preparations thereof	NA	All food-producing species	No MRL required	NA	No entry	No entry
Juniperi fructus	NA	All food-producing species	No MRL required	NA	No entry	No entry
Lauri folii aetheroleum	NA	All food-producing species	No MRL required	NA	No entry	No entry
Lauri fructus	NA	All food-producing species	No MRL required	NA	No entry	No entry
Lectin extracted from red kidney beans *Phaseolus vulgaris*	NA	All food-producing species	No MRL required	NA	For oral use only	No entry
Medicago sativa extractum	NA	All food-producing species	No MRL required	NA	For topical use only	No entry
Myristicae aetheroleum	NA	All food-producing species	No MRL required	NA	For use in newborn animals only	No entry
Piceae turiones recentes extractum	NA	All food-producing species	No MRL required	NA	For oral use only	No entry
Prunus laurocerasus	NA	All food-producing species	No MRL required	NA	For use in homeopathic veterinary medicinal products prepared according to homeopathic pharmacopoeias at concentrations in the products not exceeding one part per thousand only	No entry
Sinapis nigrae semen	NA	All food-producing species	No MRL required	NA	No entry	No entry
Solidago virgaurea	NA	All food-producing species	No MRL required	NA	For use in homeopathic veterinary medicinal products prepared according to homeopathic pharmacopoeias at concentrations corresponding to the mother tincture and dilutions thereof only	No entry
Strychni semen	NA	All food-producing species	No MRL required	NA	For oral use only at doses up to the equivalent of 0.1 mg strychnine/kg bw	No entry

(continued)

Table 7 (continued)

Pharmacologically active substance	Marker residue	Animal Species	MRL	Target tissues	Other provisions (according to Article 14(7) of Regulation (EC) No 470/2009)	Therapeutic classification
Substances used in homeopathic veterinary medicines	NA	All food-producing species	No MRL required	NA	All substances used in homeopathic veterinary medicinal products provided that their concentration in the product does not exceed one part per ten thousand	No entry

NA not applicable

References

COPA-COGECA/FEFAC (2016) EU Code of good labelling practice for compound feed for food producing animals. https://www.fefac.eu/files/69688.pdf

de Godoy MRC, Kerr KR, Fahey GC (2013) Alternative dietary fiber sources in companion animal nutrition. Nutrients 5:3099–3117

Domeneghini C, Di Giancamillo A, Arrighi S et al (2006) Gut-trophic feed additives and their effects upon the gut structure and intestinal metabolism. State of the art in the pig, and perspectives towards humans. Histol Histopathol 21:273–283

EC (2001) Directive 2001/82/EC of the European Parliament and of the Council of 6 November 2001 on the Community code relating to veterinary medicinal products (OJ L 311, 28.11.2001, p 1)

EC (2002) Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety (OJ L 31, 1.2.2002, p 1)

EC (2003a) Regulation (EC) No. 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition (OJ L 268, 18.10.2003, p 29)

EC (2003b) Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed (OJ L 268, 18.10.2003, p 1)

EC (2005) Regulation (EC) No 183/2005 of the European Parliament and of the Council of 12 January 2005 laying down requirements for feed hygiene (OJ L 35, 8.2.2005, p 1)

EC (2008a) Commission Directive 2008/38/EC of 5 March 2008 establishing a list of intended uses of animal feedingstuffs for particular nutritional purposes (OJ L 62, 6.3.2008, p 9)

EC (2008b) Commission Regulation (EC) No 429/2008 of 25 April 2008 on detailed rules for the implementation of Regulation (EC) No 1831/2003 of the European Parliament and of the Council as regards the preparation and the presentation of applications and the assessment and the authorisation of feed additives

EC (2009a) Regulation (EC) No 470/2009 of the European Parliament and of the Council of 6 May 2009 laying down Community procedures for the establishment of residue limits of pharmacologically active substances in foodstuffs of animal origin, repealing Council Regulation (EEC) No 2377/90 and amending Directive 2001/82/EC of the European Parliament and of the Council and Regulation (EC) No 726/2004 of the European Parliament and of the Council (OJ L 152, 16.6.2009)

EC (2009b) Regulation (EC) No 767/2009 of the European Parliament and of the Council of 13 July 2009 on the placing on the market and use of feed, amending European Parliament and Council Regulation (EC) No 1831/2003 and repealing Council Directive 79/373/EEC, Commission Directive 80/511/EEC, Council Directives 82/471/EEC, 83/228/EEC, 93/74/EEC, 93/113/EC and 96/25/EC and Commission Decision 2004/217/EC (OJ L 229, 1.9.2009, p 1).

ECC (1990) Council Directive of 26 March 1990 laying down the conditions governing the preparation. In: placing on the market and use of medicated feedingstuffs in the Community (90/167/EEC)

EFSA (2017a) Guidance on the assessment of the safety of feed additives for the target species. https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2017.5021

EFSA (2017b) Guidance on the assessment of the safety of feed additives for the consumer. https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2017.5022

EFSA (2017c) Guidance on the identity, characterization and conditions of use of feed additives. https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2017.5023

EFSA (2018a) Guidance on the characterization of microorganisms used as feed additives or as production organisms. https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2018.5206

EFSA (2018b) Guidance on the assessment of the efficacy of feed additives. https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2018.5274

EMA (2017) EMA/CVMP/AWP/237294/2017, Committee for Medicinal Products for Veterinary Use (CVMP), Reflection paper on off-label use of antimicrobials in veterinary medicine in the European Union, Draft, 25 July 2017

EU (2010) Commission Regulation (EU) No 37/2010 of 22 December 2009 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin (OJ L 15, 20.1.2010)

EU (2011) Commission Recommendation of 14 January 2011 establishing guidelines for the distinction between feed materials, feed additives. In: biocidal products and veterinary medicinal products (2011/25/EU)

EU (2013) Commission Regulation (EU) No 68/2013 of 16 January 2013 on the Catalogue of feed materials (OJ L 29, 30.1.2013, p 1)

EU (2017) Commission Regulation (EU) 2017/1017 of 15 June 2017 amending Regulation (EU) No 68/2013 on the Catalogue of Feed Materials

EudraLex (2015a) Volume 4—"The rules governing medicinal products in the European Union", Guidelines for good manufacturing practices for medicinal products for human and veterinary use. In EudraBook V1 May 2015/EudraLex V30 January 2015. Compendium of EU pharmaceutical law. https://ec.europa.eu/health/documents/eudralex/vol-4_en

EudraLex (2015b) Volume 5—"The rules governing medicinal products in the European Union", EU pharmaceutical legislation for medicinal products for veterinary use. In EudraBook V1 May 2015/EudraLex V30 January 2015. Compendium of EU pharmaceutical law. https://ec.europa.eu/health/documents/eudralex/vol-5_en

EudraLex (2015c) Volume 6—"The rules governing medicinal products in the European Union" Notice to applicants and regulatory guidelines for medicinal products for veterinary use. In EudraBook V1 May 2015/EudraLex V30 January 2015. Compendium of EU pharmaceutical law. https://ec.europa.eu/health/documents/eudralex/vol-6_en

EudraLex (2015d) Volume 7—"The rules governing medicinal products in the European Union" Scientific guidelines for medicinal products for veterinary use. In EudraBook V1 May 2015/EudraLex V30 January 2015. Compendium of EU pharmaceutical law. https://ec.europa.eu/health/documents/eudralex/vol-7_en

EudraLex (2015e) Volume 8—"The rules governing medicinal products in the European Union" Maximum residue limits. In EudraBook V1 May 2015/EudraLex V30 January 2015. Compendium of EU pharmaceutical law. https://ec.europa.eu/health/documents/eudralex/vol-8_en

EudraLex (2015f) Volume 9—"The rules governing medicinal products in the European Union" Guidelines for pharmacovigilance for medicinal products for human and veterinary use. In EudraBook V1 May 2015/EudraLex V30 January 2015. Compendium of EU pharmaceutical law. https://ec.europa.eu/health/documents/eudralex/vol-9_en

European Union (2018) Register of feed additives pursuant to Regulation (EC) No 1831/2003 Annex I: List of additives. https://ec.europa.eu/food/sites/food/files/safety/docs/animal-feed-eu-reg-comm_register_feed_additives_1831-03.pdf

FEDIAF (2011) Code of good labelling practice for pet food. https://www.pfma.org.uk/_assets/docs/Final%20Code%20of%20Good%20Labelling%20Practice%20Oct%202011.pdf

Franz Ch, Bauer R, Carle R, Tedesco D, et al (2005). Study on the assessment of plants/herbs, plant/herb extracts and their naturally or synthetically produced components as "additives" for use in animal production. CFT/EFSA/FEEDAP/2005/01. https://efsa.onlinelibrary.wiley.com/doi/pdf/10.2903/sp.efsa.2007.ZN-001

Garg RC (2016) Fenugreek: multiple health benefits. In: Gupta RC (ed) Nutraceutical efficacy, safety and toxicity. Academic/Elsevier, Amsterdam

Rafińska K, Pomastowski P, Wrona O et al (2017) Medicago sativa as a source of secondary metabolites for agriculture and pharmaceutical industry. Phytochem Lett 20:520–539

Regulatory Guidelines for Nutraceuticals and Food Supplements in India

P. K. Gupta

Abstract

The use of nutraceuticals and animal feed supplements is very common because animals cannot always get enough nutrients from regular meals that the owners or farmers provide. Thus, the feed supplements provide essential nutrients that are widely used for maintaining good health in animals. In some cases, if an animal does not have some specific nutrition in its diet, it may not grow properly. In India the livestock sector alone contributes nearly 25.6% of value of output at current prices of total value of output in Agriculture, Fishing and Forestry sector. India is the third-largest egg producer after China and the United States and the fourth-largest chicken producer behind China, Brazil, and the United States. The overall contribution of the livestock sector in total GDP was nearly 4.11% during 2012–2013. The animal feed industry deals with food given to animals in cattle, poultry, and aquaculture sectors as a part of animal husbandry under the control of the Department of Animal Husbandry, Dairying and Fisheries, Ministry of Agriculture. Although the animal feed industry in India is almost five decades old, this industry is still in its infancy with high reliance on imports. However, the industry is very lucrative with a large number of domestic players and various foreign multinationals striving to enter into the market. Recently, in the interest of the public, Food Safety Act of 2006 with regulation in the year 2017 is likely to be implemented very soon. The law also has a commitment to Codex Alimentarius Commission, World Trade Organization (Basic Rights and Obligations, under the SPS along with their sanitary or phytosanitary measures as per their international standards, guidelines, or recommendations. Impact of Food Safety Act of 2006) still needs to be seen after its implication in the year 2018.

Keywords

Nutraceuticals · Phytochemicals · Botanical products · Dietary supplements: probiotics · Prebiotics · Antioxidants · Mineral mixtures · Functional foods · Traditional medicine · Regulation · Health foods · Veterinary medicine · Livestock census

1 Introduction

Since ancient times, mankind has made medicines from the extracts of natural materials and has used these for various purposes. In 1989 Dr. Stephen De Felice, founder of the Foundation for Innovation in Medicine, coined the term "nutraceuticals" (the combination of nutrition and pharmaceutical), which became a newly accepted word in the *Oxford Dictionary*. Nutraceuticals have come a long way since a new trend in the care of companion animals emerged in the 1990s along with similar trends in the human sector. With the passage of the Dietary Supplement Health and Education Act of 1994, the definition of nutraceuticals has been expanded to include vitamins, minerals, herbs and other botanicals, amino acids, and dietary substances that humans use as supplements (Stauffer 1999). The term nutraceutical is not well accepted within global regulatory systems, while the term dietary supplements is. The relevance and impact of nutraceuticals have become more prominent as the present generation begins to focus more on preventive healthcare than ever before (Manoj 2015).

In India, the use of animal feed supplements is very common. These supplements provide essential nutrients that

P. K. Gupta (✉)
Toxicology Consulting Group, Bareilly, Uttar Pradesh, India
Academy of Sciences for Animal Welfare, Bareilly, Uttar Pradesh, India
Society of Toxicology of India, Ludhiana, Punjab, India
Division of Pharmacology and Toxicology, (IVRI), Bareilly, Uttar Pradesh, India
World Health Organization, Geneva, Switzerland
International Union of Toxicology, Reston, VA, USA

R. C. Gupta et al. (eds.), *Nutraceuticals in Veterinary Medicine*, https://doi.org/10.1007/978-3-030-04624-8_59

are widely used for maintaining good health in animals. In some cases, if an animal does not have some specific nutrition in its diet, it may not grow properly. The nutritional values of animal feeds are influenced not only by their nutrient content but also by many other factors such as hygiene, digestibility, and effect on intestinal health. Historically, in India multiple laws and regulations prescribed various standards regarding food, food additives, contaminants, food colors, preservatives, and labeling. This chapter briefly discusses regulatory guidelines for animal nutraceuticals and feed supplements as relevant to India.

2 Historical Background

India has one of the fastest-growing economies in the world and ranks among the top ten most popular destinations for investment. Recent policy reforms have been designed to accelerate the pace of foreign investment, and although entering the market is still a complex, multistep process, the potential of the functional foods category is exciting. At present, only a small amount of perishable agriculture products are processed (~ 2%) in comparison to 80% in the United States. The multiplicity of food regulation policy makers and enforcement agencies prevailing in different sectors of the food industry has contributed to considerable confusion among consumers, producers, retailers, and businesses and is detrimental to the growth of the functional food and nutraceutical industries (Keservani et al. 2014; Anon 2018).

By the mid-1990s, the food processing sector laws for public health were framed in a veritable grid of regulation including a multitude of state and national laws. In 1998, the Prime Minister's Council on Trade and Industry appointed a subjective group on food and agriculture industries which recommended a unified legislation under a single food regulatory authority. Public experts and members of the Standing Committee of Parliament encouraged the convergence of current food laws with single regulatory authorities accountable for public health and food safety in India. Further, integrating all acts and orders relating to food and eliminating multilevel and multi-departmental controls over food, special emphasis was given to nutraceuticals and functional food, a poorly defined segment with growing potential and implications for consumer health. In 2002, a national non-profit association was charged with determining that every food manufacturing company could provide scientific-based support for their products in order to protect consumers and to promote and defend a regulatory environment conducive to the industry in general as well as for consumer protection. In 2003, a Ministry of Health expert group report indicated the need to create new categories in present food laws for regulating functional food and dietary supplements. It was recommended that there should be mandatory safety testing for these products. In India, voluntary standards are developed by the Bureau of Indian Standards and National Standards body, which is comprised of representatives from various food sector stakeholder groups. In 2005, a number of committees, including the Standing Committee of Parliament on Agriculture, emphasized the need for a single regulatory body and integrated law. Finally, the Indian Food Safety Standard Bill 2005 was signed into law and promised a major impact on the Indian food processing industry. The Indian Food Safety and Standard Act came into enforcement in 2006 with two main objectives: to introduce a single statute relating to food and to provide for scientific development of the food processing industry. These standards basically deal with product certification, quality, system certification and testing, and consumer affairs. Efforts are being made to match Indian standards with international ones (Keservani et al. 2014; Anon 2006a).

3 Contribution of Livestock Sector in GDP

The livestock sector alone contributes nearly 25.6% of value of output at current prices of total value of output in the agriculture, fishing, and forestry sector. The overall contribution of the livestock sector in total GDP was nearly 4.11% during 2012–2013. As per records of the Government of India, the livestock census started in the country in the year 1919. The livestock census in 2012 was 19th in the series. The animal husbandry and livestock sectors are critical for the rural economy, especially for small and marginal farmers. They not only contribute to their income but are also their best insurance against any natural calamity. The total livestock population consisting of cattle, buffalo, sheep, goats, pigs, horses and ponies, mules, donkeys, camels, mithun, and yak in the country was 512.05 million in 2012. The total livestock population had decreased by about 3.33% from the previous census in 2007. The total bovine population (cattle, buffalo, mithun, and yak) was 299.9 million in 2012, which shows a decline of 1.57% over the previous census. Total sheep in the country was 65.06 million in 2012, down by about 9.07% from the 2007 census. Total number of pigs in the country has decreased by 7.54% over the previous census, and the total goat population has declined by 3.82% over the previous census. Total goats in the country were 135.17 million, and pigs in the country were 10.29 million in 2012. Horse and pony population have increased by 2.08% over the previous census and the total horses and ponies in the country was 0.62 million in 2012. Total mules in the country have increased by 43.34% over the previous census, and the total for the country was 0.19 million in 2012. Camel population has decreased by 22.48% over the previous census, and total camels in the country were 0.4 million in 2012. The total

Table 1 Percent of animal population during census year 2012

Animal	Percent of total livestock population (512,057 million)
Cattle	37.28%
Buffaloes	21.23%
Sheep	12.71%
Goats	26.40%
Pigs	2.01%
Mithun, yaks, horses	0.37%
Total	100.00 (−3.33% as compared to census in 2007)

donkey population in the country has decreased by 27.22% over the previous census, and the total in the country was 0.32 million in 2012.

In 19th livestock census, 37.28% were cattle, 21.23% buffaloes, 12.71% sheep, 26.40% goats, and 2.01% pigs. The latest census revealed 37.58%, 19.89%, 13.50%, 26.53%, and 2.10%. Mithun, yaks, and horses were 0.37% of the total livestock (Table 1). In addition, there are a good number of dogs, elephants, and rabbits. The population of dogs has decreased from 19.08 million in 2007 to 11.67 million in 2012, a decrease of 38.85%. The population of rabbits has increased from 0.424 million in 2007 to 0.592 million in 2012, an increase of 39.55%. The population of elephants has increased drastically from about 1000 to 22,000 in absolute terms. Considering the huge animal population, India has sufficient supply of nutrition products. However, the market demand and size of the market are not commensurate with the livestock population, especially in bovines (Anon 2014).

As far as the poultry industry is concerned, India is the third-largest egg producer after China and the United States and the fourth-largest chicken producer behind China, Brazil, and the United States. The total poultry population in the country has increased by 12.39% over the previous census, and total poultry in the country was 729.2 million in 2012. Poultry population in the country, along with change from the previous census, is summarized in Table 2. Poultry is the most organized sector in animal agriculture worth billions of dollars. The growth is 6–8% in layers and 10–12% in broilers per year against the growth of agriculture as a whole, which is around 2.5% (Anon 2016).

Table 2 Poultry population in the country along with change from the previous census

Bird	Population in millions (census 2012)	Percent change over the census 2007
Fowls	692,646	+12.13
Ducks	23,539	−14.85
Turkeys and others	13,025	+277.32
Total poultry	729,209	+12.39

− = decrease, + = increase

4 Indian Market and Animal Health Nutrition: Current Trends

Indians, Egyptians, Chinese, and Sumerians are just a few civilizations that have provided evidence suggesting that foods can be effectively used as medicine to treat and prevent disease. Ayurveda, the 5000-year-old ancient Indian health science, mentioned benefits of food for therapeutic purposes. As such, these nutraceuticals and functional foods for animal use can be categorized as follows:

(a) Substances which have established nutritional functions such as vitamins, minerals, amino acids, fatty acids, etc.
(b) Herbal/phytochemicals: herbs or botanical products
(c) Dietary supplements: probiotics, prebiotics, antioxidants, enzymes, etc.

The industry's production is about three million tons, which represents only 5% of the total potential, and feed exports are not very high. The feed industry has modern computerized facilities and the latest equipment for analytical procedures and least-cost ration formulation, and it employs the latest manufacturing technology. In India, most research work on animal feeds is practical and focuses on the use of by-products, the upgrading of ingredients, and the enhancing of productivity.

The country has entered into a period of liberalization and this is bound to influence the livestock industry. The per capita consumption of milk, eggs, and broiler meat will grow. Indian functional feed and feed industries are undergoing a very exciting phase of growth. It is estimated that cattle nutraceuticals/feed supplements are growing at a compound annual growth rate (CAGR) of 6%, while poultry nutraceuticals are growing at CAGR of 9%. The animal health nutrition market is driven by milk boosters such as calcium supplements, followed by mineral mixtures and fat supplements. Calcium supplements and mineral mixtures contribute 85% of the total nutraceutical market in the bovine segment, and bypass fat constitutes 4%. Probiotics, vitamin premix, and other supplements constitute the balance of 11%. Similarly, amino acid supplements and toxin binders constitute 58% of the total poultry nutraceuticals, followed by vitamin premixes 17%, growth promoters 6%, and enzymes and probiotics 6% (Balasubramaniam 2013).

The entry of new players is easy due to lack of regulations, as well as improper implementation of food safety laws.

There are about 450 animal health companies in India. It is estimated that nearly 200 of these are engaged in marketing or manufacturing nutraceutical products for bovines, canines, poultry, and sheep. There are about 12 multinational companies operating in India in the animal health and nutrition segments. This has led to high bargaining power for buyers, driving down the prices of nutraceuticals and therefore thin margins. With increasing middle class and demand for protein supplement, there is an increase in demand for milk, which is the source of protein. In the year 2015–2016, it was estimated that the demand for nutraceutical market will grow up to 1300 crore rupee by 2017–2018 (Balasubramaniam 2013).

5 Overview of Regulations of Other Countries

As in some other countries, Indian legislation doesn't actually assign any specific legal status to nutraceuticals. Nutraceuticals have different legal definitions predominantly based on two aspects:

1. Origin or source
2. Benefit(s) that it can provide to the consumer

The nomenclature of nutraceuticals also varies in different countries (Table 3): the United States describes them as

Table 3 Summary of the differences in regulations in different countries

Country	Regulation
United States of America	FDA regulates both finished dietary supplement products and dietary ingredients. FDA regulates dietary supplements under a different set of regulations than those covering "conventional" foods and drug products. Under the Dietary Supplement Health and Education Act of 1994 (DSHEA) (FDA 2017)
European Union (EU)	In EU food law, a regulatory framework for "functional foods" or "nutraceuticals" does not exist. The rules are numerous and depend on the nature of the foodstuff: (a) If a claim was made that implies a medicinal benefit for a nutraceutical product, the product will need to comply with the regulatory requirements for medicinal products, in respect of safety, efficacy, quality testing, and marketing authorization procedures (b) Claims regarding the beneficial effects of nutraceuticals can only be "health claims" and not "medicinal claims" (c) The regulatory frameworks of PARNUTS (foods prepared for particular nutritional purposes) or dietetic foods and of food supplements may be applicable to some "functional foods." The Novel Food Regulation applies to "functional foods" that are "new." More precisely, nutraceuticals are regulated as "food supplements." The regulations are governed by the European Food Safety Authority (EFSA) (Coppens et al. 2006)
Canada	Under the Natural Health Products Regulations, which came into effect on January 1, 2004, natural health products (NHPs) are defined as: (a) Vitamins and minerals (b) Herbal remedies (c) Homeopathic medicines (d) Traditional medicines such as traditional Chinese medicines (e) Probiotics (f) Other products like amino acids and essential fatty acids NHPs must be safe to use as over-the-counter products and not need a prescription to be sold. The Natural Health Products Directorate (NHPD) has changed its name to the Natural and Non-prescription Health Products Directorate (NNHPD) (Anon 2015)
Japan	In Japan functional foods/nutraceuticals are regulated by "food for specified health uses" (FOSHU) and must be approved by the Ministry of Health and Family Welfare after the submission of comprehensive science-based evidence to support the claim for the foods when they are consumed as part of an ordinary diet (Yamada et al. 2008). As per the FOSHU Act, functional food can have three functions: (a) Nutrition (b) Sensory satisfaction (c) Physiological improvements
Australia	In Australia, medicinal products/foods are referred to as "complementary medicines" and are regulated as medicines under the Therapeutics Goods Act, 1989, which was implemented in 1991. The law is governed by the Department of Health and Ageing, and the definition covers herbal medicines, vitamins and minerals, nutritional supplements, homeopathic medicines, aromatherapy products, and traditional medicines (Anon 2013)
Russia	Nutraceuticals are regulated under the term biologically active dietary supplements (BADS). The definition covers nutraceuticals (vitamins, minerals, amino acids, dietary fibers) and parapharmaceuticals (bioflavonoid, alkaloids, essential oils, polysaccharides). As per the rule, BADS are "foodstuffs with clinically proven effectiveness." They are recommended prophylactically and for the prevention of pharmaceutical therapy-induced side effects and the achievement of complete remission (Yuliva 2014)
India	As with other countries, Indian legislation doesn't actually assign any specific legal status to nutraceuticals. Government of India regulations for nutraceutical public use include the Food Safety and Standards Act (FSSA), which was passed in 2006, and Regulations, 2017, for implementation by the Ministry of Health and Family Welfare and is yet to be implemented (Anon 2006b)

"dietary supplements," in Canada they're referred to as "natural health products," and Japan lists them as "food for specified health uses (FOSHU)." These definitions can be general or specific. There are distinct definitions and regulations for dietary supplements and functional foods in the United States, Canada, and Europe, whereas in Japan, both dietary supplements and functional foods are governed by the same set of regulations. The United States and Canada list the attributes that a product must have to be called a nutraceutical, whereas Europe and Japan only provide general guidelines on the properties that a product should have to be named as such. Traditional and herbal medicines are included in the definition of dietary/nutritional supplements in Canada. Japan does not mention traditional herbal medicines under FOSHU, but the United States includes herbs and botanicals in its definition. The Indian definition for human health (as per the Food Safety and Security Act 2006) specifies the ingredients that a product should have, and the general properties of nutraceuticals derived from traditional medicines have been excluded from this definition. Table 3 briefly summarizes the differences in regulations in different countries.

6 Regulations

Food laws in every country are the basis for regulations of all kinds of foods including health food, dietary supplements, functional food, and nutraceuticals, for which specific guidelines/regulations are framed to regulate the manufacture, sale, and quality of the product to be used. As indicated previously, Indian legislation doesn't actually assign any specific legal status to the feeds/foods use for animals except from time to time some basic requirements/specifications for the regulation of mineral mixtures for supplementing animal feeds. Recently, laws for various foods for human use have been enacted and will be discussed later in this chapter.

6.1 Regulations of Mineral Mixtures for Supplementing Animal Feeds

Animal feed (including for poultry and aquaculture) plays a vital role in the food chain as feed is one of the most crucial contributors to ensuring safe, abundant, and affordable animal protein. Incessant population growth in India and rising affordability have led to a surge in demand for animal protein. The animal feed industry deals with food given to animals in cattle, poultry, and aquaculture sector as part of animal husbandry under the control of Department of Animal Husbandry, Dairying and Fisheries, Ministry of Agriculture (Anon 2009). Indian feed industry predominantly caters to cattle, sheep and goat, poultry, and aquaculture feed segment. Although animal feed industry in India is almost five decades old, the industry is still in its infancy with high reliance on imports. However, the industry is very lucrative with a large number of domestic players and various foreign multinationals striving to enter into the market.

Growth of the Indian animal feed market is propelled by a rise in demand for animal protein, a surge in dairy products consumption, and growth of the livestock population. However, there are various factors restraining growth of the market, including high import duties on feed ingredients, vague regulatory regime, volatility in raw material prices, and frequent disease outbreak. The market is characterized by leading trends such as the advent of nontraditional feed ingredients, genetically modified animal feed, and technological innovation in the industry (Anon 2017a).

The sale of cattle feed and mineral mixtures in India is regulated through the Regulation of Manufacture and Sale Order (Anon 2009) in accordance with the terms and conditions of a registration certificate issued by the Registering Authority. No person shall carry on the business of manufacture, sale, or distribution in any manner of cattle feed and mineral mixtures except under and in accordance with the terms and conditions of a registration certificate issued by the registering authority. Specifications for various feed supplements are laid down by the Bureau of Indian Standards (BIS 1983, 1992, 2002).

Provided further, a person who, at the commencement of this order, was carrying on the business of manufacture, sale, or distribution of cattle feed and mineral mixtures shall obtain a registration within the period of 3 months from the date of commencement of this order. Every holder of a registration certificate, who manufactures, sells, or distributes cattle feed and mineral mixtures, shall conform to the specifications, provided from time to time by the committee of experts on cattle feed and mineral mixture constituted by the secretary (ADF), Department of Animal Husbandry, Dairying and Fisheries, Government of India. On the advice of a committee of experts, the secretary (ADF) may issue necessary amendments as needed (Anon 2017a, b, c).

The last decade has seen healthy growth in India for both food, feed supplements and grew at with higher CAGR (6–12%), pharmaceuticals grew at around 15%. Nutraceuticals and feed supplements provide big opportunities for India with its huge human as well as animal population and need for nutrition and will ride on the growth of food, feed, and nutrition because farmers and breeders become increasingly health conscious for their livestock. It seems likely then that the nutraceutical and feed supplement market in India will experience exponential growth. Regulatory frameworks all around the globe have been primarily set up to achieve the objective of ensuring food safety and protection of consumer interests. Regulations for nutraceuticals on the market include the Food Safety and Standards Act (FSSA), which was passed in 2006, and

Table 4 List of various old state laws as well as the national laws that were consolidated to form the Food Safety and Standards Act, 2006 (FSS Act)

Export (Quality Control and Inspection) Act 1963
Solvent Extracted Oil Control Order 1967
Solvent Extracted Oil Control Order 1967
The Insecticide Act 1968
Meat Food Products Order 1973
Prevention of Food Adulteration Act (PFA) 1954 rules (Ministry of Health and Family Welfare) with last amendments in 1986
Bureau of Indian Standards Act 1986
Environmental Protection Act 1986
Pollution Control Act 1986
Milk and Milk Products Order 1992
The Infant Milk Substitutes Feeding Bottles and Infant Food (Regulation of Production, Supply) Act 1992 and Rules 1993
Agriculture Produce Act, Essential Commodities Act 1995 (Ministry of Food and Consumers Affairs)
Vegetable Oil Product Control order 1998

Regulations 2017 by the Ministry of Health and Family Welfare which may be implemented by the end of 2018 (Anon 2006a, b, 2017b). The passing of this act in India is a significant first step, but much more has to happen to eliminate the confusing overlap with various old laws and regulations (Table 4). Thus, the FSS Act has been formulated with an aim to establish a single reference point for all matters relating to regulation and supervision of food safety and standards.

6.2 Commitment Towards International Standards

FSSAI is also the National Codex Contact Point (NCCP) of India and functions as an interface between the Codex Alimentarius Commission (CAC) and India as a member country. The FSSAI has adopted some of the Codex Alimentarius international food standards which contribute to the safety, quality, and fairness of this international food trade (Anon 2017b).

India as a member country has a commitment toward the World Trade Organization. Accordingly, Article 2 (Basic Rights and Obligations) under the SPS Agreement states "Members shall ensure that any sanitary or phytosanitary measure is applied only to the extent necessary to protect human, animal or plant life or health, is based on scientific principles and is not maintained without sufficient scientific evidence." Under the same agreement, article 3 requires "to harmonize sanitary and phytosanitary measures on as wide a basis as possible, Members shall base their sanitary or phytosanitary measures on international standards, guidelines or recommendations, where they exist, except as otherwise provided for in this Agreement, and in particular in paragraph 3 of the article" (Anon 2017c).

7 Regulatory Requirements for Entry in India

With recent implementation of FSSA, nutraceutical regulations are evolving in India. There is a possibility that some of the contents are conflicting/confusing, but for this industry to take a shape, regulations must be streamlined. In order to enter the Indian nutraceutical market, some of the very important areas to focus include product evaluation, actual product analysis, procuring licenses, and developing India-specific health and label claims (Anon 2017c).

8 Product Evaluation

Under specific environmental conditions in Indian, formulations may behave differently and they may be classified incorrectly. Hence, due diligence in terms of determining a specific amount for each ingredient and the combination of ingredients becomes extremely crucial. In order to perform product assessment as per the Indian regulatory definition, it is of utmost importance to examine each active ingredient and additive in the context of permissibility, standards, and dosage of vitamins/minerals allowed as per the therapeutic, prophylactic, or RDA for Indians. Also, manufacturers are unclear whether their products will be classified as a food or food supplement or drug in the context of the Prevention of Food Adulteration Act 1954 and Rules 1955, Food Safety and Standards Act 2006, and Drugs and Cosmetics Act 1940 and Rules 1945. The Food Safety and Standards Rules 2011 highlight the regulatory enforcement structure and procedures that the central government proposes to make. The structure has a hierarchy from commissioner of food safety to the number of officers and designations such as

food safety officer, food analyst, etc., who will be involved in the product analysis process at different points (Keservani et al. 2014).

Various steps in the product analysis include:

1. Developing documents and authenticating them by the concerned authority
2. Sample collection (in the presence of witnesses)
3. Sample dispatch to concerned authority (different processes for bulk package and single package)
4. Food analysis
5. If analysis is not completed within stipulated period of time, further action plan by designated officer
6. Adjudication proceedings (holding inquiry, appeal procedure, hearing, etc.)

9 The Benefits

Nutraceuticals for veterinary use do not need strict regulations. In this respect, nutraceuticals offer significant advantages compared with the long development times and high manufacturing cost of drugs, whereas the efficacy testing of nutraceuticals requires less time for farmed species when compared to testing for humans (Varghese and Mishal 2014).

10 The Disadvantages

The major problems in regulations in India are:

1. Legal definitions for nutraceuticals/functional foods are somewhat different in different countries; for example, in India, functional foods can include herbal extracts, spices, fruits, and nutritionally improved foods or food products with added functional ingredients.
2. The requirements to make therapeutic claims are unrealistic—herbals are not patentable.
3. As there is no provision to patent, the dietary supplement label is inappropriate for nutraceuticals.
4. Problems such as quality control and unethical and criminal elements exist in this industry.

11 Role of Government and Public Sector Institutes

The government does not provide any subsidy or support for this sector. However, nutraceuticals and cattle feed segment is not taxable, which in itself is a noteworthy support extended by the government. The role of public sector institutes has been insignificant, and contribution to the industry is not up to the mark. However, the National Dairy Development Board (NDDB) and the Indian Council of Agriculture Research (ICAR) have done mineral mapping and developed mineral supplements required as per region, soil, and other conditions.

There are many technologies developed by universities, ICAR, etc. However, materializing these technologies into new products is highly localized and minimal. Mainly private players have played a larger role in this, and even multinational companies have a larger presence with new value-added products in this segment. Indian companies such as Indian Immunological Limited (IIL) have developed many nutrition products with the help of their parent organization; NDDB has entered into the market successfully.

12 Concluding Remarks and Future Directions

Some of the key challenges before the industry include regulation to bring in quality standards that will enable many companies to upgrade. The Bureau of Indian Standards (BIS) norms are being used for the use of feed supplements in animal, poultry, and aquaculture industries. However, the procedure in vogue has no control over various diseases that are affecting the animal and poultry industry, which can override all efforts to make livestock productive through nutraceuticals, improvement in breeding and genetic status of dairy animals, and integration of dairy sector. Although the Food Safety and Standard Act 2006 for human health defines functional food/nutraceuticals legally, there are still further effective regulations. Guidelines and suitable protocols are required to gain momentum for effective implementation across the nation. There is a need to clarify and formulate the regulatory framework for nutraceutical use in veterinary practice. If substantiation effectively enforced the Food Safety and Standard Act, there is the potential to open up tremendous opportunity for the functional food or nutraceutical industry. To conclude, the passing of the Food Safety and Standard Act 2006 was a significant first step, but a lot more has to happen to eliminate the overlap of old laws and regulations. The Food Safety and Standards Regulations 2017 announced in the *Gazette of India* that regulating manufacture, distribution, and sale of nutraceuticals, functional foods, and dietary supplements in India may prove useful in the implementation of food safety for all, but this has yet to be fully implemented. It is expected that this act may trigger the formation of a similar act for nutraceuticals and functional foods used in animals.

References

Anon (2006a) FICCI study on Implementation of Food Safety and Standard Act 2006: an industry perspective at. http://www.indiaenvironmentportal.org.in/Files/food_safety_study.pdf

Anon (2006b) Food Safety and Security Act of India. www.drugscontrol.org/food%20safety%20and%20standards%20Act%2006.pdf

Anon (2009) Cattle feed (Regulation of manufacture and sale) order, 2009. F. No. 2-35/2009-AHT/FF. Ministry of Agriculture, Department of Animal Husbandry, Dairying and Fisheries Government of India

Anon (2013) Overview of the regulation of complementary medicines in Australia. Australian Government, Department of Health, 25 Mar 2013. https://www.tga.gov.au/overview-regulation-complementary-medicines-australia

Anon (2014) Livestock census-2012. All India Ministry of Agriculture Department of Animal Husbandry, Dairying and Fisheries Krishi Bhawan—Report 19, New Delhi, Dated the 17 Jun 2014. http://dahd.nic.in/sites/default/files/19%20th%20Livestock%20%202012.pdf

Anon (2015) Licensed natural health products database (LNHPD)—Canada.ca, 4 May 2015. https://www.canada.ca/en/health-canada/services/drugs-health-products/natural-nonprescription/applications-submissions/product-licensing/licensed-natural-health-products-database.html

Anon (2016) Poultry production in India—the current scenario. APEDA, 10 Mar 2016. http://agriexchange.apeda.gov.in/news/NewsSearch.aspx?newsid=2205

Anon (2017a) Indian animal feed (poultry, cattle and aquaculture) market 2017 industry analysis, size, share, growth, trends and forecast by 2022. http://www.digitaljournal.com/pr/3584516

Anon (2017b) Health supplements and nutraceuticals—emerging high growth sector in India. World Food India, 3–5 Nov 2017, Ministry of Food Processing Industries, Government of India, New Delhi. http://www.ey.com/Publication/vwLUAssets/ey-health-supplements-and-nutraceuticals/$File/ey-health-supplements-and-nutraceuticals.pdf

Anon (2017c) Indian food code food categorization system, 2 Mar 2017. http://old.fssai.gov.in/Portals/0/Pdf/INDIAN_FOOD_CODE(25-06-2012).pdf

Anon (2018) Potential for functional foods in the Indian market. 30 Aug to 1 Sept 2018, India. Expo Centre, Greater Noida, Delhi-NCR, India https://www.figlobal.com/india/visit/news-and-updates/potential-functional-foods-indian-market

Balasubramaniam KV (2013), Animal nutra market will grow to 1,300 cr by 2017–2018, 23 Feb 2015, Bangalore Bureau Report. www.nuffoodsspectrum.in/inner_view_single_details_print.php?page

BIS (1983) Mineral mixtures for supplementing cattle feed specification IS 10672. Bureau of Indian Standards, Government of India

BIS (1992) Mineral mixtures for supplementing poultry feed specification IS 5672. Bureau of Indian Standards, Government of India

BIS (2002) Mineral mixtures for supplementing cattle feed specification IS 1664. Bureau of Indian Standards, Government of India

Coppens P, de Silva MF, Pettman S (2006) European regulations on nutraceuticals, dietary supplements and functional foods: a framework based on safety. Toxicology 221:9–74

FDA (2017) Dietary supplements—overview of dietary supplements and FDA's role in regulating them. 29 Nov 2017. https://www.fda.gov/Food/DietarySupplements/

Keservani, R K, Sharma Anil K, Ahmad F, Baig Mirza E (2014) Nutraceutical and functional food regulations in India In: Nutraceutical and functional food regulations in the United States and around the world by Debasis Bagchi (2nd edn), Food Science and Technology, 2014, Elsevier: Amsterdam327-342. https://doi.org/10.1016/B978-0-12-405870-5.00019-0

Manoj PK (2015) Cattle feed industry in India: a macro perspective. Int J Bus Manag Soc Sci (IJBMSS) IV(10 (I)):96–101. (ISSN-P: 2249-7463). https://www.Researchgate.Net/Publication/280253947_Cattle_Feed_Industry_In_India_A_Macro_Perspective

Stauffer JE (1999) Nutraceuticals. Cereals Food World 44(2):115–116

Varghese T, Mishal P (2014) Scrutinizing the term 'nutraceutical'—a global regulatory perspective. Nutraceut Bus Rev. 21 Jul 2014. https://www.nutraceuticalbusinessreview.com/. . ./Scrutinising_the_term_nutraceutical

Yamada K, Sato-Mito M, Nagata J, Umegaki K (2008) Health claim evidence requirements in Japan. J Nutr 138:1192S–1198S

Yuliva V (2014) Russia: biologically active supplements. Export.gov, June 2014. http://files.export.gov/x_4455523.pdf

Uses and Regulatory Guidelines for Nutraceuticals in China

Jianhua Sun, Zhongqi Jiang, Feng Wang, and Likun Gong

Abstract

In China, nutraceuticals are not regulated as veterinary drugs but are approved as feed additives, feed premix, or feed. The use of additives in animal feed is supervised by the Ministry of Agriculture, which is responsible in evaluating, regulating, and publishing a catalog of feed additives, with advised dosing and limitations. However, detailed information is usually available in the product insert from the related company. Nutraceuticals traditionally include amino acids, vitamins, minerals, enzymes, live microorganism, antioxidants, and others. Herbal medicine additives are popular and therefore frequently used in animal feed. Among them are garlic, tea polyphenol, the extract of *Eucommia ulmoides* leaves, the extract of *Epimedium*, and the extract of *Perilla* seed. Additives are largely applied in intensive farming by a nutritionist, without the need of a prescription from a veterinarian. However, more veterinarians are increasingly prescribing nutraceuticals for pets. In this chapter, we will discuss the classification and regulatory rules of commonly used nutraceuticals approved in China for a better understanding of the regulatory provisions and rational application scenarios.

Keywords

Nutraceuticals · Regulatory guideline · China

J. Sun (✉) · L. Gong
Academy of Sciences, Shanghai Institute of Materia Medica, Center for Drug Safety Evaluation and Research, Pudong, Shanghai, P.R. China
e-mail: jhsun@mail.cdser.simm.ac.cn

Z. Jiang
Department of Veterinary Medicine, College of Animal Science, Zhejiang University, Hangzhou, Zhejiang, P.R. China

F. Wang
Amlan Trading (Shenzhen) Company, Ltd., Shenzhen, Guangdong, P. R. China

1 Introduction

A nutraceutical is a pharmaceutical-grade and standardized nutrient (Sarris et al. 2016) that has been defined as "the use of micronutrients, macronutrients, and other nutritional supplements as therapeutic agents" (AVMA 1999). However, in China, nutraceuticals do not exist as a regulatory category and are controlled as dietary supplements (premix), feed additives, or feeds. Animal nutraceuticals are regulated by the Ministry of Agriculture (MOA) which has published an essential list of feed additives for animals (Notice No. 105, 1999). This list was updated in MOA Notice No. 1224 (2009), in which the source, animals, recommended dosage, and limitations of the feed additives were all included. Eight years later, the document was updated in Notice No. 2625 (2017), in which more additives and specified limitations were included.

Since China joined the World Trade Organization (WTO) in 2001, many corporations gained access to the Chinese veterinary drug market. Within the pet market, among the categories of feed supplements available, several are from Japan, Germany, and the UK. As compared with the USA, China's animal medication industry is relatively young. However, the potential is significant not only for China's rapid economic growth but for the increasingly larger consumer demand as well.

In all, the use of nutraceuticals for animals is a relatively recent practice in China. While nutraceuticals are commonly added in livestock feeds, especially during vaccination, breeding, and egg- or milk-producing period, these dietary supplements are also becoming popular in small animal veterinarians. However, there are still some companion animal veterinarians that refuse to use nutraceuticals due to insufficient data on efficacy and safety. In addition, animal owners' acceptance of nutraceuticals may influence this decision.

R. C. Gupta et al. (eds.), *Nutraceuticals in Veterinary Medicine*, https://doi.org/10.1007/978-3-030-04624-8_60

2 Classification

Nutraceuticals are registered as animal feed premix, feed additives, or feed, rather than veterinary drugs. This classification applies as guidelines for the approved feed additives, and the feed additives were divided into amino acids, vitamins, minerals, enzymes, life microorganisms, flavoring and appetizing substances, antioxidants, and others (Notice No. 2045, 2013a, b). It is worth mentioning that as nutraceuticals, herbal medicine or their bioactive constituents are popular and frequently used in China. For example, garlic (flavoring and appetizing substance), tea polyphenol (antioxidants), and the extracts from *Eucommia ulmoides* leaves, *Epimedium*, or *Perilla* seeds are among them.

Nutraceuticals used for pets are generally cataloged according to their functions. Common products on the market include those for joint repair treatment with the main ingredients of glucosamine, chondroitin sulfate, green-lipped mussel nutrient, and vitamin and those for improving parorexia and growth promotion which contained vitamins and trace elements. In addition, products used to prevent hair dry/peeling, to promote recovery of the natural color of hair, and to increase pigmentation with lecithin combined with natural seaweed powder are also often applied. Moreover, probiotics, microelement tablets, and calcium preparations containing crushed bone, vitamin D3, calcium lactate, and calcium gluconate are also available for selection.

3 Uses of Chinese Native Nutraceuticals

3.1 Garlic (Allimin)

Garlic (*Allium sativum* L.) originated in China and is a popular ingredient for cooking due to its strong smell and delicious taste. Garlic is nutritious with rich mineral substances and trace elements. Moreover, garlic possesses various biological properties, such as immunomodulatory, anticancer, antimicrobial, antihypertensive, and antiatherosclerotic (Lin et al. 2017; Lou et al. 2018; Sheppard et al. 2018; Dubey et al. 2017; Gonen et al. 2005). Therefore, as a powerful functional food, garlic has been accepted and widely applied for more than 5000 years, both for as a food additive and for treating a wide variety of diseases.

Garlic contains allimin ($C_6H_{10}OS_2$), which is metabolized by alliinase from alliin, and is responsible for garlic's pungent flavor and aroma as well as its therapeutic benefit (Alhashim and Lombardo 2018). However, allicin is an unstable compound that is only briefly present in fresh garlic after it has been crushed. In fact, sulfur compounds, for example ajoene, may play an important role in garlic's health benefits (Demeule et al. 2004; Geng et al. 1997; Kaschula et al. 2016).

Garlic powder is traditionally and extensively used in the feeds of fish, chickens, pigs, cows, and even cynomolgus monkeys. The diet supplemented with garlic at 1.0–1.5 g/kg significantly improved performance in boiler chickens (Jimoh et al. 2013) and at 20 g/kg enhanced the resistance of African catfish fingerlings to *A. hydrophila* infection (Eirna-liza et al. 2016). As for garlic extract, the dose required depends on its allicin content. For instance, a feed with 200 mg kg garlic extract (20% allicin) could raise vaccination efficacy of *Clostridium perfringens* toxoid in rabbits (Abu El Hammed et al. 2016). Garlic powder and the structural formula of its most representative bioactive constituents are shown in Fig. 1 (structure originate from Martins et al. 2016).

3.2 Tea Polyphenol

Tea polyphenols (TPs), which contain epigallocatechin gallate (EGCG), have several beneficial properties, including anticancer, antioxidant, antidiabetic, antihypertensive, antimicrobial, and antimetabolic syndrome effects, as well as the ability to improve fertility in humans and animals (Posadino et al. 2017; Rahman et al. 2018). The structural formula of EGCG is shown in Fig. 2.

Green tea polyphenol is valuable for pigs, chickens, and other animals. For example, an extract containing 0.2% w/v polyphenols can lessen sperm injury resulting from freeze-thawing in swine (Kitaji et al. 2015), and green tea polyphenols fed at 50 and 100 mg/kg body wt for 20 days could alleviate obesity in broiler chickens via regulation of the lipid-metabolism-related genes and transcription factor expression (Huang et al. 2013).

Although tea polyphenol is a luxury for animals, there is still a huge quantity of extract to be gained from pruning and coarse old leaves of tea trees. Details of health benefits of tea polyphenol can be found in several reviews (Williamson 2017; Bag and Bag 2018; Mao et al. 2017).

3.3 Extract of *Eucommia ulmoides* Leaves

Cortex *Eucommia* (Du zhong, in Chinese), the bark of *Eucommia ulmoides* Oliver (Eucommiaceae), has been used in traditional Chinese medicine for a long time. *Eucommia ulmoides* leaves contain iridoids, lignans, flavonoids, phenols, and terpenes, and as a result, the extract of *Eucommia ulmoides* leaves has favorable effects against not only hypertension, obesity, and gastric ulcer; they also have antioxidative, anti-sedative, and anti-hypnotic properties (Deyama et al. 2001; Li et al. 2017; Hirata et al. 2011).

The extract of *Eucommia ulmoides* leaves and structural formula of chlorogenic acid are shown in Fig. 3.

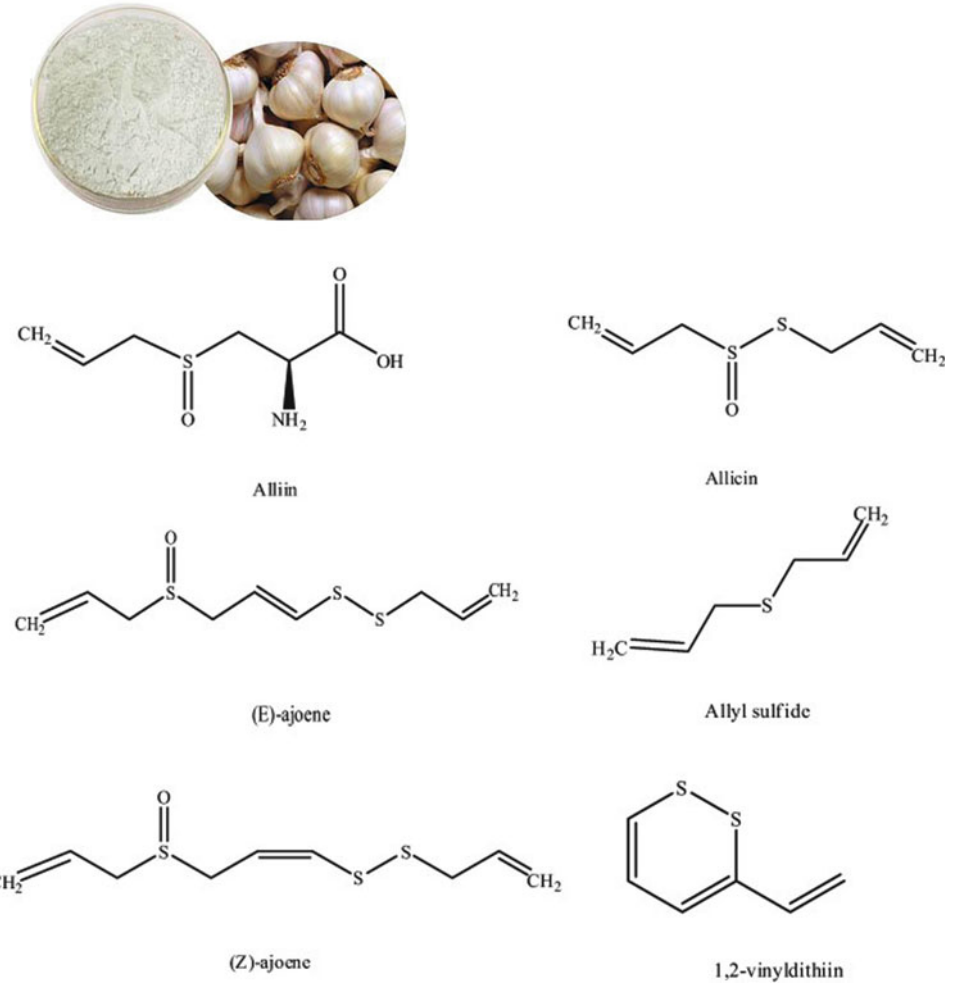


Fig. 1 Stereochemical structure of the most representative bioactive constituents from *Allium sativum* L.: alliin, allicin, allyl sulfide, (E)-ajoene, (Z)-ajoene, and 1,2-vinyldithiin

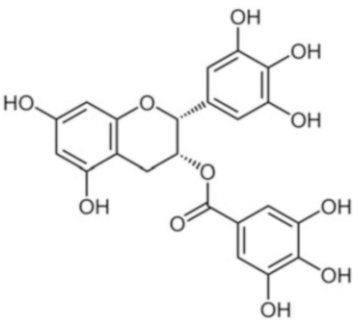


Fig. 2 Structural formula of epigallocatechin gallate

In rats and pigs, 5% of *Eucommia ulmoides* leaf extract added to a high-fat diet can reduce blood pressure and weight (Hosoo et al. 2017). Similarly, the same extract level can improve growth performance, blood indices, and meat quality for growing pigs (Lee et al. 2009).

The leaves of *Eucommia ulmoides* are also dried and used in tea for humans. Although of lower quality, the leftover

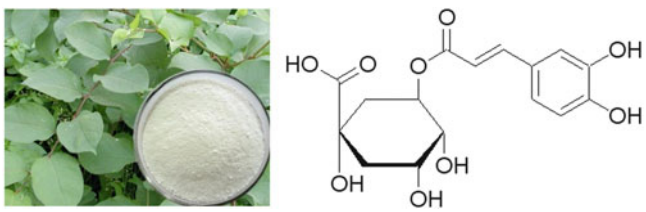


Fig. 3 *Eucommia ulmoides* and structural formula of chlorogenic acid

extract could be applied to animal feed. Currently, the recommended dose of the *Eucommia ulmoides* leaf extract in animal feeds ranges from 80 to 200 mg/kg.

3.4 Extract of *Epimedium*

As a common traditional Chinese medicine, *Epimedii folium* has been used for treating erectile dysfunction, postmenopausal syndrome, and osteoporosis for thousands of years.

Epimedium's main component is icariin (8-prenyl derivative of kaempferol 3,7-*O*-diglucoside), and it can protect the ischemic myocardium in dogs and prevent glucocorticoid-induced femoral head necrosis properties in rats. Furthermore, the extract possesses the properties of antioxidant activity and immunomodulatory effect (Wang et al. 1998, 2011; Yuan et al. 2016). The extract of *Epimedium* leaves and structural formula of icariin are shown in Fig. 4.

The extract of *Epimedium* is a double-edged sword, which may only be used in suitable animals during an appropriate period. The safe dose in feed is 150 g/kg recommended by the manufactures for breeding livestock or boiler chickens.

3.5 Extract of Perilla Seed

Perilla seed is obtained from the plant *Perilla frutescens* L. which is commonly called Zisu in Chinese, Cha-jo-ki in Korean, and Shiso in Japanese. Amino acids, linolenic acid, rosmarinic acid, and vitamin E are the main active substances in perilla seeds, and they lower blood lipids, inhibit bacteria, and are anti-inflammatory and antioxidative (Wang et al. 2016). Senavong et al. (2016) reported that an extract of perilla leaf and seed oil showed a neuroprotective effect by decreasing oxidative stress and inhibiting tau-protein hyperphosphorylation in vitro.

Müller-Waldeck et al. (2010) described that several genotypes of *P. frutescens* L. showed remarkably high contents of carotenoids, especially lutein, and various phenolic compounds. However, they also contain perilla ketone, a potent lung toxin (Guerry-Force et al. 1988). Although the toxic dose of perilla ketone in humans is unknown, it is recommended that perilla ketone-containing perilla species not be consumed. Therefore, for the safety of animals, the amount of perilla ketone in the extract of perilla seed need to be strictly controlled via perilla genotypes identification and optimization of the extraction methods.

The figure of perilla seed extract and the structural formula of alpha-linolenic acid are shown in Fig. 5. The structural formula of perilla ketone is shown in Fig. 6.

The addition of 250–350 mg/kg *Perilla frutescens* seed extracts to the diet significantly increased production performance of growing finishing pigs (Chu et al. 2011). In addition, 300 mg/kg *Perilla frutescens* seed extracts added to the diet considerably increased the laying rate, average daily egg production, and immune function of laying hens during the late laying peak period. This addition improved immune function of finishing cattle as well (Shi et al. 2015; Zhang et al. 2011). Currently, the manufacturer recommends adding a daily dose of 20–50 mg/kg *Perilla frutescens* seed extracts to animal feed.

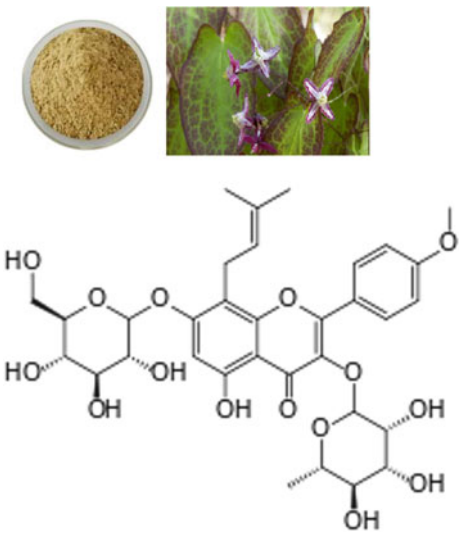


Fig. 4 *Epimedium* leaves and structural formula of icariin

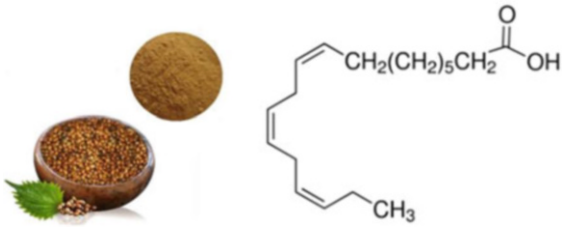


Fig. 5 Perilla seeds and structural formula of alpha-linolenic acid

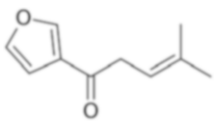


Fig. 6 Structural formula of perilla ketone

4 Regulatory Guidelines of Nutraceuticals in China

4.1 List of Current Version of Regulatory Guidelines

The State Council is the supreme administrative agency for animal feed and feed additives in China. MOA is responsible for the management of animal feed and feed additives. Their published Notice and Decree gives guidance for animal feed production, import, and use of feed and feed additives.

(a) *Regulation for Management of Feed and Feed Additive*
State Council, Decree No.609, 2011
(b) *Approved Feed Additive*
MOA, Notice No. 2045, 2013a, b
(c) *Regulation of Safe Use for Feed Additives*
MOA, Notice No. 2625, 2017
(d) *Regulation of Feed and Feed Additives Produce*
MOA, Decree No. 3, 2012a
MOA, Decree No. 5, 2012b
(e) *Measures for Registration and Management of Imported Feed and Feed Additives*
MOA, Decree No. 2, 2014

4.2 Regulation for Management of Feed and Feed Additive

This regulation is an administrative decree issued by the prime minister. General outline of feed and feed additives management (production, management, and use) are included in this regulation, ensuring the quality and safety of animal products and maintenance of public health (Decree No. 609, 2011).

The responsibilities of MOA have also been stipulated, such as publication of the approved feed additives and feed ingredients. Moreover, the procedures for importing feed additives are also specified, and penalties for violations have furthermore been laid out. In short, this is a basic law for the management of animal health products.

4.3 Approved Feed Additive

The main ingredients of animal nutraceuticals must be included in the approved feed additive. In 1999, a total of 173 additives were permitted for use in animal feed, which included vitamins, minerals, and trace elements, feed grade enzymes, feed grade probiotics, antioxidants, and others (Notice No.105, 1999). More and more feed additives, such as oregano and carvacrol (*Origanum aetheroleum*), were added, and some imported products were also approved in Notice No. 318 (2003) and Notice No. 658 (2006).

In Notice No. 1126 (2008), several feed additives were added, and 24 new feed additives were listed, although they are still in the period of protection.

In Notice No. 2045 (2013a, b), the current version of approved feed additives, garlic, tea polyphenol, and the extracts from *Eucommia ulmoides* leaves, *Epimedium grandiflorum* Morr, and *Perilla* seeds are all included.

4.4 Regulation of Safe Uses for Feed Additives

Further guidance on the safe uses of feed additives was issued in Notice No. 1224 (2009); the source, approved animals, recommended dosage, and the maximum limitations were all included. These guidelines were updated in Notice No. 2045 (2013a, b).

As the highest administrative regulation, Notice No. 2625 (2017) stipulates the use of most animal feed additives. However, the contents are relatively brief and incomplete. For example, vitamin E is only given permission for use, but no dosage recommendation is given. This leads to different doses being published in animal trials and recommendations.

4.5 Regulation of Feed and Feed Additives Produce

In China, if the product is to be produced, it must first obtain a production license for feed or feed additives. Enterprises must apply to MOA for a production license of feed additives in accordance with MOA regulation Decree No. 3 (2012a). The evaluation committee of the production license for feed and feed additives of MOA will be responsible for the technical review for license approval.

After obtaining the production license, the enterprise must apply for a license number from the people's government at the provincial level according to MOA Decree No. 5 (2012b). In the application, product ingredients, product quality standard, test method of product ingredients, label style of product, and product description should be included. In a word, production is not permitted product license and license number are obtained.

4.6 Import of Feed and Feed Additives

In China, enterprises must apply to MOA for an import approval number before importing animal nutraceuticals in accordance with MOA Decree No. 2 (2014). Furthermore, application for a feed or feed additive that has never been used in China requires more data, such as safety evaluation test reports.

Foreign enterprises need to apply to MOA for import registration for first time export of feed or feed additives to China. Several reports about the product are required, including the analysis and evaluation report on the possible impact of the residue of the feed additive on human health, the stability test report, and the environmental impact report. If

maximum limitation is required, the maximum value and the method for the determination of effective components shall also be provided.

5 Concluding Remarks and Future Directions

The science behind the use of nutraceuticals is profoundly complicated by species, age, and state of disease. The veterinarian's knowledge of nutraceutical use is significantly less compared to that of usual veterinary drugs. Although the uses of nutraceuticals are becoming popular, sufficient animal tests and mechanism research need be conducted to better understand both their efficacy and safety.

For a large number of nutraceuticals, no recommended dosages are available and depend on manufacturer's standards. Current regulatory guidelines of MOA need to be further specified. Some herbal medicines have shown strong efficacy, but are not without potential toxicity (Gupta 2016; Gupta et al. 2018). When nutraceuticals are used in animals as therapeutic medicines, they need to be evaluated for any possible toxicity for both animal and human health.

Acknowledgment The authors would like to thank Ziqiang Wang for his technical assistance in preparation of this chapter.

References

Abu El Hammed W, Soufy H, El-Shemy A et al (2016) Use of allicin as feed additive to enhance vaccination capacity of Clostridium perfringens toxoid in rabbits. Vaccine 34(17):2000–2007

Alhashim M, Lombardo J (2018) Mechanism of action of topical garlic on wound healing. Dermatol Surg 44(5):630–634

American Veterinary Medical Association (1999) Guidelines for alternative and complementary veterinary medicine. In: AVMA directory and resource manual (48th edn). American Veterinary Medical Association, Schaumburg, IL

Bag A, Bag N (2018) Tea polyphenols and prevention of epigenetic aberrations in cancer. J Nat Sci Biol Med 9(1):2–5

Chu XH, Hu JP, Wang ZG et al (2011) Effect of feeding of perilla seed extracts on growing finishing pigs. Acta Agric Zhejiang 23 (3):514–516

Demeule M, Brossard M, Turcotte S et al (2004) Diallyl disulfide, a chemopreventive agent in garlic, induces multidrug resistance-associated protein 2 expression. Biochem Biophys Res Commun 324(2):937–945

Deyama T, Nishibe S, Nakazawa Y (2001) Constituents and pharmacological effects of Eucommia and Siberian ginseng. Acta Pharmacol Sin 22(12):1057–1070

Dubey H, Singh A, Patole AM et al (2017) Antihypertensive effect of allicin in dexamethasone-induced hypertensive rats. Int Med Res 6 (1):60–65

Eirna-liza N, Saad CR, Hassim HA, et al (2016). The effects of dietary inclusion of garlic on growth performance and disease resistance of African catfish (*Clarias gariepinus*) fingerlings against *Aeromonas hydrophila* infection. J Environ Biol 37(4 Spec No):817–824

Geng Z, Rong Y, Lau BH (1997) S-allyl cysteine inhibits activation of nuclear factor kappa B in human T cells. Free Radic Biol Med 23 (2):345–350

Gonen A, Harats D, Rabinkov A et al (2005) The antiatherogenic effect of allicin: possible mode of action. Pathobiology 72(6):325–334

Guerry-Force ML, Coggeshall J, Snapper J et al (1988) Morphology of noncardiogenic pulmonary edema induced by Perilla ketone in sheep. Am J Pathol 133(2):285–297

Gupta RC (ed) (2016) Nutraceuticals: efficacy, safety and toxicity. Academic Press/Elsevier, Amsterdam, 1022 p

Gupta RC, Srivastava A, Lall R (2018) Toxicity potential of nutraceuticals. In: Nicolotti O (ed) Computational toxicology: methods and protocols. Springer Nature, New York, NY, pp 367–394

Hirata T, Kobayashi T, Wada A et al (2011) Anti-obesity compounds in green leaves of Eucommia ulmoides. Bioorg Med Chem Lett 21 (6):1786–1791

Hosoo S, Koyama M, Watanabe A et al (2017) Preventive effect of Eucommia leaf extract on aortic media hypertrophy in Wistar-Kyoto rats fed a high-fat diet. Hypertens Res 40(6):546–551

Huang J, Zhang Y, Zhou Y et al (2013) Green tea polyphenols alleviate obesity in broiler chickens through the regulation of lipid-metabolism-related genes and transcription factor expression. J Agric Food Chem 61(36):8565–8572

Jimoh AA, Ibitoye EB, Dabai YU et al (2013) In vivo antimicrobial potentials of garlic against Clostridium perfringens and its promotant effects on performance of broiler chickens. Pak J Biol Sci 16 (24):1978–1984

Kaschula CH, Hunter R, Cotton J et al (2016) The garlic compound ajoene targets protein folding in the endoplasmic reticulum of cancer cells. Mol Carcinog 55(8):1213–1228

Kitaji H, Ookutsu S, Sato M et al (2015) Preincubation with green tea polyphenol extract is beneficial for attenuating sperm injury caused by freezing-thawing in swine. Anim Sci J 86(11):922–928

Lee SD, Kim HY, Song YM et al (2009) The effect of Eucommia ulmoides leaf supplementation on the growth performance, blood and meat quality parameters in growing and finishing pigs. Anim Sci J 80(1):41–45

Li X, Tang Z, Fei D et al (2017) Evaluation of the sedative and hypnotic effects of astragalin isolated from Eucommia ulmoides leaves in mice. Nat Prod Res 31(17):2072–2076

Lin XL, Hu HJ, Liu YB et al (2017) Allicin induces the upregulation of ABCA1 expression via PPARγ/LXRα signaling in THP-1 macrophage-derived foam cells. Int J Mol Med 39(6):1452–1460

Lou Z, Wei QQ, Wang DW et al (2018) Effect of allicin on proliferation and apoptosis of KG-1 cells and its molecular mechanism. Zhongguo Zhong Yao Za Zhi 43(12):2612–2617

Mao X, Gu C, Chen D et al (2017) Oxidative stress-induced diseases and tea polyphenols. Oncotarget 8(46):81649–81661

Martins N, Petropoulos S, Ferreira IC (2016) Chemical composition and bioactive compounds of garlic (Allium sativum L.) as affected by pre- and post-harvest conditions: a review. Food Chem 211:41–50

Müller-Waldeck F, Sitzmann J, Schnitzler WH et al (2010) Determination of toxic perilla ketone, secondary plant metabolites and antioxidative capacity in five Perilla frutescens L. varieties. Food Chem Toxicol 48(1):264–270

Posadino AM, Phu HT, Cossu A et al (2017) Oxidative stress-induced Akt downregulation mediates green tea toxicity towards prostate cancer cells. Toxicol in Vitro 42:255–262

Rahman SU, Huang Y, Zhu L et al (2018) Therapeutic role of green tea polyphenols in improving fertility: a review. Nutrients 10(7):E834

Sarris J, Murphy J, Mischoulon D et al (2016) Adjunctive nutraceuticals for depression: a systematic review and meta-analyses. Am J Psychiatry 173(6):575–587

Senavong P, Kongkham S, Saelim S et al (2016) Neuroprotective effect of perilla extracts on PC12 cells. J Med Assoc Thail 99(Suppl 4): S256–S264

Sheppard JG, McAleer JP, Saralkar P et al (2018) Allicin-inspired pyridyl disulfides as antimicrobial agents for multidrug-resistant Staphylococcus aureus. Eur J Med Chem 143:1185–1195

Shi YL, Gu XH, Huang Y et al (2015) Effect of perilla frutescens seed extracts on performance, reproductive hormone and immune function of laying hens during the late laying peak period. Chin J Anim Nutr 27(5):1519–1526

The Ministry of Agriculture of the People's Republic of China (1999) The approved feed additives. Notice No.105. http://jiuban.moa.gov.cn/zwllm/tzgg/gg/200210/t20021016_14634.htm

The Ministry of Agriculture of the People's Republic of China (2003) The approved feed additive. Notice No.318. https://wenku.baidu.com/view/93dd81d533d4b14e85246859.html

The Ministry of Agriculture of the People's Republic of China (2006) The approved feed additive. Notice No.658. http://www.moa.gov.cn/govpublic/XMYS/201006/t20100606_1535045.htm

The Ministry of Agriculture of the People's Republic of China (2008) Feed additive catalog. Notice No.1126. http://www.moa.gov.cn/gk/tzgg_1/gg/201006/t20100606_1535108.htm

The Ministry of Agriculture of the People's Republic of China (2009) Specification for safe use of feed additives. Notice No.1224. http://www.moa.gov.cn/govpublic/XMYS/201006/t20100606_1535173.htm

The Ministry of Agriculture of the People's Republic of China (2012a) Management measures for approval number of feed additive and additive premix feed products. Decree No.3 in 2012. http://jiuban.moa.gov.cn/zwllm/tzgg/bl/201205/t20120508_2619500.htm

The Ministry of Agriculture of the People's Republic of China (2012b) Management measures for the license number of feed additives and feed additive premix. Decree No.5 in 2012. http://www.gov.cn/gongbao/content/2012/content_2218048.htm

The Ministry of Agriculture of the People's Republic of China (2013a) The approved feed additives. Notice No.2045. http://jiuban.moa.gov.cn/zwllm/tzgg/gg/201401/t20140103_3730193.htm

The Ministry of Agriculture of the People's Republic of China (2013b) The approved feed additives. Notice No.2045. http://www.moa.gov.cn/nybgb/2014/dyq/201712/t20171219_6104350.htm

The Ministry of Agriculture of the People's Republic of China (2014) Measures for the registration and management of imported feed and feed additives. Decree No.2 in 2014. http://jiuban.moa.gov.cn/zwllm/tzgg/bl/201401/t20140120_3743426.htm

The Ministry of Agriculture of the People's Republic of China (2017) Specification for safe use of feed additives. Notice No.2625. http://www.moa.gov.cn/nybgb/2018/201801/201801/t20180129_6135954.htm

The State Council of the People's Republic of China (2011) Regulations for management of feed and feed additives. Decree No.609. http://www.gov.cn/flfg/2011-11/15/content_1993910.htm

Wang C, Li Y, Wang Y (1998) A review of pharmacological study on Epimedium grandiflorum Morr and its active constituents. Zhongguo Zhong Yao Za Zhi 23(3):183–185

Wang JZ, Gao HY, Wang KZ et al (2011) Effect of Epimedium extract on osteoprotegerin and RANKL mRNA expressions in glucocorticoid-induced femoral head necrosis in rats. Nan Fang Yi Ke Da Xue Xue Bao 31(10):1714–1717

Wang J, Liu M, Wu Y et al (2016) Medicinal herbs as a potential strategy to decrease methane production by rumen microbiota: a systematic evaluation with a focus on Perilla frutescens seed extract. Appl Microbiol Biotechnol 100(22):9757–9771

Williamson G (2017) The role of polyphenols in modern nutrition. Nutr Bull 42(3):226–235

Yuan JR, Wang CF, Song J et al (2016) Effect of Epimedii Herba alcohol extract on inhibition of lung tumor growth and immunomodulatory. Zhongguo Zhong Yao Za Zhi 41(1):112–117

Zhang WH, Dong GZ, Wu YX et al (2011) Effects of perilla seed extracts on performance and immune function of finishing cattle. Chin J Anim Nutr 23(3):473–479

Regulation of Nutraceuticals in Australia and New Zealand

Rhian B. Cope

Abstract

In the Australian and New Zealand regulatory landscape, nutraceuticals fall at the potentially very complex human food-medicine interface. Both countries have a very complex set of legislation, legislative instruments, regulations, and guidance documents. In addition, multiple different regulatory bodies and agencies may be involved. In Australia, nutraceuticals may also be subjected to various state and territorial legislation and regulatory processes in addition to the Federal system. For the most part, three main regulatory agencies are likely to be involved: the Australian Therapeutic Goods Administration which is part of the Australian Commonwealth Department of Health, the transnational agency Food Standards Australia New Zealand, and Medsafe New Zealand. To organizations that are unused to the Australian and New Zealand systems, the requirements and processes can seem like a Gordian knot. Thus, this chapter hopes to provide an introduction to these regulatory ecosystems and to demystify some of the important concepts.

Keywords

Nutraceuticals · Veterinary nutraceuticals · Regulation in Australia and New Zealand

1 Introduction

Whether or not products at the human food-medicine interface are regulated as foods or medicines in Australia and New Zealand remains a somewhat complex issue. Within Australia, these products potentially fall either under the Australian Therapeutic Goods Act 1989 where they may be evaluated as a complimentary medicine or a medicine. In New Zealand, they may be regarded as a drug, or they may fall under the Dietary Supplements Regulations 1985, which, in turn, falls under the Food Act 2014. However, depending on the health effects claimed, they may potentially be regarded as a "novel food" or a "food for special medical purposes" under the Food Standards Code, administered by the transnational agency Food Standards Australia New Zealand and by a second-tier layer of legislation at the New Zealand and Australian state and territory level. In New Zealand, they could also be classified as a dietary supplement which is regulated under the Dietary Supplements Regulations 1985, which fall under the Food Act 2014. It could also be regulated as a supplemented food as per the New Zealand Food (Supplemented Food) Standard 2016.

As can already be comprehended by reading the above paragraph, the decisions on what laws, regulatory processes, and regulatory standards apply to a specific nutraceuticals product in Australia and New Zealand can be very confusing.

The objective of this chapter is to (hopefully) demystify some of these issues.

2 What Is and Is Not a Therapeutic Good in Australia

In Australia, a product that is ingested (i.e., swallowed) is regulated as either a food or as a therapeutic good (i.e., a medicine, a complementary medicine, or a medical device). The key issues of whether or not a product is a food or a medicine are the nature of the health benefit claims made by the product. Critically, just because a product is ingested by mouth and makes some type of health-based claim does not automatically make it a therapeutic good. Likewise, just because the physical form of the product is "medicine-like" (e.g., a capsule, a tablet, a powder, etc.) also does not

R. B. Cope (✉)
Australian Pesticides and Veterinary Medicines Authority, Armidale, NSW, Australia
e-mail: rhian.Cope@apvma.gov.au

R. C. Gupta et al. (eds.), *Nutraceuticals in Veterinary Medicine*, https://doi.org/10.1007/978-3-030-04624-8_61

automatically make it either a therapeutic good, a dietary supplement, or a food.

Therapeutic goods in Australia are regulated by 26 different Acts, Regulations, and Legislative Instruments (!). Within Australia, a therapeutic good is specifically defined in Section 3 of the Therapeutic Goods Act 1989 act as the following:

> therapeutic goods means goods:
>
> (a) that are represented in any way to be, or that are, whether because of the way in which the goods are presented or for any other reason, likely to be taken to be:
> (i) for therapeutic use; or
> (ii) for use as an ingredient or component in the manufacture of therapeutic goods; or
> (iii) for use as a container or part of a container for goods of the kind referred to in subparagraph (i) or (ii); or
> (b) included in a class of goods the sole or principal use of which is, or ordinarily is, a therapeutic use or a use of a kind referred to in subparagraph (a) (ii) or (iii);
>
> and includes biologicals, medical devices and goods declared to be therapeutic goods

The above is generally interpreted to cover any product for use in humans in connection with:

1. Preventing, diagnosing, curing, or alleviating a disease, ailment, defect, or injury
2. Influencing inhibiting or modifying a physiological process
3. Testing the susceptibility of persons to a disease or ailment
4. Influencing, controlling, or preventing conception
5. Testing for pregnancy
6. Helping protect the skin from the damaging effects of UV radiation

This also includes things that:

1. Are used as an ingredient or component in the manufacture of therapeutic goods
2. Are used to replace or modify of parts of the anatomy
3. Make therapeutic claims on a label or in advertising (including packaging)

The following dichotomous key works through the seven fundamental questions that producers of nutraceuticals for the Australian market need to answer in order to determine where their product falls on the therapeutic good-nutraceutical interface.

1. Is the product for oral use in humans?
   (a) Yes: Go to 2.
   (b) No: The issue of the food-medicine interface does not apply and the product may be a therapeutic good.
2. Has the Secretary of the Australian Commonwealth Department of Health declared that particular goods or classes of goods are therapeutic goods (called a Section 7 Declaration under the Therapeutic Goods Act 1989)?
   (a) Yes: The issue of the food-medicine interface does not apply and the product is a therapeutic good.
   (b) No: Go to 3.
3. Has the Secretary of the Department of Health declared that particular goods or classes of goods are foods (called a Section 7AA declaration under the Therapeutic Goods Act 1989)?
   (a) Yes: The product is not a "therapeutic good." It is likely to be "food" within state/territory food regulation legislation and/or regulated under other state/territory legislation.
   (b) No: Go to 4.
4. Does the product fit into one of the standards in the Food Standards Code (food additive, vitamins and minerals, processing aid, novel foods, etc.; see http://www.foodstandards.gov.au/code/Pages/default.aspx)
   (a) Yes: The product is not a "therapeutic good." It is likely to be "food" within state/territory food regulation legislation and/or regulated under other state/territory legislation.
   (b) No: Go to 5.
5. Does the product have a tradition of use as a food for humans in Australia and the form of the product has not been substantially altered (e.g., not purified, refined, compositionally altered, etc.)?
   (a) Yes: The product is not a "therapeutic good." It is likely to be "food" within state/territory food regulation legislation and/or regulated under other state/territory legislation.
   (b) No: Go to 6.
6. Do any of the following apply: (a) is the product represented in *any way* to be for therapeutic use (is it a therapeutic good or a therapeutic device)?; (b) likely to be *taken for therapeutic use* because of the way that it is presented?; and (c) likely to be *taken for therapeutic use* for any other reason?
   (a) Yes: The product is a therapeutic good and is assessed and regulated as per the Therapeutic Goods Act 1989.
   (b) No: Go to 7.
7. Is the product in a class of goods the sole or principal use of which is, or ordinarily is, a therapeutic use?
   (a) Yes: The product is a therapeutic good and is assessed and regulated as per the Therapeutic Goods Act 1989.
   (b) No: If it is not a biological or medical device, the product is not a "therapeutic good." It may be "food" within state/territory food regulation legislation.

If the above dichotomous key determines that the product is a therapeutic good, then some specific regulatory consequences apply:

1. The Therapeutic Goods Act 1989 enables the Therapeutic Goods Administration to regulate therapeutic goods that are imported into Australia and/or those which are shipped, transported, or sold across state or territory borders. It does not regulate products that are formulated or compounded within a state or territory that are not shipped, transported, or sold across a national, state, or territory border (e.g., compounded and sold by a practitioner only within a state or territory of Australia, also called extemporaneously compounded). Extemporaneously compounded substances are regulated by state and territory authorities.
2. Therapeutic goods intended solely for the purpose of export are required to be listed (not registered) on the Australian Register of Therapeutic Goods before export is commenced.
3. The Therapeutic Goods Administration may take against the importer, exporter, manufacturer, or supplier if the product is not included in the Australian Register of Therapeutic Goods (ARTG) or is otherwise not exempt or approved under the Therapeutic Goods Act 1989.
4. If the product may be a health risk to the public (e.g., it contains substances that are only available when prescribed by a health professional), the TGA can publish an alert to the public, and if necessary, order a recall of the product.
5. If a product is not a therapeutic good and it is in the ARTG, the TGA can take action under Section 9F of the Therapeutic Goods Act 1989 to remove the product.

3 What is a Complementary Medicine in Australia?

Some classes of nutraceuticals, which meet the definition of a therapeutic good, may be classified as a complementary medicine in Australia. As a general rule, the regulatory rigor applied to complementary medicines is lower than that applied to a medicine or medical device. The pre-market approval evaluations of complementary medicines are typically focused on product safety and manufacturing quality. The standard of evidence to demonstrate efficacy is much lower than that required for a medicine or drug: the focus of the pre-market evaluation is on human safety and manufacturing quality and consistency. They may be listed on the ARTG if certain requirements are met.

A complementary medicine is defined as a therapeutic good consisting wholly or principally of one or more designated active ingredients, each of which has a clearly established identity and each of which has a traditional use. Designated active ingredients may include (but are not limited to):

1. An amino acid
2. Charcoal
3. A choline salt
4. An essential oil
5. A homeopathic preparation
6. A microorganism, whole or extracted, except a vaccine
7. A mineral including a mineral salt and a naturally occurring mineral
8. A mucopolysaccharide
9. Nonhuman animal material (or a synthetically produced substitute for material of that kind) including dried material, bone and cartilage, fats and oils, and other extracts or concentrates
10. A lipid, including an essential fatty acid or phospholipid
11. A substance produced by or obtained from bees, including royal jelly, bee pollen, and propolis
12. A sugar, polysaccharide, or carbohydrate
13. A vitamin or provitamin

Complementary medicines are regulated as per the Australian Regulatory Guidelines for Complementary Medicines (https://www.tga.gov.au/publication/australian-regulatory-guidelines-complementary-medicines-argcm). As discussed above, extemporaneously compounded and dispensed substances may be exempt from Australian Federal Regulation under specific conditions; however, they may be subject to regulation by state and territory authorities.

4 What Is and Is Not a Therapeutic Product in New Zealand and How Are They Regulated?

Therapeutic products and medical devices in New Zealand are potentially regulated by up to 19 different pieces of legislation (!). New or changed medicines and related products that are classifiable as therapeutic products require pre-market evaluation to establish safety, quality, and efficacy as well as being subjected to a post-market assessment process. The term "therapeutic product" is a comprehensive term that is applied to products that are intended to be used in or on humans for a therapeutic purpose as defined by Section 4 of the Medicines Act 1981. A therapeutic product is designed to:

1. Prevent, diagnose, monitor, alleviate, treat, cure, or compensate for a disease, ailment, defect, or injury.
2. Influence, inhibit, or modify a physiological process.
3. Test the susceptibility of persons to a disease or ailment.

4. Influence, control, or prevent conception.
5. Test for pregnancy
6. Investigate, replace, or modify parts of the human anatomy.

Under the New Zealand approach, products are regarded as having a therapeutic purpose if:

1. The product contains one or more ingredient(s) that have a pharmacological action.
2. A therapeutic purpose is claimed for the product (usually on the label or in promotional material).
3. A therapeutic purpose is implied for the product (usually on the label or in promotional material).
4. The product contains a medicine listed in the First Schedule to the Medicines Regulations or a Notice in the New Zealand Gazette issued under Section 106 of the Medicines Act 1981 (unless the product is in a form that cannot be administered to a human being for a therapeutic purpose).

In order for a product not to be regarded as having a therapeutic purpose, the label and promotional material must (at the very least) avoid the following:

1. A trade name that conveys an intended therapeutic purpose
2. Words such as remedy, medicated, or therapeutic
3. Statements that a product will/can/may prevent or treat a disease or condition or give relief from symptoms of a disease or condition
4. Statements of traditional therapeutic use or use by ethnic groups for a therapeutic purpose
5. Directions for use that infer a therapeutic purpose such as "dosing instructions" or instructions to "apply to the affected area"
6. Statements to the effect that the law prevents the supplier from making therapeutic claims that they consider they should be able to make about the product

Critically, nutritional statements (i.e., statements regarding a normal biochemical or nutritional characteristic of a product) are not regarded as therapeutic claims.

Section 2 of the Medicines Act 1981 treats herbal remedies as a subcategory of therapeutic products provided that they do not contain a prescription, restricted or pharmacy-only medical ingredient. To be defined as an herbal remedy, the product must be derived from a plant material that has been dried, crushed, subjected to aqueous or ethanolic extraction, or be a mixture of the plant-derived material with an inert substance.

Section 94 of the Medicines Act 1981 also identifies another class of substances termed "related products." These are products that are primarily foods, dentifrices, or cosmetics that also have a therapeutic purpose. Related products much not contain a prescription, restricted or pharmacy-only medicine ingredient.

In New Zealand, dietary supplements are controlled by the Dietary Supplements Regulations 1985. These products are defined by Regulation 2 as an edible substance, in a controlled dosage form, which is intended to supplement the intake of substances normally derived from food. These substances must not be marketed or promoted for a therapeutic purpose.

5 What is a Novel Food in Australia and New Zealand

Some nutraceuticals that are not therapeutic goods or therapeutic products may be classified as a novel food as per Australia New Zealand Food Standards Code—Standard 1.5.1. Like complementary medicines, these products require pre-market approval before sale and use. Typically, a very extensive and detailed pre-market evaluation is carried out by Food Standards Australia New Zealand, a transnational agency. Additional regulation may occur at the Australian state and territory level or in New Zealand.

A novel food is a substance or proposed food ingredient that has no history of traditional use. The term "no history of traditional use" is specifically defined as follows (https://www.legislation.gov.au/Details/F2017C00324):

> (a) a food that does not have a history of human consumption in Australia or New Zealand; or
> (b) a substance derived from a food, where that substance does not have a history of human consumption in Australia or New Zealand other than as a component of that food; or
> (c) any other substance, where that substance, or the source from which it is derived, does not have a history of human consumption as a food in Australia or New Zealand.
> (d) The presence of a food in a food for special medical purposes or the use of a food as a food for special medical purposes does not constitute a history of human consumption in Australia or New Zealand in relation to that food for the purposes of this section.

The pre-market evaluation of novel foods in Australia typically involves an in-depth and detailed assessment of the public health and safety considerations having regard to:

1. The potential for adverse effects in humans
2. The composition or structure of the food
3. The process by which the food has been prepared
4. The source from which it is derived
5. Patterns and levels of consumption of the food
6. Any other relevant matters

Typical categories of novel foods may include (a) plants or animals and their components; (b) plant or animal extracts; (c) herbs, including extracts; (d) dietary macro-components; (e) single chemical entities; (f) microorganisms, including probiotics; and (g) foods produced from new sources or by a process not previously applied to food.

6 Concluding Remarks and Future Directions

Taking a helicopter view, the critical issue that must be addressed by a nutraceutical producer in Australia and/or New Zealand is whether or not a therapeutic claim will be made by the product. Although the Australian and New Zealand regulatory landscapes are complex, the first overriding principle in both systems is that if a product makes any sort of therapeutic claim in any form of media (labeling, packaging, promotional, advertising, etc.) regarding the substance, then it will be potentially regulated as a therapeutic good or therapeutic product. The second overriding principle is that if the product contains any drug or pharmaceutical substance (including anything that is chemically related to a drug or pharmaceutical active substance), then it will potentially be regulated as a therapeutic good or therapeutic product. The third overriding principle is that, irrespective of what regulatory category a nutraceutical falls under in Australia and New Zealand, the human safety and quality characteristics of the product remain paramount. Both countries have very substantial legal and regulatory processes for the restriction and/or removal of products that have human safety concerns. If nutraceutical producers have any doubts about the legalities of their products and how they may be regulated, they should contact the Australian Therapeutic Goods Administration, Medsafe New Zealand, or Food Standards Australia New Zealand *before* producing, importing, or marketing their products.

References

Commonwealth of Australia Therapeutic Goods Act 1989. https://www.legislation.gov.au/Series/C2004A03952

Therapeutic Goods Administration Australia. TGA basics. https://www.tga.gov.au/tga-basics

Therapeutic Goods Administration Australia. How therapeutic goods are regulated in Australia. https://www.tga.gov.au/how-therapeutic-goods-are-regulated-australia

Therapeutic Goods Administration Australia. Food-Medicine Interface Guidance Tool (FMIGT). https://www.tga.gov.au/food-medicine-interface-guidance-tool-fmigt

Medsafe New Zealand. Introductory Regulatory Guidance. http://www.medsafe.govt.nz/regulatory/regguidance.asp

Medsafe New Zealand. Categorisation of products. http://www.medsafe.govt.nz/regulatory/categorisation-of-products.asp

The Association of New Zealand Advertisers. Therapeutic advertising pre-vetting service. https://www.anza.co.nz/Category?Action=View&Category_id=262

Food Standards Australia New Zealand. Regulation of novel foods. http://www.foodstandards.gov.au/industry/novel/Pages/default.aspx

Food Standards Australia New Zealand. Guidance tool for determining whether a food is novel or not. http://www.foodstandards.gov.au/industry/novel/documents/Guidance%20Tool%20-%20for%20website%20_2_.pdf

Food Standards Australia New Zealand. Advisory Committee Novel Foods. http://www.foodstandards.gov.au/industry/novel/novelcommittee/Pages/default.aspx

Regulatory Guidelines for Nutraceuticals and Dietary Supplements for Animals in Turkey

Ayhan Filazi and Begüm Yurdakok-Dikmen

Abstract

Due to global trend in healthy food from farm to fork which compromises human health, food safety, and environmental protection, consumers today are more sensitive to contaminant-free foods of animal origin. These contaminants are mainly fertilizers, biotechnological additives, agricultural products, pesticide residues, veterinary medicines, chemical additives, genetically modified products, and contaminants arising from product processing, maintenance, transport, and storage. To increase both the quantity of animal products and the hygiene quality and standards of those goods, feed additives are being widely used. The ability to identify additive substances used in animal nutrition—in both feed and feedstuffs—is very important when investigating the effects of these substances on human health. In Turkey, nutraceuticals are listed as "feed/food supplements" in Law on Veterinary Services Plant Health Food and Feed. All legislation and guidelines being implemented for this purpose in Turkey comply with European Union legislation. This chapter provides information on regulations pertaining to the approval of feed additives used in Turkey, as well as those pertaining to the launch and control of those additives.

Keywords

Animal · Feed supplement regulatory guidelines · Legislation · Nutraceuticals · Turkey

1 Introduction

Feed, which is used in animal nutrition, constitutes an important link in the food production chain. Feed produced to meet animal nutrition requirements features a balanced and appropriate mixture and plays an important role in increasing animal production and bringing about economical animal breeding. Animal-breeding targets are about obtaining maximum efficiency per animal; these targets need to be met if animal-derived food quantities are to be reliable, so as to protect public health. An important point in obtaining safe animal-derived food is the reliable production of feed in accordance with hygiene rules. To ensure farm-to-table food safety, feed needs to be produced, transported, stored, and used in accordance with good production practices and hygiene rules at all stages, from primary production to final product acquisition (Dorne and Fink-Gremmels 2013).

Livestock production comprises an important part of Turkey's agricultural sector and economy. The growing demand for animal products on account of increased population, along with growing export demands, has increased the expectations placed on both local breeders and large companies vis-à-vis sustainable clean/green/ethical production. Turkey has a distinct advantage, situated in the center of three different climate zones and three different kinds of soil conditions with a unique geography leading to optimal growth of various plant- and animal-derived products.

According to the Turkish Statistical Institute's (2017) animal production statistics, there were increases in bovine, small ruminant, and poultry numbers, along with their products, relative to 2016. The numbers of livestock in Turkey, by species, are as follows: bovine (cattle and buffalo), 16,105 million heads; small ruminant (sheep and goats), 44,312 million heads; broiler chickens, 221,245 million units; and laying hens, 121,556 million units (Turkish Statistical Institute 2017). These large numbers indicate the application of intensive management systems supported by major technological innovations and structural changes. With these systems, sustainable development is directly linked to sustainable animal nutrition and disease control practices—practices that should be carefully applied to ensure safe, efficient, and high-quality animal production yields. Disease prevention and control management to protect the economy

A. Filazi (✉) · B. Yurdakok-Dikmen
Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, Ankara University, Ankara, Turkey

R. C. Gupta et al. (eds.), *Nutraceuticals in Veterinary Medicine*, https://doi.org/10.1007/978-3-030-04624-8_62

and enhance food security are expected to be promoted in the form of drug-free production systems, mainly in response to resistance issues and public demand. Meanwhile, cost-effective methods are still in high demand among producers. Given the increased threat of antimicrobial resistance in treating both human and animal health in Turkey (Yilmaz et al. 2016), alternative strategies by which to retain optimal production strategies and cope with increasing numbers of animals have emerged. Nutraceuticals play an important role in drug-free production systems, since their anti-inflammatory, antioxidant, and microbiota-modulating effects are key to conserving animal health.

During the process of harmonizing Turkish legislation relating to food and feed safety with European Union (EU) legislation (Regulation 178/2002/EC, 2002), the first Veterinary Services Plant Health Food and Feed law (Law No 5996, 2010) were prepared.

The secondary legislation on the feed issue prepared in accordance with this Law is as follows:

1. The Regulation on Feed Hygiene (27 December 2011)
2. The Regulation on Animal By-products and Derived Products not Intended for Human Consumption (24 December 2011)
3. The Regulation on Placing on the Market and Feed Use (27 December 2011)
4. The Communiqué on Undesirable Substances in Animal Feed (19 April 2014)
5. The Regulation on the Official Control of Feed and Food (17 December 2011)
6. The Regulation on Feed Additives for Use in Animal Nutrition (18 July 2013)
7. The Communiqué as Regards the Preparation and the Presentation of Applications and the Assessment and The Authorisation of Feed Additives (27 May 2016)

These legislations were all prepared in compliance with the European Unions and updating regularly according to the EU legislation. This chapter is aimed to give information about regulatory guidelines for nutraceuticals and dietary supplements in animals in Turkey.

2 Applications Relating to Feed Hygiene Regulation

Feed hygiene regulation (Regulation 2011/28155, 2011a) has been prepared as per EU Regulation No. 183/2005/EC, the purposes of which are to provide the general rules concerning feed hygiene and the conditions required for the registry and approval of feed enterprises, enable feed traceability, and make necessary arrangements regarding these subjects. According to this Regulation, feed enterprises are divided into two categories—namely, registered enterprises and approved enterprises. According to *The Regulation on Animal By-products and Derived Products Not Intended for Human Consumption* (Regulation 2011/28152, 2011), rendering enterprises (meat–bone meal, chicken meal, fish meal, etc.) and enterprises that produce cat or dog food are obligated to obtain approval from the relevant competent authorities. Apart from these, all other enterprises are required to be registered. As of July 2018, approximately 13,600 feed enterprises were approved and registered in Turkey (Table 1). In the mixed-feed sector in 2017, nearly 23 million tons of mixed feed were produced in total, most of which was broiler chicken feed (Ministry of Agriculture and Forestry 2018).

Feed enterprises are obligated to comply with the hygiene rules specified in this Regulation, at every stage of production, operations, and distribution (excluding primary production). According to this Regulation, feed operators are obligated to establish and implement a feed safety system that is based on Hazard Analysis and Critical Control Points (HACCP) principles in production. When operating in accordance with the HACCP principles, it is critical to identify and control those hazards that may have adverse effects on the reliability of any animal feed produced and marketed; in this way, safe and reliable feed production is assured (Regulation 2011/28155, 2011a).

With risk assessment, the probability of occurrence of a physical, chemical, or biological factor or situation that can have a negative impact on feed safety, as well as the intensity

Table 1 Number of registered and approved feed enterprises in Turkey[a]

Operating type	Number of enterprises	Approved/ registered
Mixed feed-producing enterprises for food animals	322	Approved
Enterprises that produce their own mixed feed	108	Approved
Feed additive-producing enterprises	15	Approved
Premix-producing enterprises	100	Approved
Cat-dog food-producing enterprises	29	Approved
Rendering enterprises	77	Approved
Feed additive and premix sales enterprises	1002	Approved
Mixed-feed-producing enterprises for food animals	193	Registered
Enterprises that produce their own mixed feed	443	Registered
Block mineral (licking block)-producing enterprises	23	Registered
Feed additive- and premix-producing enterprises	28	Registered
Retail storage and sales shops	11,237	Registered
Fish meal-producing enterprises	23	Registered
Total	13,600	Registered

[a]Reference: Ministry of Agriculture and Forestry 2018

of probable impacts, is evaluated, where a risk score of danger is determined. By scoring dangers in this way, it is possible to assign greater importance to those risks with higher scores. After classifying dangers in terms of their scores, if a danger with a high-risk score were to occur, it would be important to apply control measures to either eliminate the danger or mitigate it to an acceptable level. This process can be readily applied to hazards as they pertain to feed and feedstuffs.

3 Applications Relating to the Regulation on Placing on the Market and Feed Use

This Regulation (Regulation 2011/28155, 2011b) was prepared in accordance with EU Regulation No. 767/2009/EC, with the aims of ensuring top-level feed safety, protecting animal and public health, adequately informing users and consumers, and regulating conditions related to the market launch of feed, as well as the quality thereof. According to this Regulation, feed that is not safe cannot be launched to the market. Feed enterprises have an obligation to ensure that the feed they launch to the market is safe and that it is labeled, packed, and put on the market in accordance with their features and intended usage. This Regulation also specifies that all feed content components be specified on the product labeling, along with their weight ratios (in descending order). Compliance with this feed Regulation demands that all feed be labeled in this manner before being launched to the market.

4 Applications Relating to the Communiqué on Undesirable Substances in Animal Feed

This Communiqué (Communique 2014/11, 2014) is prepared in accordance with EU Directive No. 2002/32/EC. "Undesirable substances" in animal feed are materials that are in the feed apart from pathogenic elements and which pose potential hazards for animal and human health and the environment or which may have a negative impact on animal production. This Communiqué specifies the maximum allowable limits for substances not desired in the feed. As these substances constitute a chemical hazard within the audit scope of the Communiqué, inspections are carried out by searching for and analyzing inorganic contaminants and nitrogenous compounds, mycotoxins, plant toxins, organic chlorinated compounds (including dioxins and PCBs), and harmful botanical contaminations. Also inspected are approved feed additives that cannot help but be transported with other feeds. If the values determined in the course of this analysis exceed the maximum limits imposed by the Communiqué, the feed is considered "unsafe feed," and it cannot be neither used to feed animals nor placed on the market.

5 Applications Relating to the Regulation on the Official Control of Feed and Food

This Regulation (Regulation 2011/28145, 2011) is prepared in accordance with EU Regulation No. 882/2004/EC. To ensure safe food and feed supplies, this Regulation aims to help control all production and distribution stages from the field to the table and to promote investigations regarding whether or not the conditions specified in the legislation are being fulfilled. Feed inspections executed as per this Regulation are carried out within the context of the annual feed control plan. Inspections are also executed in response to notifications and complaints. Inspections carried out within the framework of the feed control plan are scheduled while mainly considering the results of investigations conducted in the previous year, as well as the enterprise's production capacity, its feed varieties, possible product hazards, risks incurred by such hazards on animal and human health and the environment, and similar factors. Risk analysis is conducted each year, and the conditions underlying them are also revised each year. During those inspections, feed enterprises are investigated with respect to compliance with hygiene conditions, and whether the feed being produced and/or launched to the market is controlled in terms of compliance with feed safety regulations. Feed samples taken during feed safety inspections are analyzed on a risk basis (i.e., against chemical and biological hazards) and with respect to labeling compliance. On a risk basis, analyses are undertaken with respect to undesired substances in the feed (e.g., heavy metals, mycotoxins, anticoccidials, pesticides, and dioxins), substances that are prohibited from being added to feed (certain animal-derived proteins in ruminant feed, antibiotics), microbiological contamination (*Salmonella* or *Enterobacteriaceae*), and those concerning biosafety legislation compliance. In particular, the frequency of risk-based control testing and analysis is expected to be increased each year.

6 Applications Relating to the Regulation on Feed Additives for Use in Animal Nutrition

In Turkey, processes relating to feed additives are carried out as per the provisions of *The Regulation on Feed Additives for Use in Animal Nutrition* (Regulation 2013/28711, 2013), which aims to protect human and animal health, animal welfare, and the environment. This Regulation sets forth procedures and principles for the authorization, inspection,

and labeling of feed additive products and premixtures that are destined for the market. This Regulation does not apply to veterinary medicinal products, with the exception of coccidiostats and histomonostats used as feed additives. Additionally, this Regulation is based on articles 21, 22, 24, 25, 26, and 43 of Law 5996, the basic legislation in Turkey on food quality control/food safety, consumer protection, *Codex Alimentarius*, international trade, inspection, offenses/penalties, animal feed/feedstuffs, animal health, animal welfare, plant protection, and pests/diseases. It is also prepared in accordance with EU Regulation No. 1831/2003/EC. Processes relating to the approval of a new feed additive substance not stated in the recorded list of feed additive substances are carried out as per *The Communiqué as Regards the Preparation and the Presentation of Applications and the Assessment and The Authorization of Feed Additives* (Communique 2016/15, 2016). This Communiqué also complies with EU Regulation No. 429/2008/EC.

Regarding feed additive substances and premixes, controls are carried out especially with regard to searches for heavy metals and antibiotics and to ensure compliance with label information regulations. The use of antibiotics as feed additive substances has been prohibited since 2006. However, in cases where disease is seen in an animal, exceptions are allowed—in which case, if a treatment drug is used and administered in tandem with its feed, approved feed-producing enterprises may produce feed containing antibiotics but only under a veterinarian's prescription. Such veterinary medical premixes must be approved by the relevant competent authorities.

Feed additives are used in animal production to improve product capacity and to ensure hygiene quality and standards compliance. Feed additives are generally provided from abroad by companies that produce mixed feeds or trade in feed additives in pure, concentrated, or premixed forms. Feed additives that are imported in pure or concentrated forms are presented for use after they have been diluted to certain ratios with an appropriate carrier at premix preparation facilities. Feed additives can be added as a single additive premix with a mixed feed carrier substance, in the form of mixtures containing more than one effective substance; in the form of special premixes, such as vitamin concentrates; as mixed premixes containing vitamin-trace minerals or amino acids; or as minerals or complementary feed containing macro and trace elements. Feed additives that are used in mixed feed for poultry animals at significant ratio levels (for various purposes) are generally used at lower ratios in mixed feed for bovine and ovine animals, so as to contain more vitamin/trace element premixes.

Feed additives are substances with organic or inorganic structures that are added to feed to ensure optimal nutrition substance consumption, increase animal product quantity, improve feed use by aiding with digestion and metabolism, protect animal health, influence animal product quality positively, facilitate the preparation and storage of feed, or derive economic benefits in other ways (Regulation 2013/28711, 2013). Although they are used at lower ratios in mixed feed, their effectiveness and importance are much higher.

According to EU Regulation No. 2013/28711 and Turkish Legislation, the term "feed additives" refers to substances, microorganisms, or preparations, other than feed material and premixtures, that are intentionally added to feed or water in order to perform, in particular, one or more of the following functions:

1. Favorably affect feed characteristics
2. Favorably affect animal product characteristics
3. Favorably affect the color of ornamental fish and birds
4. Satisfy the nutritional needs of animals
5. Favorably affect the environmental consequences of animal production
6. Favorably affect animal production, performance, or welfare, particularly by affecting the gastrointestinal flora or digestibility of feedstuffs
7. Have a coccidiostatic or histomonostatic effect

Today, a large number of additives are used in support of animal nutrition. In Turkey, only the use of EU-approved feed additives is allowed (European Union Register of Feed Additives 2018). Table 2 lists the categories of feed additives approved by the relevant competent authority (Ministry of Agriculture and Forestry 2018).

6.1 Technological Additives

6.1.1 Preservatives

These substances or microorganisms protect feed against deterioration, as caused by either microorganisms or their metabolites. Some of the materials currently used as preservatives are lactic acid, acetic acid, propionic acid, sorbic acid, DL-malic acid, potassium sorbate, calcium acetate, sodium propionate, calcium propionate, ammonium propionate, ammonium formate, calcium lactate, citric acid, orthophosphoric acid, potassium sorbate, sodium diacetate, calcium acetate, sodium formate, potassium diformate, calcium formate, fumaric acid (poultry and pigs, young animals fed with milk replacers, other animal species), sodium bisulfate (pets and other non-food-producing animals other than cats and mink, cats, mink), sodium nitrite (dogs, cats), sodium benzoate 140 g/kg + propionic acid 370 g/kg + sodium propionate 110 g/kg (pigs, poultry, bovines , sheep, goats,

Table 2 Additives used in feeds in Turkey

Group	Feed additives
1) Technological additives	a) Preservatives b) Antioxidants c) Emulsifying and stabilizing agents, thickeners, and gelling agents d) Acidity regulators e) Binders, anticaking agents, and coagulants f) Substances that control radionuclide contamination g) Silage additives
2) Sensory additives	a) Colorants b) Flavoring compounds
3) Nutritional additives	a) Vitamins, provitamins, and chemically well-defined substances bearing similar effect b) Trace element compounds c) Amino acids, their salts, and analogs d) Urea and its derivatives
4) Zootechnical additives	a) Digestibility enhancers b) Gut flora stabilizers c) Substances which favorably affect the environment d) Other zootechnical additives
5) Coccidiostats and histomonostats	–

rabbits, horses), and *Lactobacillus fermentum* + *L. plantarum* + *L. rhamnosus* (dogs). The use of formaldehyde as a feed preservative has been banned since February 7, 2018.

6.1.2 Antioxidants

Antioxidants are substances that prolong the storage life of feedstuffs and feed materials by protecting them against oxidation-induced deterioration. Some antioxidants are synthetic; others are natural, and in recent years, their use has increased. Materials used for this purpose include ascorbic acid, sodium ascorbate, calcium ascorbate, ascorbyl palmitate, tocopherol extracts from vegetable oils, tocopherol-rich extracts from vegetable oils (delta rich), alpha-tocopherol, propyl gallate, butylated hydroxyanisole, butylated hydroxytoluene, and ethoxyquin. Some aromatic plants (e.g., sage, rosemary, laurel, clove, thyme, cumin, peppermint, and cinnamon) have been used as antioxidants.

6.1.3 Emulsifying and Stabilizing Agents, Thickeners, and Gelling Agents

Emulsifiers are substances that make it possible to form or maintain within feedstuffs a homogeneous mixture of two or more immiscible phases. These are used to provide a homogenous distribution of feed, and they are typically added to feed in an oil form to provide energy. Stabilizers are substances that make it possible to maintain the physiochemical state of feedstuffs; they are used to increase the stability of vitamins, essential amino acids, and certain medicines that are added to feed in very small amounts. Thickeners, meanwhile, are substances used to increase the viscosity of feedstuffs, while gelling agents are substances that give feedstuffs more texture through the formation of a gel. Such substances include lecithins (only as emulsifying agent), sodium alginate [fish; pets and other non-food-producing animals (nonfood fur animals)], agar [pets and other non-food-producing animals (nonfood fur animals)], carrageenan [pets and other non-food-producing animals (nonfood fur animals)], locust bean gum (Carob gum), guar gum, tragacanth, acacia (Gum arabic), xanthan gum, polyoxyethylene (20)-sorbitan monooleate, microcrystalline cellulose, methylcellulose, ethyl cellulose, hydroxypropyl cellulose, hydroxypropyl methylcellulose, carboxymethylcellulose, glyceryl polyethyleneglycol ricinoleate, polyethyleneglycol ester of fatty acids from soya oil [calves], sorbitan monolaurate, and cassia gum (dogs, cats).

6.1.4 Acidity Regulators

Acidity regulators are substances that adjust feedstuff pH. DL- and L-Malic acid (cats, dogs), sodium hydroxide (cats, dogs, ornamental fish), and sodium bisulfate are used for this purpose.

6.1.5 Binders, Anticaking Agents, and Coagulants

Binders are substances that increase the tendency of feedstuff particles to adhere to one another, while anticaking agents reduce this tendency. Coagulants allow liquid feedstuffs to darken to a semisolid clotting state. Some substances used for these purposes are sodium ferrocyanide, potassium ferrocyanide, silicic acid (precipitated and dried), colloidal silica, kieselgur (diatomaceous earth, purified), calcium silicate (synthetic), sodium aluminosilicate (synthetic), kaolinitic clays (free of asbestos), natural mixtures of steatites and chlorite, vermiculite, sepiolite, sepiolitic clay, lignosulphonates, natrolite-phonolite, clinoptilolite of volcanic origin (pigs, poultry), and perlite.

6.1.6 Substances That Control Radionuclide Contamination

Substances used to control radionuclide contamination suppress the absorption of radionuclides or promote their excretion. Substances used as radioactive cesium binders (^{137}Cs and ^{134}Cs) include ferric (III) ammonium hexacyanoferrate (II) [ruminants (domestic and wild); calves prior to the state of rumination; lambs prior to the state of rumination; kids prior to the state of rumination; pigs (domestic and wild)] and bentonite [all animal species].

6.1.7 Silage Additives

Silage additives are substances (including enzymes or microorganisms) incorporated into feed to improve silage production. Enzymes used for this purpose include

alpha-amylase (from *Aspergillus oryzae* or *Bacillus amyloliquefaciens* or *Bacillus subtilis*), beta-glucanase (from *Aspergillus niger*), cellulase (from *Aspergillus niger* or *Trichoderma longibrachiatum*), and xylanase (from *Trichoderma longibrachiatum*). The microorganisms used in the production of the silage are *Enterococcus faecium*, *Lactobacillus brevis*, *L. buchneri*, *L. plantarum*, *L. fermentum*, *L. casei*, *L. kefiri*, *L. diolivorans*, *L. paracasei*, *L. rhamnosus*, *Propionibacterium acidipropionici*, preparation of *L. plantarum*, and *Pediococcus pentosaceus*. In addition, organic acids and chemicals such as ammonium propionate, potassium sorbate, formic acid, sodium formate, propionic acid, sodium propionate, ammonium propionate, and sodium benzoate are also used in the production of silage.

6.2 Sensory Additives

6.2.1 Colorants

Colorants are substances that add or restore feedstuff color; these, when fed to animals, add colors to food of animal origin and when consumed can favorably affect the color of ornamental fish or birds. They are commonly used in the poultry sector to improve the color of chicken meat and eggs. For this purpose, plant materials (e.g., yellow corn, alfalfa, corn gluten, and red pepper powder) containing various levels of coloring materials, as well as carotenoids such as lutein, zeaxanthin, capsanthin, and lycopene extracted from natural sources, are used. Synthetic sources such as ethyl ester of beta-apo-8′-carotenoic acid, citranaxanthin, tartrazine, sunset yellow, and Ponceau 4 R (dogs, cats) are also used. Substances that enhance or restore the color of feedstuffs include quinoline yellow (non-food-producing animals), azorubine (carmoisine) (cats and dogs), and patent blue V (non-food-producing animals).

Substances that, when fed to animals, add colors to food of animal origin include astaxanthin dimethyldisuccinate (salmon, trout), red carotenoid-rich *Paracoccus carotinifaciens* (salmon, trout), canthaxanthin (chickens for fattening and minor poultry species for fattening, laying poultry and poultry reared for laying), and astaxanthin (fish, crustacean).

Finally, substances that favorably affect the color of ornamental fish or birds include canthaxanthin (ornamental fish and ornamental birds except ornamental breeder hens; ornamental breeder hens) and astaxanthin (ornamental fish). Other colorants are tartrazine (grain-eating ornamental birds, small rodents, ornamental fish), sunset yellow FCF (grain-eating ornamental birds, small rodents, ornamental fish), Ponceau 4 R (ornamental fish), erythrosine (ornamental fish), indigotine (ornamental fish), beta-carotene (canaries), and red iron oxide (ornamental fish).

6.2.2 Flavoring Compounds

Flavoring compounds are substances that, when added to feedstuffs, enhances feed smell or palatability. Some of the substances allowed for this purpose are indole, beta-alanine, taurine, citronellol, methoxyacetophenone, phenylmethanethiol (cats, dogs), alpha-methylcinnamaldehyde, diphenyl ether, alpha-hexylcinnamaldehyde, ethyl phenylacetate, isobutyl phenylacetate, 3-methylbutyl phenylacetate, phenethyl phenylacetate, methyl cinnamate, ethyl cinnamate, benzyl cinnamate, phenethyl acetate, phenethyl butyrate, cinnamyl acetate, phenylacetic acid, phenylpropanal, cinnamyl alcohol, cinnamaldehyde, ethyl butyrate, geranyl formate, geranyl propionate, neryl propionate, geraniol, and nerol.

6.3 Nutritional Additives

6.3.1 Vitamins, Provitamins, and Chemically Well-Defined Substances Bearing Similar Effects

Salts and the analogs of amino acids and vitamins or provitamins are added to feed to meet animals' nutritional requirements. For this purpose, substances such as vitamins A, B, C, D, E, and K and their salts, calcium-D-pantothenate L-carnitine, folic acid, omega-3 and omega-6, etc., among others, are used.

6.3.2 Trace Element Compounds

Among the trace elements added to feed are iron, selenium, potassium, copper, cobalt, calcium, manganese, zinc, molybdenum, and their salts.

6.3.3 Amino Acids, Their Salts, and Analogs

Some of the allowable substances that fall under this category are lysine; L-threonine, L-histidine, and L-tryptophan produced through fermentation with *Escherichia coli*; and L-arginine, L-valine, and sodium DL-methionine produced by *Corynebacterium glutamicum*.

6.3.4 Urea and Its Derivatives

These additives are generally used as protein sources, and they are added to the diets of ruminant animals that have functional rumen. The most commonly used compounds are urea, biuret, urea-phosphate, and diureidoisobutane.

6.4 Zootechnical Additives

6.4.1 Digestibility Enhancers

These substances enhance diet digestibility, through action on target feed materials when fed to animals. For this

purpose, polysaccharides (lipases, phytases, pectinase, amylase, and cellulase) from various fungi (e.g., *Trichoderma reesei*, *T. viride*, and *Aspergillus niger*) or from certain bacteria (e.g., *Bacillus subtilis*) are used to break down polysaccharides that cannot be digested in the intestinal environment of poultry. Certain enzymes (cellulase, xylanase, endoglucanase, exoglucanase, amylase, and proteases) are also used to improve digestion and improve rumen performance.

6.4.2 Gut Flora Stabilizers

Gut flora stabilizers have a positive effect on gut flora. Substances whose chemical properties have been so described, or microorganisms that have a positive effect on intestinal microflora, help comprise this group. Enzymes, probiotics, prebiotics, essential oils, and plant extracts are used to regulate digestive and intestinal microflora, and these additives are multifunctional. The microorganisms most commonly used to produce probiotics are *Lactobacillus* and *Streptococcus* bacteria, which produce lactic acid. *Saccharomyces cerevisiae* from yeasts and *Aspergillus niger* and *A. oryzae* from fungi are also widely used in commercial probiotic production. Some probiotics are *Saccharomyces cerevisiae*, *Enterococcus faecium*, *Pediococcus acidilactici*, *Lactobacillus acidophilus*, *Bacillus subtilis*, *B. amyloliquefaciens*, *B. licheniformis*, *Clostridium butyricum*, *Bifidobacterium animalis* ssp. *animalis* + *Lactobacillus salivarius* ssp. *salivarius* + *E. faecium*, *E. faecium* + *L. acidophilus* + *L. helveticus* + *L. delbrueckii* ssp. *lactis* + *L. delbrueckii* ssp. *bulgaricus* + *Streptococcus thermophilus*, and *L. lactis* + *Carnobacterium divergens* + *L. casei* + *L. plantarum* + *S. cerevisiae.*

Prebiotics are intact nondigestible feed additives that increase the number and activity of beneficial bacteria living in the intestines and which thus positively improve animal health. The most commonly used prebiotics are mannan oligosaccharides, fructooligosaccharides, chitosan oligosaccharides, and beta-glucans.

Herbal extracts and essential oils—both of which are natural in recent times—offer antimicrobial efficacy, promote growth, and improve feed efficiency. Aromatic plants, extracts, and oils such as thyme, rosemary, sage, clove, laurel, cinnamon, cumin, coriander, ginger, mustard, garlic, and mint are used as additives.

6.4.3 Substances That Favorably Affect the Environment

Substances that favorably affect the environment include substances used to reduce the formation of methane gas in ruminants, as well as phytase enzyme, which has been shown to enhance plant phosphorus utilization in poultry. Methane production by rumen microorganisms leads to the attenuation of both methane gas production (i.e., from the feed energy) and pollution from the methane emitted into the atmosphere. To prevent methane production, oils, chloral, hemiacetate products of starch and halogenated compounds (tetrachloride methylene chloride, bromochloromethane), and anticoccidials (e.g., lacoloside and monensin) are used. Another supplement is phytase, which is used to increase plant phosphorus use among poultry. The enzyme phytase is used both to increase vegetative phosphorus use and prevent phosphorus pollution by reducing phosphorus levels in groundwater and the soil.

6.4.4 Other Zootechnical Additives

Toxin binders are used to prevent mold growth in feed. For this purpose, compounds such as organic acids (propionic, sorbic, benzoic, and acetic acids), organic acid salts (such as calcium propionate and potassium sorbate), organic dyes, and copper sulfate are used. Adsorbent materials such as polyvinylpolypyrrolidone polymers, aluminum silicate compounds, activated charcoal, hydrate sodium calcium aluminosilicate (HSCAS), bentonite, perlite, diatomaceous earth, and zeolite are used as toxin binders in feeds.

Buffer substances are typically used in ruminants to prevent pH attenuation in the rumen—a condition that typically stems from the use of large amounts of intense feed. In recent years, sodium bicarbonate has been widely used for this purpose; also used are buffer materials such as various salts from volatile fatty acids, phosphate salts, ammonium chloride, and sodium sulfate.

Chitosan is a product obtained by deacidifying chitin into a nontoxic and bioavailable biopolymer structure that resembles cellulose found in arthropoda (crabs and shrimp); it is useful, in that it promotes enhanced digestibility, growth performance, energy and protein utilization, and rumen fermentation.

6.5 Coccidiostats and Histomonostats

Coccidiostats and histomonostats are substances administered to kill or otherwise inhibit protozoa. They are substances used to protect *Eimeria* species placed in the intestines of poultry from protozoa that can cause coccidiosis (i.e., bloody diarrhea). Some substances are allowed by law to be used (e.g., monensin sodium, decoquinate, robenidine hydrochloride, lasalocid A sodium, halofuginone hydrobromide, narasin, salinomycin sodium, maduramicin ammonium alpha, diclazuril, semduramicin sodium, nicarbazin, and diclazuril); however, there are withholding periods for each of these substances, and adherence to such regulations is essential.

7 Concluding Remarks and Future Directions

Turkey has great potential with respect to animal production. Nutraceuticals are being widely used to increase the number of animal products generated and improve hygiene quality and standards adherence. The ability to determine the substances added both to feed and to products obtained for animal nutrition purposes is essential, as doing so is critical to determining the effects of these substances on human health. All guidelines and pieces of legislation being applied in Turkey for this purpose comply with current European Union legislation.

References

Commission Regulation 429/2008/EC (2008) Commission Regulation of 25 April 2008 on detailed rules for the implementation of Regulation (EC) No 1831/2003 of the European Parliament and of the Council as regards the preparation and the presentation of applications and the assessment and the authorisation of feed additives. Official Journal of the European Union, 22 May 2008, L 133:1–65

Communique 2014/11 (2014) The Communique on undesirable substances in animal feed. Official Journal of Turkey Republic, 19 April 2014/28977

Communique 2016/15 (2016) The Communique as regards the preparation and the presentation of applications and the assessment and the authorisation of feed additives. Official Journal of Turkey Republic, 27 May 2016/29724

Directive 2002/32/EC (2002) Directive of The European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed. Official Journal of the European Communities, 30 May 2002, L 140:10–21

Dorne JL, Fink-Gremmels J (2013) Human and animal health risk assessments of chemicals in the food chain: comparative aspects and future perspectives. Toxicol Appl Pharmacol 270(3):187–195

European Union Register of Feed Additives (2018) European Union Register of Feed Additives pursuant to Regulation (EC) No 1831/2003. https://ec.europa.eu/food/safety/animal-feed/feed-additives/eu-register_en. Accessed 19 July 2018

Law No 5996 (2010). Law on veterinary services, phytosanitary, food and feed. Official Journal of Turkey Republic, 13 June 2010/27610

Ministry of Agriculture and Forestry (2018) Yem Isletmeleri Listesi. https://www.tarimorman.gov.tr/Konu/1719/Yem-Isletmeleri-Listesi. Accessed 19 July 2018

Regulation 178/2002/EC (2002) Regulation of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. Official Journal of the European Communities, 01 February 2002, L 31:1–24

Regulation 183/2005/EC (2005) Regulation of the European Parliament and of the Council of 12 January 2005 laying down requirements for feed hygiene. Official Journal of the European Union, 8 February 2005, L 35:1−22

Regulation 767/2009/EC (2009) Regulation of the European Parliament and of Council of 13 July 2009 on the placing on the market and use of feed, amending European Parliament and Council Regulation (EC) No 1831/2003 and repealing Council Directive 79/373/EEC, Commission Directive 80/511/EEC, Council Directives 82/471/EEC, 83/228/EEC, 93/74/EEC, 93/113/EC and 96/25/EC and Commission Decision 2004/217/EC. Official Journal of the European Union, 1 September 2009, L 229:1–28

Regulation 882/2004/EC (2004) Regulation of the European Parliament and of the Council of 29 April 2004 on official controls performed to ensure the verification of compliance with Regulation 178/2002/EC (2002) Regulation of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. Official Journal of the European Communities, 1 February 2002, L 31:1–24

Regulation 2011/28145 (2011) The regulation on for official control of feed and food. Official Journal of Turkey Republic, 17 December 2011/28145

Regulation 2011/28152 (2011) The regulation on animal by-products and derived products not intended for human consumption. Official Journal of Turkey Republic, 24 December 2011/28152

Regulation 2011/28155 (2011a) The regulation on feed hygiene. Official Journal of Turkey Republic, 27 December 2011/28155

Regulation 2011/28155 (2011b) The regulation on the placing on the market and use of feed. Official Journal of Turkey Republic, 27 December 2011/28155

Regulation 2013/28711 (2013) The regulation on feed additives for use in animal nutrition. Official Journal of Turkey Republic, 18 July 2013/28711

Turkish Statistical Institute (2017) Animal production statistics, 2017. Ankara. http://www.turkstat.gov.tr/PreHaberBultenleri.do?id=27704. Accessed 25 July 2018

Yılmaz EŞ, Aslantaş Ö, Önen SP, Türkyılmaz S, Kürekci C (2016) Prevalence, antimicrobial resistance and virulence traits in enterococci from food of animal origin in Turkey. LWT – Food Sci Technol 66:20–26

Uses and Regulation of Nutraceuticals for Animals in the Philippines

Jacob Anderson C. Sanchez and Geraldine C. Sanchez

Abstract

The Philippines is an archipelagic country in Southeast Asia with many requirements for food animals. Traditional veterinary practices are still thriving, and their application to animal health exists among rural communities. Herbal treatments for animals listed in this chapter are mostly used for gastrointestinal disorders such as diarrhea, constipation, bloating, and dysentery. These herbs which are known to possess medicinal benefits for animals were put together based on applications of herbs in treating human diseases. The top ten medicinal plant species approved by the Philippine Department of Health-Food and Drug Administration (DOH-FDA) for therapeutic purposes are lagundi for cough and asthma, sambong as an anti-urolithiasis, ampalaya for lowering of blood sugar and as an antidiabetic, garlic as antihypercholesterolemia, guava for oral/skin antiseptic, tsaang gubat for mouthwash, yerba buena as an analgesic or antipyretic, niyog-niyogan as an antihelminthic, acapulco as an antifungal, and ulasimang-bato as an anti-hyperuricemia. The Philippine research and regulatory bodies include the Department of Agriculture (DA), DA-Bureau of Animal Industry (DA-BAI), Department of Health (DOH)-Philippine Institute of Traditional and Alternative Health Care (PITAHC), DOH-FDA, and Philippine Natural Health Products Industry (PNHPI). In 2017, a total of 497 companies were accredited by DA-BAI; however, these are not categorized based on whether or not they sell synthetic drugs or nutraceuticals. The use of nutraceuticals for veterinary purposes in the Philippines is still in its infancy. It is imperative for veterinary universities in the Philippines to innovate the utilization of food or feeds for animals with certain medicinal benefits. In response to this growing need, the Pampanga State Agricultural University, through the Department of Agriculture-Bureau of Agricultural Research, has established the Nutraceutical Research Laboratory (NRL).

Keywords

Uses and regulation · Nutraceutical · Food animals · Philippines

1 Introduction

The Philippines is an archipelagic country in Southeast Asia with many requirements for food animals. Except for poultry, the production of pork, beef, chevon, mutton, and duck meat largely depends on backyard farmers. Therefore, it is common that traditional veterinary practices are still thriving and their application in animal health exists among rural communities. Herbal treatments for animals listed in this chapter are mostly used for gastrointestinal disorders such as diarrhea, constipation, bloating, and dysentery. These herbs which are known to possess medicinal benefits for animals were put together based on applications of herbs in treating human diseases. The top ten medicinal plant species approved by the Philippine Department of Health-Food and Drug Administration (DOH-FDA) for therapeutic purposes are lagundi (*Vitex negundo*) for cough and asthma, sambong (*Blumea balsamifera* L.) as an anti-urolithiasis, ampalaya (*Momordica charantia* L.) for lowering of blood sugar and an antidiabetic, garlic (*Allium sativum*) as anti-hypercholesterolemia, guava (*Psidium guajava*) for oral/

J. A. C. Sanchez
College of Arts and Sciences (CAS), Pampanga State Agricultural University, Magalang, Pampanga, Philippines

Nutraceutical Research Laboratory, Pampanga State Agricultural University (PSAU), Magalang, Pampanga, Philippines

G. C. Sanchez (✉)
Nutraceutical Research Laboratory, Pampanga State Agricultural University (PSAU), Magalang, Pampanga, Philippines

College of Veterinary Medicine (CVM), Pampanga State Agricultural University (PSAU), Magalang, Pampanga, Philippines

R. C. Gupta et al. (eds.), *Nutraceuticals in Veterinary Medicine*, https://doi.org/10.1007/978-3-030-04624-8_63

skin antiseptic, tsaang gubat (*Carmona retusa*) for mouthwash, yerba buena (*Mentha arvensis*) as an analgesic or antipyretic, niyog-niyogan (*Quisqualis indica*) as an anthelminthic, acapulco (*Cassia alata*) as an antifungal, and ulasimang-bato (*Peperomia pellucida*) as an anti-hyperuricemia. The Philippine research and regulatory bodies include multi-stakeholders such as the Department of Agriculture (DA), DA-Bureau of Animal Industry (DA-BAI), Department of Health (DOH)-Philippine Institute of Traditional and Alternative Health Care (PITAHC), DOH-FDA, and the Philippine Natural Health Products Industry (PNHPI). In 2017, a total of 497 companies were accredited by DA-BAI; however, these are not categorized as to whether or not they sell synthetic drugs or nutraceuticals. Most are still relying on commercially available synthetic drugs either produced locally or imported. It can be said that nutraceuticals for veterinary purposes in the Philippines are still in its infancy. Therefore, it is imperative for veterinary universities in the Philippines to innovate by doing research in relation to utilization of food or feeds with certain medicinal benefits in animals (Abelarde 2013). In response to this growing need, the Pampanga State Agricultural University, through the Department of Agriculture-Bureau of Agricultural Research, has established the Nutraceutical Research Laboratory (NRL). This chapter describes the uses and regulations of nutraceuticals in the Philippines.

2 Regulation of Nutraceuticals

The Philippines is an archipelagic country with 7107 islands where provinces are either landlocked or surrounded by different bodies of water. Its people are comprised of different ethnicities and religions on major islands, namely, Luzon, Visayas, and Mindanao. While there are a variety of other factors, it is the geographical, social, and religious factors (FAO 2002) that influence the Philippine animal industry.

People in Luzon are mostly Christians, and their primary source of meat is poultry, pork, and beef. Visayas is made up of several islands making them a prolific producer of fishery products. Mindanao is a combination of Christians and Muslims. It is also a rich source of fisheries, but their production system is focused on *halal foods*. Therefore, research initiatives are focused to meeting the increasing demand for and consumption of meat products.

The bureaus and research agencies that focus on these commodities are the Department of Agriculture (DA), DA-Bureau of Agricultural Research (DA-BAR), DA-Bureau of Animal Industry (DA-BAI), Philippine Carabao Center (PCC), DA-Livestock Biotechnology Center (DA-LBC), and Department of Science and Technology-Philippine Council for Agriculture, Aquatic, and Natural Resources Research and Development (DOST-PCAARRD).

Meanwhile, the Philippine Statistics Authority (PSA) has reported that in 2016, the volume of livestock and poultry production was highest for pork, followed by chicken, beef, carabeef, and chevon, with the least in duck meat (PSA 2018). The major sources of meat in the country are derived from food animals such as large ruminants (cattle and carabao), small ruminants (goats and sheep), swine, and poultry (chicken and duck) (BAI 2015).

Although efforts to promote animal health by the government and private sector are in place, with reference to current statistics of the PSA, the Philippine animal industry remains largely dependent upon backyard farmers rather than commercial producers with the exception of the poultry industry. Therefore, it is common that traditional veterinary practices are still thriving and their application in animal health exists among rural communities.

The Food and Agriculture Organization (FAO) of the United Nations and the Regional Animal Production and Health Commission for Asia and the Pacific (APHCA) documented that even before the arrival of modern medicine and western veterinary medications, Filipinos had already been using herbs for treatment of animal diseases (FAO 1992). It is believed that a trial-and-error method by "herbolarios" or traditional faith healers was used in the application of any herb, and this knowledge and skill was passed on to the next generation. Through the years, medicinal plants that worked well are still being used, while those that did not were forgotten.

Contrary to popular belief, herbs which are known to possess medicinal benefits for animals were actually put together based on applications of herbs in treating human diseases. In fact, a document produced by the FAO tabulated the summary of plants used, prescription indications, and regions where these are reported or practiced. A majority of these herbs are used in the treatment of gastrointestinal disorders.

For example, in treating diarrhea, the following plants are used: guava (*Psidium guajava*), caimito (*Chrysophyllum cainito*), banana (*Musa* sp.), bamboo (*Bambusa spinosa*), duhat (*Syzygium cumini*), sambong (*Blumea balsamifera*), and lagundi (*Vitex negundo*). Their leaves are prepared into a decoction by boiling in water and added to the drinking water of pigs (FAO 1992).

In treating constipation, the milk extract from meat of coconut (*Cocos nucifera*) is given to pigs, cattle, and carabao. Also, the leaves of kangkong (*Ipomoea aquatica*) or fresh fruit of papaya (*Carica papaya*) are given as food to animals.

For bloating in cattle, carabao, goats, and sheep, the meat of a matured coconut is prepared into an extracted juice and given orally to the affected animal. Another practice is a

decoction of luya (*Zingiber officinale*), vinegar, and water orally administered to goats.

Dysentery is treated with a decoction of banaba (*Lagerstroemia speciosa*) leaves and given as a drench to pigs, carabao, and cattle. The fruit of chico (*Achras zapota*) is mixed with animal feed, and the leaves of tawa-tawa (*Euphorbia hirta*) and bangka-bangkaan (*Rhoeo discolor*) or fresh flowers of alibangbang (*Bauhinia malabarica*) are infused in water and drenched daily to the animal.

Although the medicinal plants, as mentioned in the introduction part of this chapter, are already approved by the DOH-FDA, their utilization is mostly as a food or dietary supplements for humans, and they are labeled as "no approved therapeutic claims." Still, there are some attempts by local pharmaceutical industries to manufacture and commercialize nutraceuticals for veterinary purposes.

In order to preserve and promote the utilization of the aforementioned medicinal plants for health and nutraceutical purposes, the Philippines enacted the Republic Act 8423 otherwise known as the Traditional and Alternative Medicine Act (TAMA) of 1997 (LawPhil Project 2018). This act created the Philippine Institute of Traditional and Alternative Health Care (PITAHC) to accelerate the development of traditional and alternative health care in the Philippines, providing for traditional and alternative health-care development fund and for other purposes. The PITAHC is an agency of the Department of Health (DOH).

The mandate of the PITAHC is to promote quality, safety, efficacy, accessibility, and acceptable traditional and complementary medicine (T&CM) products, technologies, and modalities, as well as the protection of Philippine traditional medicine (PTM). These mandates are strengthened by the Governance Commission for GOCCs (GCG) in 2012 giving emphasis that PITAHC is a research institution whose budgetary affairs shall now be under the Department of Budget and Management (DBM). However, in a meeting of the PITAHC Board of Trustees in October 2015, they affirmed that the thrust of PITAHC is primarily in research and not in manufacturing.

Meanwhile, the Department of Agriculture-Bureau of Animal Industry accredits and recognizes veterinary drugs and product manufacturers or distributors in the country (BAI 2018). There are a total of 497 accredited companies, but these are not categorized whether they sell synthetic drugs or nutraceuticals. Most are still relying on commercially available synthetic drugs either locally produced or imported. Hence, it can be said that nutraceuticals for veterinary purposes in the Philippines are still in its infancy.

To accelerate the market of nutraceuticals for veterinary purposes, there has recently been an effort from industries to organize themselves and form the Philippine Natural Health Products Industry (PNHPI), which is represented by the Chamber of Herbal Industries of the Philippines (CHIPI). The CHIPI is an association of companies that manufacture, develop, research, distribute, and trade the supply of herbal products. It also drafted the PNHPI Roadmap FY 2014–2030 which categorized natural health products into (a) natural ingredients (plant parts, extracts, oil, semi-processed) and (b) finished products for human, household, veterinary, and green fertilizer/pesticide use.

To address the limitations in the regulations of nutraceuticals or functional foods, the DOH-FDA drafted the Administrative Order on the "Rules and Regulations Governing Nutrition and Health Claims of Pre-packaged Food Products Distributed in the Philippines" (Bagchi 2014). At present, nutraceutical products are evaluated based on research evidence through the regulations of the DOH-Food and Drug Administration, formerly Bureau of Food and Drugs (BFAD). It issues several rules and guidelines on probiotics, formulation, labeling, and technical specifications which serve as its basis for evaluation.

Product information and other requirements must include (1) list of ingredients, (2) physical, chemical, and microbiological characteristics: (3) presentation of actual sample for sensory evaluation; (4) presentation of sample label in which the nutraceutical claims are given emphasis on the active ingredient but not on the finished product; (5) shelf life and methods to determine such; and (6) a description of the manufacturing process (Tecson-Mendoza 2007). Several studies are also conducted by the Department of Science and Technology-Food and Nutrition Research Institute (DOST-FNRI) including various state universities and colleges. Research on nutraceuticals is essentially multidisciplinary covering basic agriculture, clinical pharmacology, toxicology/mutagenicity, dosage formulation, manufacturing research, and phytochemistry (Mallillin and Bautista-Batallones 2004).

Several studies on nutraceuticals have been conducted, but many of these remain unpublished and underutilized. This may be linked to the small number of industries which adopt and utilize such technologies. However, the growing interest of the public for buying healthy alternatives has resulted in a number of small to medium enterprises (SMEs) in the country to develop nutraceutical products (capsules, healthy beverages, and teas) which are all for human consumption but not for veterinary uses. Therefore, it is imperative for veterinary universities in the Philippines to also innovate by doing investigations in relation to utilization of food or feeds for animals with certain medicinal benefits.

Fig. 1 Kauna-Unahang Nutraceutical Research and Development Facility Sa Buong Bansa, Pinasinayaan Pampanga

In response to this growing need, the Pampanga State Agricultural University, through a funding grant from the Department of Agriculture-Bureau of Agricultural Research, has established the Nutraceutical Research Laboratory (NRL) (Fig. 1). This laboratory has tissue culture, microbiology, and an animal facility to conduct experiments using laboratory mice. The laboratory, headed by Dr. Geraldine C. Sanchez, project leader of NRL, has been permitted to operate as an animal model research facility from the Bureau of Animal Industry.

The NRL has already produced several research outputs on the antidiabetic properties of mushrooms (*Pleurotus florida*, *Coprinus comatus*, *Ganoderma lucidum*, *Volvariella volvacea*), red mold rice, and brown rice extracts; anticlastogenic of red mold rice, pigeon pea, black rice, turmeric, and mulberry; and hypocholesterolemic of pigeon pea. Currently, the following experiments are being conducted: (1) the role of *Moringa* on estrous cyclicity and follicular dynamics, (2) soybean as fertility enhancer among native pigs, and (3) anticlastogenecity of *Malabar* nightshade.

3 Concluding Remarks and Future Directions

The Philippines is an archipelagic country comprised of people of multiethnic backgrounds. The country has a rich history of using herbal medicines, yet it lacks evidence-based rationale and concept. In response to this growing need, investigations are carried out at various universities, including the Pampanga State Agricultural University, through a funding grant from the Department of Agriculture-Bureau of Agricultural Research. The Nutraceutical Research Laboratory (NRL) has taken the lead. The future of nutraceutical use in the prevention and treatment of animal diseases seems bright in the Philippines.

References

Abelarde L (2013) The Philippine natural health products industry roadmap. Retrieved from http://industry.gov.ph/wp-content/uploads/2015/05/7th-TID-Mr.-Abelardes-Presentation-on-Natural-Health-Products.pdf on July 18, 2018

Bagchi D (2014) Nutraceutical and functional food regulations in the United States and around the world, 2nd edn. Academic, London. ISBN:978-0-12-405-870-5

BAI (2015) Bureau of Animal Industry. Annual Report of the Philippine Bureau of Animal Industry. Retrieved online from http://www.bai.gov.ph on April 11, 2018

BAI (2018) Bureau of Animal Industry. Issuance of Feed/Veterinary Drugs and Products (VDAP) Commodity Clearance/Certificate of Free Sales. Retrieved from http://www.bai.da.gov.ph/index.php/regulatory/item/314 on April 11, 2018

FAO (1992) Food and Agriculture Organization. Traditional Veterinary Medicine in the Philippines. FAO Regional Office for Asia and the Pacific, Bangkok, Thailand

FAO (2002) Food and Agriculture Organization. The diverse functions of livestock: an Asia-Pacific perspective. Retrieved from http://www.fao.org/docrep/005/AC448E/ac448e06.htm

LawPhil Project (2018) Republic Acts of 1997. Retrieved from the website of Arellano Law Foundation database of Philippine laws at https://www.lawphil.net/statutes/repacts/ra1997/ra1997.html

Mallilin AC, Bautista-Batallones C (2004) Review of country status on functional foods: Philippines: Report of the regional expert consultation of the Asia-Pacific network for food and nutrition on functional foods and their implications in the daily diet, FAO Corporate Document Repository, RAP Publication 2004/33.

PSA (2018) Philippine Statistics Authority. Selected Statistics on Agriculture. Retrieved from http://www.psa.gov.ph

Tecson-Mendoza EM (2007) Development of functional foods in the Philippines. Food Sci Technol Res 13(3):179–186

Regulatory Guidelines for Nutraceuticals in South Africa

V. Naidoo and E. Mokantla

Abstract

South Africa, as a country, does not include the term 'nutraceutical' within its drug-controlling legislation by direct definition. Nonetheless, substances that can be considered nutraceuticals are available as complementary medicines, animal feed and/or stock remedies. Generally, the available veterinary remedies are controlled by the National Department (Ministry) of Agriculture for over-the-counter use, especially since the country is constrained by the total number of veterinarians in the country. Examples of available substances include arnica oil, chondroitin and specified probiotic antimicrobial strains. As with allopathic medicines the regulatory systems in South Africa take into account the safety, quality and efficacy of the product under consideration for market authorisation. However unlike allopathic medicines, where a complete global set of information is required in the registration dossier, the registration requirements for the complementary medicines and foodstuffs place predominant focus on quality in manufacture while adopting a more stepped approach for safety and efficacy, i.e. the greater the risk(s) associated with a specific claim, the greater the scientific information that will be required to support said claims of efficacy and associated safety.

1 Introduction

South Africa, as a country, does not include the term 'nutraceutical' within its drug-controlling legislation by direct definition. However, when no direct medical claims are made for a nutraceutical, it is registrable either as a complementary medicine or as an animal feed under one of two regulatory acts administered by the National Department [Ministry] of Health (DoH) and by the National Department [Ministry] of Agriculture, Forestry and Fisheries (DAFF), respectively, with the latter predominating. Table 1 lists some examples of products available in South Africa that can be generally regarded as veterinary nutraceuticals.

V. Naidoo (✉)
Faculty of Veterinary Science, Department of Paraclinical Science, University of Pretoria, Pretoria, South Africa
e-mail: vinny.naidoo@up.ac.za

E. Mokantla
Department of Agriculture, Forestry and Fisheries, Pretoria, South Africa

2 Legislative Framework in South Africa

South Africa is a vast country (1.22 million square kilometres in area) that can be best described as semi-arid, making it most suitable for livestock production, which is carried out in approximately 70% of the country. The 2010 census documented the following estimates of production animal numbers in both the commercial sector and the rural farming sector (Scholtz et al. 2013): 13.6 million beef cattle, 1.4 million dairy cattle, 24.6 million sheep, 7 million goats, 3 million ranched wildlife animals, 1.1 million pigs, 113 million broiler chickens, 31.8 million layer chickens and 1.6 million ostriches. It was also estimated that nearly 5 million households provide homes to over 8 million cats and dogs (Durham 2011). Despite the size of the country and its population of 55 million people, the country is served by only approximately 3500 veterinarians. Further to this, a large portion of the country is still rural, with the communities in these areas being constrained in terms of the type of veterinary services they can afford. To overcome the limitation in veterinary services and to mitigate the cost of veterinary treatment versus the value of the animal, the country has adopted a dual regulatory system whereby prescription medicines are limited to veterinary use through the DoH, while the DAFF makes a large number of medication and feedstuffs available over the counter, without any veterinary intervention, for direct use by the farmer.

R. C. Gupta et al. (eds.), *Nutraceuticals in Veterinary Medicine*, https://doi.org/10.1007/978-3-030-04624-8_64

Table 1 Examples of veterinary nutraceuticals available in South Africa

Brands/products	Ingredient(s)	Uses
Eukanuba, Hills, Royal Canin	Prescription veterinary diets	Management of pets with urinary conditions, dermatological conditions, obesity, arthritis, etc.
Calmeze	L-Tryptophan, vitamin B_6, L-theanine, vitamin B_1, vitamin B_3	Calmeze can be mixed into the animal's food or given directly by mouth prior to events that are likely to cause fear or anxiety
Allerderm	Skin lipid complex, glycotechnology, defensin technology	Helps maintain skin barrier integrity and the skin's natural microbial balance
Arthridese	Homeopathic remedy	To assist in relief of chronic inflammation and arthritic discomfort
Canigest	Rapeseed oil, kaolin, fructo-oligosaccharides, mannan-oligosaccharides, pectin, glutamine, roast meat flavouring, *Enterococcus faecium*	Complementary feedstuff for dogs and cats, combining a probiotic, prebiotic, glutamine, kaolin and pectin; it is particularly suitable for feeding to dogs to assist in nutritional management of digestive tract upsets
Digesteaze	Amylase, protease, lipase	Enzyme supplement for exocrine pancreatic insufficiency, derived from fresh porcine pancreas
Equifox Pharmacalm Plus	L-Tryptophan	Concentrated calming paste for use in emergencies to help horses cope with stressful situations without removing the 'edge' required for performance
Fart Eze	Enzyme, probiotic, yucca extract	Supplement for control of flatulence and faecal odour in dogs
GCS Joint Care Advanced Chews	Collagen type 11, omega-3, chondroitin sulphate, glucosamine, green-lipped mussel extract, MSM	Nutritional supplement that supports joint health in dogs
GCS Joint Care Advanced Powder	Glucosamine, chondroitin sulphate, manganese, ascorbic acid	Nutritional supplement to assist management of degenerative joint conditions in dogs
Fulvic Force	Fulvic acid	Helps in the growing of superior-quality birds that are healthier, are bigger and produce finer-quality meat; reduces the need for antimicrobial drugs; helps produce eggs that are larger and have thicker shells with far less cracking
Clostat	*Bacillus subtilis* strain PB6	Helps to maintain the balance of microflora in the intestinal tract of poultry

MSM methylsulphonylmethane

This control falls under two acts: the Medicines and Related Substances Control Act 1965 (Act No. 101 of 1965, which is administered by the DoH and is known commonly as the Medicines Act) and the Fertilizers, Farm Feeds, Agricultural Remedies and Stock Remedies Act 1947 (Act No. 36 of 1947, which is administered by the DAFF and is commonly known as the Stock Remedies Act). Both acts follow the same manner of control and are supported by both regulations and guidelines, which are periodically reviewed. In general, South Africa follows International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (VICH) guidelines as a result of the country's observer status in the VICH programme. Both acts also share a similar governance structure in that registrations require complete evaluation of a submitted dossier by technical advisers, who hold qualifications in the fields of chemistry, veterinary pharmacology, toxicology, microbiology, virology and/or clinical veterinary medicine in all applicable major species as required. The only major difference between the two acts is evident in the intended use of the products. Since the Medicines Act tends to control products restricted to the veterinary professional (veterinary medicines), that act ensures that every product is supported by a full scientific package insert. In contrast, the Stock Remedies Act, of which the main function is to allow over-the-counter availability of products to non-veterinary professionals, places major focus on ensuring that the supporting package insert/leaflet is written in clear, simple, nonambiguous language that makes it easy to follow the instructions. Further, the act places major emphasis on ensuring that the outer packaging and accompanying marketing material include no items or markings (direct or indirect) that could misrepresent how to use the product.

The Medicines Act controls the following categories of substances:

- *Medicine*: This means any substance or mixture of substances that is used or purports to be suitable for use—or that is manufactured or sold for use—in diagnosis, treatment, mitigation, modification or prevention of disease or an abnormal physical or mental state (or symptoms thereof) in man; or in restoring, correcting or modifying any somatic, psychic or organic function in man. Medicines are controlled by means of the schedules

Table 2 Schedules of the Medicines Act in South Africa

Schedule numbers[a]	General categories	Control requirements	Examples
S0	Over-the-counter medicines	Available for sale in any shop without restriction; may be advertised to the public	Aspirin, paracetamol, antiseptics
S1	Pharmacy over-the-counter medicines	Available over the counter from any pharmacy without requiring consultation with a pharmacist; may be advertised to the public	Topical preparations of local anaesthetics, probiotics above a defined CFU value, medical dewormers
S2	Pharmacy prescription medicines	Available from any pharmacy following consultation with a pharmacist; may not be advertised to the public	Medical NSAIDs, NSAID/codeine products, contraceptive medications
S3	Prescription-only medication	Chronic medication requiring a prescription from a medical doctor or veterinarian	Veterinary NSAIDs, hormone replacement therapies
S4		General group of medicines prescribed by a medical doctor or veterinarian	Antimicrobials, systemic medications
S5		Potentially dependence-producing medicines	Anaesthetics, tranquillisers, sedatives, behavioural modifiers
S6		Dangerous dependence-producing medicines	Opioids, synthetic opioids, veterinary euthanasia formulations
S7	Banned substances	Undesirable medicinal substances	Amphetamines, cannabis, cannabinoids, cocaine, phencyclidine
S8	Medicines available for use only with a special permit	Restricted-use medicines	Any S7 substance

CFU colony-forming unit, *NSAID* nonsteroidal anti-inflammatory drug
[a]Since S2–S8 medicines and substances are not directly available to the public, they may not be advertised to the public

of the act (S0–S8), with different access controls per schedule. In general, S0 covers over-the-counter human medicines, S1 and S2 cover medicines that are prescribable by a pharmacist, S3–S6 medicines are under the control of a medical or veterinary doctor, S7 medicines are banned substances and S8 medicines are banned substances with restricted medical use potential (Table 2).

- *Veterinary medicine*: This means any substance or mixture of substances that is used or purports to be suitable for use—or that is manufactured or sold for use—in connection with vertebrates, for treatment, diagnosis, prevention or cure of any disease, infection or other unhealthy condition; or for maintenance or improvement of health, growth, production or working capacity; or for curing, correcting or modifying any somatic or organic function; or for correcting or modifying behaviour. Veterinary medicines fall under same scheduling system mentioned above for medicines, with the exception that the schedules make provision for exclusion of active ingredients under the control of the Stock Remedies Act.
- *Complementary medicine*: This means any substances or mixture of substances that originates from a plant, fungus, alga, seaweed, lichen, mineral, animal or other substance as determined, and that is used or purports to be suitable for use—or that is manufactured or sold for use—in maintaining, complementing or assisting innate healing power or physical state or mental state; or to diagnose, treat, mitigate, modify, alleviate or prevent disease or illness (or symptoms or signs thereof) or an abnormal physical or mental state in a human being or animal; and is used as a health supplement or in accordance with the following disciplines: herbal substance/preparation; traditional Chinese, Ayurvedic and Unani Tibb substances; and homeopathic substances and aromatherapy. Complementary medicines are generally meant for over-the-counter sales and are freely available to the public at most health shops, supermarkets and pharmacies.
- *Health supplement*: This means any substance, extract or mixture of substances that may supplement the diet or have a nutritional physiological effect. This category includes prebiotics, probiotics and other supplements sold in pharmaceutical dosage forms not usually associated with a foodstuff, and excludes injectables. As is the case for complementary medicines, health supplements can be sold from any retail outlet.
- *Medical device*: This means any instrument, appliance, material, machine, apparatus, implant or diagnostic reagent that is used or purports to be suitable for use—or that is manufactured or sold for use—in diagnosis, treatment, mitigation, modification, monitoring or prevention of disease or abnormal physical or mental states (or symptoms thereof); or for restoring, correcting or modifying any somatic, psychic or organic function; or for diagnosis or prevention of pregnancy; and that does not achieve its purpose through chemical, pharmacological, immunological or metabolic means.

Table 3 Products that can be considered nutraceuticals and their registration process in South Africa

Legal categories	Act numbers	Descriptions	Examples
Complementary medicines	101/65	Generally substances that are not controlled as allopathic medicines within the schedules of the act, with a primary function of supporting the immune system and thereby promoting wellness	Homeopathic remedies, Western herbalism, Eastern medicines
Health supplements	101/65	Generally substances that exert physiological effects through nutritive effects	Probiotics, glucosamine
Stock remedies	36/47	Medicinal substances that are usually available for over-the-counter sale for animal use	Arnica oil, camphor cream
Farm feeds and pet foods	36/47	Substances that support animal nutrition and those that have a physiological effect in a manner that does not make them stock remedies	Green-lipped mussel, chondroitin, glucosamine
Zootechnical additives	36/47	Enzymes and/or probiotics used for management of gut health in animals	Defined bacterial strains

The Stock Remedies Act controls the following categories of substances:

- *Fertilisers*: This includes any substance that is intended or offered to be used for improving or maintaining the growth of plants or the productivity of the soil.
- *Agricultural remedies*: This includes any chemical substance or biological remedy, or any mixture or combination of substances or remedies, that is intended or offered for use in destruction, control, repelling, attraction or prevention of any undesired microbe, alga, nematode, fungus, insect, plant, vertebrate, invertebrate or any product thereof.
- *Farm feed*: This includes any substance obtained by a process of crushing, gristing or grinding; any substance obtained by addition to, or removal from, another substance; any condimental food, vitamin or mineral substance or other substance that possesses, or is alleged to possess, nutritive properties; any bone product that is intended or sold for feeding of domestic animals or livestock; or any stock lick or substance that can be, and is, used as a stock lick, whether or not such a stock lick or substance possesses medicinal properties. It also includes 'complete pet food', which is a pet food that contains all necessary nutrients in the correct quantities and proportions for the physiological needs of the animal. It is within this category that veterinary nutraceuticals are controlled in South Africa and, as is the case for the complementary medicines (as mentioned above), they are available for open sale from any retail outlet.
- *Stock remedies*: This includes any substance that is intended or offered to be used in connection with domestic animals, livestock, poultry, fish or wild animals (including wild birds) for diagnosis, prevention, treatment or cure of any disease, infection or other unhealthy condition; or for maintenance or improvement of health, growth, production or working capacity. Stock remedies generally cover medicines used for treatment of ectoparasites, endoparasites or tickborne diseases; certain antimicrobials; and injectable vitamins/minerals. This category also includes complementary veterinary medicines such as arnica and aromatic oils. In general, stock remedies are meant for open sale at any retail outlet. However, there are some restrictions on certain products, since a degree of veterinary supervision is required for their use.
- *Zootechnical additives*: This includes digestibility-enhancing substances, gut flora stabilisers (which include microorganisms or other chemically defined substances that have a positive effect on the gut flora) and substances that favourably affect the environment (Table 3).

3 General Control of Veterinary Nutraceuticals

As is evident from the above, veterinary nutraceuticals can fall under the control of the DoH or the DAFF, depending on which category the medicine falls into. Not surprisingly, the registering company can choose which act they register their product under. In general, the DAFF remains the main point of registration of nutraceutical remedies and is estimated to control 90% of all medications available for use in animals in the country. The latter is linked to the focus of the DAFF on animal health, and this department has controlled medicinal substances for a substantially longer period than the DoH. Also, the registration of complementary medicines with the DoH as a medicine category is relatively new and came into effect only in 2013.

3.1 Registration Requirements

As with medicines, the regulatory systems in South Africa take into account the safety, quality and efficacy of a product being considered for market authorisation. However unlike

medicines (and stock remedies), for which a complete global set of information is required in the registration dossier, the registration requirements for complementary medicines and foodstuffs place the predominant focus on quality in manufacturing, with adoption of a more stepped approach for safety and efficacy, in that the greater the risk, the greater the study requirements are to support the said claims of safety and efficacy. A major difference is readily evident for health supplements, for which claims of efficacy are not allowed, and these products need to be labelled with the following wording: 'Health supplements are intended only to complement health or supplement the diet'.

Registration of Complementary Medicines (Act 101 of 1965)

The following requirements are in place for complementary medicines:

- *Quality control*: The DoH requires compliance with good manufacturing practice (GMP), good laboratory practice (GLP) and good agricultural and collection practices (GACP) in the manufacturing of complementary medicines, according to the guidelines stipulated by the South African Health Products Regulatory Authority (a new body that replaced the Medicines Control Council of South Africa in 2018). The regulations further stipulate that to be regulated as a complementary medicine, a product needs to be free of any substances contained in the schedules of the act—that is, it cannot contain or be co-formulated with an active pharmaceutical ingredient; be used as a means to avoid being controlled as a medicine (or veterinary medicine); or generally contain any chemically defined isolated constituent of a plant, fungus, alga, seaweed, lichen, animal or mineral, or a combination of such constituents, that has a pharmacological effect. It is, however, allowed for a complementary medicine to contain inactive ingredients as long as they are classified as being Generally Regarded as Safe (GRAS) by the US Food and Drug Administration (FDA).
- *Safety*: It is required for complementary medicines to demonstrate their safety, which may be determined through a literature review and/or submission of original study data, as per other pharmaceutical active ingredients. The amount of information required will depend on the documented historical use of the product and the risks associated with its use.
- *Efficacy*: For registration, appropriate evidence in support of efficacy for the proposed indication(s) and claims needs to be submitted, depending on the nature of the said claims. Claims are divided into those made for minor disorders and those made for major disorders. Disorders considered minor are usually linked to a product that has nonspecific indications and usually a history of traditional use supporting the said indication. Major indications are claims made towards the treatment of a specific disease (e.g. treatment of diabetes), which generally require more information to prove efficacy. For high-risk claims or where traditional use has not been established, efficacy needs to be supported by appropriate preclinical and/or clinical evidence.

Registration of Farm Feeds (and Pet Foods) (Act 36 of 1947)

The following requirements are in place for farm feeds and pet foods:

- *Manufacturing*: The act makes mention that the place of manufacturing, packing, marking, labelling and/or storage needs to be kept orderly and clean and supported by equipment that is both suitable for the purpose and maintained in good working order so as to allow for uniform distribution of the feedstuff in the mixed food. In terms of the quality system, the feed manufacturer needs to establish critical points in manufacturing and ensure that they are controlled. The regulations make special mention that feeds and feedstuffs used in manufacturing must be protected against damage, contamination and deterioration; and that different additives and premixes must be kept separate from each other to avoid cross-contamination.
- *Quality*: Quality control on foods is not limited to manufacturing standards; it also considers interactions and is very specific that a formulation should not contain any ingredient(s) that could lead to the loss of one or more of the nutrients in the product. Since products may be of animal origin, the quality standard indicates that animal-derived products must first be sterilised so that any contaminating microorganisms are reduced to a level at which they pose no danger to the health or productive capacity of the animals fed on the said product, or no longer pose a disease risk to the country. The act also contains a list of prohibited substances that may not be included in animal feeds.
- *Efficacy*: The requirements firstly limit feeds to a physical form that can be consumed by oral ingestion. In general, when a food is to be registered for a particular nutritional purpose, information needs to be generated to support its precise use so as to demonstrate that the particular nutritional purpose attributed to the product is appropriate. For microorganisms specifically, the name and taxonomic classification of each microorganism needs to be provided, according to the latest published information in the International Codes of Nomenclature (ICN). In addition, all relevant morphological, physiological and molecular

characteristics necessary to provide the unique identification of the strain and the means to confirm its genetic stability needs to be described.

- *Safety*: It is a requirement that the safety of the target animals of the feed or the ingredients used are supported with appropriate data. A material safety data sheet must be provided. If necessary, measures for prevention of occupational risks and means of protection during manufacturing, handling, use and disposal shall be proposed.

4 Concluding Remarks and Future Directions

South Africa, as a country, has a dual regulatory system to allow for availability of medicines, which was developed to allow for treatment of animals by animal owners and farmers within a landscape that is constrained by the veterinary capacity of the country. Within this dual regulatory system, although products that can be considered nutraceuticals are not considered, the country does allow for their registration within one of the subcategories within the drug control acts, with the majority of products used in animals being controlled as farm and pet foods.

References

Durham L (2011) Embrace pets as part of the family. Supermarket & Retailer, September: 39–43.

Fertilizers, Farm Feeds, Agricultural Remedies and Stock Remedies Act (1947) (Act No. 36 of 1947). http://www.nda.agric.za/doaDev/sideMenu/ActNo36_1947/act36.htm. Accessed 2018

Medicines and Related Substances Control Act (1965) (Act No. 101 of 1965). www.mccza.com. Accessed 2018

Scholtz MM, van Ryssen JBJ, Meissner HH, et al. (2013) A South African perspective on livestock production in relation to greenhouse gases and water usage. S Afr J Anim Sci 43(3): 247–254. http://www.scielo.org.za/scielo.php?script=sci_arttext&pid=S0375-15892013000300003&lng=en.

Correction to: Evaluation of Safety and Efficacy of Nutraceuticals Using *Drosophila* as an *in vivo* Tool

Anurag Sharma, Clinton D'Souza, Vipin Rai, and Subash Chandra Gupta

Correction to:
Chapter 49 in: R. C. Gupta et al. (eds.),
***Nutraceuticals in Veterinary Medicine*,**
https://doi.org/10.1007/978-3-030-04624-8_49

The original version of Chap. 49 was inadvertently published with the incorrect chapter title "Evaluation of Safety and Efficacy of Nutraceuticals Using *Drosophila* as an *in vitro* Tool" whereas it should be "Evaluation of Safety and Efficacy of Nutraceuticals Using *Drosophila* as an *in vivo* Tool". The chapter has now been corrected and approved by the author.

The updated online version of this chapter can be found at
https://doi.org/10.1007/978-3-030-04624-8_49

R. C. Gupta et al. (eds.), *Nutraceuticals in Veterinary Medicine*, https://doi.org/10.1007/978-3-030-04624-8_65

Index

R. C. Gupta et al. (eds.), *Nutraceuticals in Veterinary Medicine*, https://doi.org/10.1007/978-3-030-04624-8

F