Veerasamy Sejian · Surinder Singh Chauhan ·
Chinnasamy Devaraj · Pradeep Kumar Malik ·
Raghavendra Bhatta *Editors*

Climate Change and Livestock Production: Recent Advances and Future Perspectives

Springer

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ISBN 978-981-16-9835-4 ISBN 978-981-16-9836-1 (eBook)
https://doi.org/10.1007/978-981-16-9836-1

This Springer imprint is published by the registered company Springer Nature Singapore Pte Ltd.
The registered company address is: 152 Beach Road, #21-01/04 Gateway East, Singapore 189721, Singapore

Foreword

Livestock farming and allied practices provide a source of income to a major proportion of people across the globe and contribute to the global agricultural gross domestic product. Livestock are of prime importance for ensuring food security and serve as the preferred protein source for the growing human population. Climate change is a global challenge and its impact on the livestock has attracted the attention of policymakers to work on suitable strategies and also to fund research in this area. It is therefore necessary to propagate relevant information to all the climate change researchers across the globe, thereby enabling the adoption of a multidisciplinary and multi-institutional collaborative approach to tackle climate change and sustain livestock production.

I am informed that a workshop is being organized under the project, "Climate Change and Livestock Production: Current Scenario and Way Forward" at ICAR-National Institute of Animal Nutrition and Physiology, India, in association with the University of Melbourne, Australia, to disseminate knowledge on various established concepts of climate change impacts on livestock production and also to inform the participants on the various advances in this field.

The organizers are bringing out the proceedings of this workshop in the form of a book entitled "Climate Change and Livestock Production: Recent Advances and Future Perspective." I hope that the book will serve as resource material for the policymakers to implement new policies to sustain future livestock production in the changing climate scenario. It is expected that researchers, including students, teachers, and other practitioners in the area will greatly benefit from the deep insights provided by experienced authors in this book.

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Preface

Climate change is no longer a myth; its causes, outcome, and projected trends have brought the entire globe to recognize this as a major concern. The rising global surface temperatures, increasing incidences of environmental hazards, drought, disease outbreaks, pandemics, severe economic losses, and future projections have made it very essential to address this issue on priority. Livestock production is one such area gaining immense importance for its significant role in supporting the global agricultural gross domestic product, human livelihood, and food security. This sector is also of huge relevance from a climate change perspective for its significant impact on climate change through greenhouse gas emissions. Hence there is an increasing interest for researchers in this subject.

Climate change and livestock production can be considered as a field of science that is being established with various concepts and subsections. While some of the classical concepts of impact of climate change on livestock production are well established, there are also increasing advancements in this field of science. Dissipating such knowledge among the widely spread scientific fraternity would further enhance in the adoption, validation, improvisation, and further propagation of the knowledge. This forms the basis of this volume entitled "**Climate Change and Livestock Production: Recent Advances and Future Perspectives**."

We believe that this volume serves as a mini-encyclopedia containing most of the critical concepts of climate change and livestock production with the latest information. Furthermore, it can also serve as a preamble for all the students, researchers, scientists, and policymakers across the globe who are either established or beginners in the field of climate change and livestock production. In addition, this volume also contains classic reference of world-class standard involving the contributions from nearly six scientists who have been listed in the recently published database on the world's top 2% scientists, released by Stanford University, USA. Moreover, the authors of the chapters of this volume are distributed across the globe covering many continents such as Asia, Oceania, Europe, Africa, and South America. The volume is an exemplary compilation of 23 chapters which can be broadly categorized into six parts. The first part on impact of climate change on livestock will introduce the readers to an overview on varied sectors of livestock production that is severely impacted due to climate change. This is followed by a list of chapters addressing the impact of heat stress on different adaptive responses in livestock. From there, the volume elevates to part III which lists out some of the methods to quantify heat stress response in livestock. Though there are a number of established methodologies to assess impact of climate change on livestock production, this part brings information on some of the recent advances in this field.

The volume also comprises a list of chapters covered in part IV that would especially attract the varied policymakers as it contains the strategies to ameliorate heat stress impacts in livestock. As mentioned earlier, livestock not only gets severely affected due to climate change but also contributes to it. This unique volume also has gathered some chapters by established researchers on the topic pertaining to enteric methane emission and amelioration which is covered under part V of this volume. Lastly, reserving the best for the last, eminent researchers have channelized their vast experience to compile chapters pertaining to the adaptation strategies and future perspectives of climate change and livestock production. The chapters under this part have great promise to be of interest to a wide range of readers, beginning from students to young budding researchers, established scientists from multiple disciplines, and also the ultimate policymakers. Reference materials pertaining to climate change and livestock production are scanty and obsolete. Therefore, by addressing systematically and comprehensively all major aspects of climate change and livestock production particularly concentrating on some of the recent advances and future perspectives, this volume is a useful resource material in understanding the various intricacies in this field of science.

The contributors of various chapters are world-class professionals with vast experience in the chosen field supported by several peer-reviewed publications. The Editorial Committee takes this opportunity to thank all the contributors from different parts of the world for their dedication in drafting, timely submission, and for sharing their rich knowledge and experience with others. The efforts of many others, all of those cannot be individually listed, were also very pertinent in completing this relevant and important volume.

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Bangalore, India — Chinnasamy Devaraj
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About the Editors

Veerasamy Sejian MVSc, PhD, is a Principal Scientist at ICAR-National Institute of Animal Nutrition and Physiology (NIANP), Bangalore, India. His central thrust area of research is on "Climate Change and Livestock Production." He is the team leader in establishing the concept of "Multiple Stresses Impacting Small Ruminants." His current research focuses on identifying molecular markers for different environmental stresses in small ruminants with the primary focus to develop agroecological zone-specific thermotolerant breeds. He has published more than 160 peer-reviewed articles, 99 book chapters, 285 invited/lead papers, 175 conference papers, and 22 technical manuals.

Further, he has published three International Springer books. He did his first postdoctorate at The Ohio State University, USA. He was bestowed with Endeavour Research Fellowship by the Australian Government to pursue a postdoctorate at The University of Queensland, Australia. For his outstanding contribution to animal science, the Indian Council of Agricultural Research (ICAR) has bestowed him with the prestigious Lal Bahadur Shastri Outstanding Young Scientist Award in 2012. Further, Career360, an academic organization in India, had selected him to be one of the top 10 scientists in India under the environmental sciences category during 2017–2018. He is also listed in the world's top 2% scientists by Stanford University, USA, during 2020 and 2021. He is also listed in the world's top 1% of scientists by PubMed based on the last 10 years of publications in heat stress. In addition, he is also serving as Field Editor in Springer's International Journal of Biometeorology, Associate Editor in Elsevier's Small Ruminants Research, and Academic Editor in PLOS Climate. He has developed four technologies and also has one patent granted.

Surinder Singh Chauhan DVM, PhD, is the Senior Lecturer in the Department of Veterinary and Agricultural Sciences, The University of Melbourne, Australia, and has 12 years of research and teaching experience in veterinary and animal sciences in three countries, including Australia, India, and the USA. He obtained his Bachelor of Veterinary Science and Animal Husbandry and Master of Veterinary Science from CSK, Himachal Pradesh Agriculture University, Palampur, India. He has served the

State of Himachal Pradesh, India, for more than 10 years and has provided his services as Senior Veterinary Officer and Veterinary Officer in various Civil Veterinary Hospitals and Livestock Breeding Farms. He has also served as Assistant Professor in The Department of Veterinary Physiology and Livestock Production and Management, College of Veterinary and Animal Sciences at the CSK, Himachal Pradesh Agriculture University, Palampur, India. He moved to Australia in 2011 following his selection for the prestigious Australian Leadership Award (Ph.D. scholarship) from the Australian Govt and completed his Ph.D. under the guidance of Prof Frank Dunshea, Faculty of Veterinary and Agricultural Sciences, The University of Melbourne in 2014. Later, he completed his Postdoctorate in Animal Science (Muscle Biology and Meat Science) at The Ohio State University, Columbus, USA. He has presented his research at various national and international conferences, including 7 keynote papers, and has published more than 70 refereed research papers. At The University of Melbourne, he is leading the Australia–India Collaborations for heat stress mitigation and Sustainable Livestock production funded by the Australian Government Australia–India Council.

Chinnasamy Devaraj MVSc, PhD, is a Scientist at the Division of Bioenergetics and Environmental Sciences, ICAR-National Institute of Animal Nutrition and Physiology (NIANP), Bangalore, India. His central thrust area of research is on “Energy Metabolism, Environmental Physiology, and Livestock Production.” His current research focuses on unraveling the physiological role of adipokines associated with energy metabolism in small ruminants. The Indian Council of Agricultural Research (ICAR) has awarded Junior Research Fellowship based on All India entrance for pursuing M.V.Sc program (2004–2006) at National Dairy Research Institute, Karnal, India. He has also received ICAR Award for Outstanding Interdisciplinary Team Research in Agricultural and Allied Sciences (Animal and Fisheries Sciences) for 2007–2008. He has published more than 160 research articles, including 25 peer-reviewed articles, 5 book chapters, 45 invited/lead papers, 74 conference papers, and 7 technical manuals. Presently, he is serving as a reviewer for the International Journal of Biometeorology and Journal of Agrometeorology.

Pradeep Kumar Malik MSc, PhD, is presently working as Principal Scientist at the ICAR-National Institute of Animal Nutrition and Physiology, Bengaluru. He was an Assistant Professor at Navsari Agricultural University, Navsari, and Senior Scientist at NIANP. He has accomplished International Training at the CSIRO, Queensland, Australia, and postdoctoral fellowship under the Endeavour Research Program of the Australian Government at the University of Queensland, Australia, in 2014–2015. His primary research area is enteric methane measurement and amelioration in livestock. He is handling a multicentric project on “Estimation of Methane Emissions under Different Feeding Systems and Development of Mitigation Strategies.” He is also associated with the ILRI-ICAR collaborative project on “Methane Emissions and its Mitigation” and the DBT-DFG Project on “Contamination of Feed and Fodders with Heavy Metals and Agrochemicals Impact on Milk Composition, Rumen Microbes, and Methanogenesis in Dairy Cattle along with the

Rural-Urban Interface of Bengaluru." He has developed a state-wise national inventory on enteric methane emissions from Indian livestock using primary data on methane production potential. He has created two farmers' friendly anti-methanogenic products, "Harit Dhara" and "Tamarin Plus," and filed four patents. He has published more than 50 research papers in international and national journals of repute, two international books by CABI and Springer in livestock production and climate change, and 40 book chapters. He also has 264 NCBI submissions in his name. He was also the recipient of the prestigious Australian Ambassador Award in 2015 by the Australian High Commission in India.

Raghavendra Bhatta MVSc, PhD, is currently the Director of ICAR-National Institute of Animal Nutrition and Physiology (NIANP), Bengaluru, Karnataka, India. During his research career spanning 27 years, he has done extensive research work involving small ruminant nutrition concerning "plant phenolics." He has developed the All-India state-wise inventory on enteric methane emission based on primary data and has evolved simple, eco-friendly strategies for enteric methane reduction in livestock. He was awarded the prestigious Japan Society for the Promotion of Science (JSPS) postdoctoral fellowship (2004–2006) at NILGS, Japan. He was invited to present theme papers at the Greenhouse Gases and Animal Agriculture (GGAA) Conference at Zurich, Switzerland in 2005; GGAA 2010 conference in Banff, Canada; and GGAA 2016 conference in Melbourne, Australia. He is the Fellow of the National Academy of Agricultural Sciences, the National Academy of Veterinary Science, and the National Academy of Dairy Science, India. He is the recipient of several research awards, including the prestigious Sir CV Raman State Award from the Karnataka State Council for Science and Technology, Govt. of Karnataka, and the Rafi Ahmed Kidwai Award for Outstanding Research in Agricultural Sciences, 2019 by ICAR. He is recognized as one of the world's top 2% scientists by Stanford University during 2020 and 2021. He has published more than 100 research articles in journals and presented more than 100 papers in conferences of national and international reputation and has authored 3 books, one each in Cambridge University Press, Centre for Agriculture and Bioscience International, and Springer. He has written 45 chapters in books and has two patents to his credit.

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Part I

Impact of Climate Change on Livestock

Impact of Climate Change on Animal Production and Welfare

1

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V. Sejian et al. (eds.), *Climate Change and Livestock Production: Recent Advances and Future Perspectives*, https://doi.org/10.1007/978-981-16-9836-1_1

Abstract

This chapter addresses in detail the various impacts of climate change on livestock production and welfare. Among all the environmental stressors, heat stress is of major concern as it has the most deleterious impact on livestock production especially in tropical countries which hold a major proportion of the world's livestock population. One of the primary responses exhibited by animals under stress, especially heat stress, is decline in feed intake. This direct impact results in a series of events that is responsible for significant reduction in the productive potential of animals. The decreased feed intake in animals during heat stress has been proved to explain nearly half of the milk yield reduction in dairy cattle. Meat production is another critical economic trait which again is significantly altered due to heat stress. In dairy cattle, heat stress has been proven to alter the milk composition impairing its quality and ultimately the economic value. Reduction in milk fat and protein content in sheep and goat milk due to heat stress negatively influence the production of high quality cheese. Similarly, the meat quality traits and its governing genes expression patterns are also compromised in heat stressed animals. Further, climate change also induces changes in welfare variables governing behavior, physiological, endocrine, and metabolic as well as immune system related variables. In addition, climate change also affects the animal health particularly the vector borne diseases. Thus, climate change and its impact on animal production, health and welfare are of great relevance that needs to be addressed efficiently to sustain animal production in the changing climate scenario.

Keywords

Climate change · Health · Heat stress · Livestock · Milk production · Welfare

Abbreviations

%	Percentage
ACTH	Adrenocorticotropic hormone
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
BHBA	β-hydroxy butiric acid
BUN	Blood urea nitrogen
CAPN	Calpain
CAST	Calpastatin
CD	Cluster of differentiation
CRH	Corticotrophin releasing hormone
CRYA	Crystallin alpha
DFD	Dark, firm and dry
DGAT	Diacylglycerol acyltransferase

GH	Growth hormone
GSH	Glutathione
HPA	Hypothalamo-pituitary-adrenal
HSP	Heat shock protein
IFN	Interferon
Ig	Immunoglobulin
IGF-1	Insulin-like growth factor-1
IL	Interleukin
LDL	Low density lipoprotein
MDA	Malondialdehyde
MSTN	Myostatin
NEFA	Non-esterified fatty acids
PSE	Pale, soft and exudate
RVF	Rift valley fever
SAM	Sympathetic-adrenal-medullary
SOD	Superoxide dismutase
T_3	Triiodothyronine
T_4	Thyroxine
THI	Temperature humidity index
TLR	Toll-like receptor
TNF	Tumor necrosis factor
TSH	Thyroid stimulating hormone
USD	United States dollar
VFA	Volatile fatty acid
WHC	Water holding capacity

1.1 Climate Variables Affecting Animal Production and Welfare

The global human population is prediction to increase by 33% from 7.2 to 9.6 billion by the end of 2050. This would thereby rise the demand for agricultural products that is predicted to increase by 70% (Rojas-Downing et al. 2017). However, there is no increase in the total global land area for cultivation, rather it remains nearly the same since 1991. This therefore reflects the intensification efforts initiated to increase the production levels. Among the agricultural sector, livestock products are thought to play a vital role in ensuring food security especially with the rising human demand for animal protein. The livestock sector therefore has adopted several intensified practices so as to boost their production levels. Livestock production however is influenced by a number of factors, climate change being one among them. Apart from having a direct impact, the indirect impact of climate change on livestock production like reduction in fodder, pasture, water scarcity and increased disease outbreaks, are also of great concern. Furthermore, these deleterious consequences also impair the animal's well-being thereby creating a global issue on animal welfare.

Climate change is no longer a myth, in fact having known the impact of climate change on the ecosystem, leaders from across the globe have formed numerous joint ventures to ameliorate and mitigate the adverse impact of climate change. The livestock sector holds a unique peculiarity for being both a contributor to climate change and also affected by it. Air temperature, humidity, wind velocity and solar radiation are among the prime climate variables that affect livestock production. Furthermore, among all the environmental stressors, heat stress is of major concern as it has the most deleterious impact on livestock production especially in tropical countries which hold a major proportion of the world's livestock population.

Animals possess innate abilities to combat the adverse climatic variabilities, however these impose serious negative influence on their production potential. Apart from production decline and economic losses, another vital aspect that is often overlooked is the well-being of the animal. The impact of climate change on animal welfare is a less explored area that needs much attention. The increasing occurrences of climatic adversities, both in frequency and intensity, rapid spread of diseases, lack of quality feed and fodder, along with the intensified production practices to increase production, all of these together exert a lot pressure on all livestock species. Hence it is the need of the hour to propagate the urge to ensure a balance optimum production amidst the drastically changing climate scenario and animal welfare.

1.2 Effects of Climate on Production Parameters

1.2.1 Yield

In the United States alone, heat stress was reported to cause an estimated financial loss of nearly 900 million USD/year, 300 million USD/year and 300 million USD/year in dairy, beef and swine industry respectively. This would quantify the relevance to address the rising concern of climate change and livestock production. One of the primary responses exhibited by animals under stress, especially heat stress, is decline in feed intake. This direct impact results in a series of events that is responsible for significant reduction in the productive potential of animals. Significant decline in a number of economic traits like milk yield, meat yield, body weight, body condition score, egg production and so on, as a consequence of heat stress have been reported in livestock and poultry.

In a study conducted in Sub-Saharan African climate of Tanzania, the impact of heat stress on milk production was assessed among the small holder dairy cattle populations (Ekine-Dzivenu et al. 2020). Based on the results obtained, heat stress was proved to cause a decline in milk production by 4.16% up to 14.42% across the temperature humidity index (THI) groups (61–86). Furthermore, a W-shaped response pattern of milk yield across THI scale was observed in this study. The proportion of decline in milk yield and also its trend across THI may vary depending on the breed, region, management and several other factors. However, this is an inevitable fact that heat stress results in a significant decline in milk yield and also its components like fat yield, lactose, etc. Furthermore, the decreased feed intake in animals during heat stress has been proved to explain nearly half of the milk yield reduction in dairy cattle. Similar reduction in milk yield as a consequence of heat stress has been reported in buffalo, and goats too.

Meat production is another critical economic trait which again is significantly altered due to heat stress. This area however has been least explored in livestock species. Unlike the milk variables, the impact of heat stress on meat production cannot be measured immediately. Significant reduction in growth rate, feed conversion efficiency, body weight, body condition score, carcase weight, omental fat and loin yield, due to heat stress have been reported in beef cattle, sheep and goats. Likewise, heat stress also has a deleterious impact on egg production in layers. The impact of heat stress on meat production and body characteristics varies among species, breed, sex and age. Nevertheless, the decline in production caused is severe that needs to be addressed efficiently.

Based on the recently published report by Rahimi et al. (2021), the frequency of dangerous heat stress events and the average number of days under heat stress for livestock reared in East Africa was projected to increase by 2100. Additionally, the report also suggested that with the projected rising global surface temperature, most of East Africa would be unsuitable to support exotic swine, dairy cattle and poultry production. This is of serious concern from the food security point of view and also from the animal welfare perspective.

1.2.2 Quality

The livestock sectors stands strong for the massive economic returns obtained; this is governed by several factors, the quality of product being one among the most vital factors. Apart from negatively influencing the yield, heat stress also compromises on the quality of livestock products obtained. In dairy cattle, heat stress has been proven to alter the milk composition impairing its quality and ultimately the economic value. In small ruminants, milk quality is a huge determinant for cheese production. Reduction in milk fat and protein content in sheep and goat milk due to heat stress negatively influence the production of high quality cheese. Moreover, the plasma mineral imbalance caused during high ambient temperatures also impairs the quality of milk and its products.

Studies on impact of heat stress on meat quality are less documented in most livestock species like beef cattle, sheep, goat and pigs. Most of the studies on this aspect have primarily focused on poultry meat and hence there is increasing research on this line in other livestock species since the recent past. The impact of heat stress on meat quality traits varies among the livestock species that could be possibly attributed to the varied thermo-tolerance ability in these species and their breeds. However, despite these variations, to summarize the impact of heat stress on meat quality, animals subjected to acute heat stress prior to slaughter were reported to stimulate muscle glycogenolysis. As a consequence of this impact, meat from such animals usually undergoes a condition known as pale, soft and exudative (PSE) meat having low water holding capacity (WHC). On the other hand, chronic heat stress is reported to result in dark, firm and dry (DFD) meat in animals which is characterized by high ultimate meat pH and high WHC. Irrespective of the type of heat stress, its impact on animals negatively affects the quality of meat, be it PSE or DFD, reducing its shelf life, consumer preference and food safety (Gonzalez-Rivas et al. 2020).

The impact of heat stress on meat quality in animals has been explained via a number of physiological processes occurring in the muscles. However, advanced studies have also reported the associations of a number of molecular mechanisms which involve the expression of a number of genes. In a recent study reported by Devapriya and co-workers (2021), to assess the meat quality of indigenous Kodi Aadu goats subjected to heat stress, a number of novel genes influencing meat quality were identified. The authors placed on record the vital role of myostatin (*MSTN*), calpain 1 (*CAPN1*), calpain 2 (*CAPN2*), calpastatin (*CAST*), diacylglycerol acyltransferase 1 (*DGAT1*), crytallin alpha (*CRYA*), heat shock protein 27 (*HSP27*), *HSP40* and *HSP90* genes to influence the meat quality variables in goats on exposure to heat stress.

1.3 Effects of Climate on Welfare Parameters

As mentioned earlier, alterations in the climatic conditions have an unequivocal impact on livestock production. Though this is of major concern in the tropical regions, it's an alarming issue also for animals reared in the temperate regions wherein the summers are getting relatively warmer over the years. Additionally, the evolving human lifestyle is also exerting pressure regarding farm animal welfare. Apart from intensification of livestock production, the outcomes of climate change, especially heat stress, also significantly deter the welfare of animals. These can be assessed based on several variables such as environmental indices for heat stress, behavioral responses exhibited by animals under heat stress, physiological alterations caused especially due to failure to cope with the deleterious heat stress effects and also based on their health status (Sejian et al. 2011).

1.3.1 Behavior

Heat stressed animals exhibit a number of behavioral alteration so as to adapt to heat stress. As a matter of fact behavioral adaptations are stated to be the first response adopted by animals to combat the deleterious impact of climatic alterations. Among the behavioral responses, shade seeking and reduced feed intake have been documented as the quick and profound responses observed in heat stressed animals. While shade seeking can be observed only in extensive rearing, reduction in feed intake has been reported both in extensively and intensively reared animals (Sejian et al. 2021). The primary objective of reducing feed intake during heat stress can be put forth as an adaptive response exhibited by heat stressed animals so as to reduce their internal metabolic heat production.

Significantly higher water intake and increased drinking frequency are also the classical response observed in heat stressed animals. This sign is of welfare issue especially in extensively reared animals as they may have to travel longer distances in search of water which adds on to the stress. The concept of multiple stresses reported by Sejian et al. (2018) reflects a major animal welfare issue in animals wherein heat stress, in addition with nutritional and walking stress, has a multifold impact on the animals' response and production when compared to any of these stressors alone.

Alteration in standing time and lying time are also important behavioral responses established in heat stressed animals. As a general concept, heat stressed animals have been reported to spend more time standing which enables them to re-orient their body so as to avoid the direct solar radiation and also from the surface radiations emitted mainly from the ground. Similarly, altered urinating frequency, defecating frequency and rumination time are among the other behavioral responses exhibited by animals under heat stress. Additionally, wallowing is a unique behavioral response observed only in buffaloes during heat stress. Furthermore, increased aggression and restlessness are other behavioral signs indicating severe stress in animals.

The expression of all the above mentioned behavioral alteration depend highly on the type of species, breed, their age, stage of production and also management practices followed. Furthermore, marked variations in the exhibition of these responses have been reported in cattle, sheep and goats. Though there are a number of causes for these variations, climate resilience is among the prime determinants influencing these responses in animals.

1.3.2 Physiology

1.3.2.1 Endocrine

The endocrine system, involved in the production of hormones, play a significant role in thermoregulation in animals. Stress induced especially due to environmental alterations trigger the hypothalamo–pituitary–adrenal axis (HPA axis) which is the predominant endocrine regulator for stress response in animals. The activation of the HPA axis stimulate the production and release of a series of hormones comprising the corticotrophin-releasing hormone (CRH), adrenocorticotropic hormone (ACTH) that finally leads to the secretion of the classical stress marker, cortisol. Apart from this, the thyroid hormones, triiodothyronine (T_3) and thyroxine (T_4), that are associated with metabolic responses are also altered in heat stressed animals. These hormones play a crucial role to maintain thermogenesis in animals especially from the metabolic response point of view. Additionally, heat stress also leads to altered hormonal profile in other homeostasis, metablosim and production associated hormones like aldosterone, adrenalin/noradrenalin, melatonin, prolactin, oestrogen, leptin, growth hormone (GH), insulin-like growth factor 1 (IGF-1) and many more.

Several studies have associated the alteration in such endocrine variable with heat stress in livestock.

In a study led by Li et al. (2020) to assess the seasonal dynamics in buffaloes under hot and humid climate, high THI was associated with significant increase in serum cortisol concentration in non lactating Nili Ravi buffaloes. While the plasma T_3, ACTH, insulin and GH concentrations were observed to be lower in buffaloes during the summer season when compared to spring, autumn and winter seasons. In another study by Pragna et al. (2018), comparatively assessed the summer season induced rhythmic alterations in metabolic activities in three indigenous goats breeds. Based on their results heat stress was reported to significantly reduce the plasma thyroid stimulating hormone (TSH) concentration in Malabari and Salem Black goats exposed to heat stress when compared to their respective control. Additionally the plasma T_3 concentration was also significantly reduced in heat stressed Malabari goats when compared to their control. Interestingly none of these hormone were significantly altered in the third breed, Salem Black, thereby reflecting the better climate resilience ability.

Several other studies in cattle, sheep, pigs and poultry depicted altered hormonal profile in heat stressed animals. Moreover, a number of hormonal markers for heat stress have also been established. Though there are no well established threshold limits for the concentration of such hormones in livestock species, their relative levels with respect to animals under thermo-neutral conditions can definitely reflect the stress status of the animal.

1.3.2.2 Metabolic

The phenomenon of reduction in feed intake to reduce metabolic heat generation has already been explained in the previous sections. Metabolic responses exhibited by animals under heat stress can be assessed by screening their enzyme profile. This not only provides representative information on the activity of other tissues and organs during heat stress but can also reveal the wellness of the animal. Acid phosphatase, alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), non-esterified fatty acids (NEFA), β-hydroxybutiric acid (BHBA) and volatile fatty acids (VFAs) are some of the established heat stress associated metabolic markers in livestock.

The alteration of metabolic variables and impaired production during heat stress was reported in lactating sows by He et al. (2019). The study revealed significant increase in serum insulin, creatinine, BUN and plasma NEFA in heat stressed sows (Landrace x Large White) that were during late gestational stage. The authors also reported significant alterations in a number of metabolites involved in glycerolipid metabolism, β-alanine metabolism, and pantothenate and CoA biosynthesis in pregnant sows. Their study summarized heat stress to elevate thermal response in late gestational sows that significantly reduced their productive performance and most importantly, enhanced lipid and protein catabolism.

In another study, Joo and co-workers (2021) assessed the alterations in blood metabolites (biochemical metabolities, enzymes and minerals) in Holstein and Jersey dairy cows exposed to heat stress. Apart from reporting an evident impact of heat stress on the metabolic profile in dairy cattle, the authors also reported breed variations for some of the variables. Such variations were linked to differences in productivity, metabolism and disease vulnerability between the two breeds. The blood metabolities significantly altered due to heat stress included albumin, protein, glucose, BUN, total cholesterol, LDL cholesterol, ALT, AST, creatine kinase, calcium, sodium, potassium, chloride, magnesium and phosphorus. Such alterations in the metabolic profile were assessed in several other livestock species. This thereby highlight the severity of distress the animals would be undergoing as a consequence of heat stress. Therefore, it is of utmost importance to work on ameliorative and mitigation measures to combat the adverse effect of heat stress in livestock production both from the production and animal welfare perspective.

1.3.2.3 Immunity

Impact of climate change on immune status in animals is another crucial yet least explored concept. Heat stressed animals are prone to a number of diseases that indirectly reduces their productivity and also adds up to the existing stress. Immune suppression in heat stressed animal is mainly associated to the activation of the HPA and sympathetic-adrenal–medullary (SAM) axes (Inbaraj et al. 2016). The final end products excreted through these axes, glucocorticoids and catecholamines, suppress/inhibit the synthesis and release of cytokines and other products having a vital role in immune defense. Scientific studies exploring the impact of heat stress in immune system of animals have been gaining more momentum over the past few years. A number of immunity associated markers have been reported in livestock species. Some of these include altered hematological profile, interleukin-2 (IL-2), IL-4, IL-5, IL-6, IL-12, interferon γ (IFNγ), tumor necrosis factor-α (TNF-α), toll-like receptors (TLR) 1–10, IgG, IgA, and so on.

In the previously stated study by Joo et al. (2021), the impact of heat stress was also assessed on immune cells of lactating Jersey and Holstein cows. Similar to their findings on the blood metabolite profile, an evident breed variation was observed apart from heat stress influence on the differential immune cell population of PBMCs. Heat stress was observed to significantly increase the proportion of B cell (CD4–CD21+) only in Holstein cows, while that of monocytes (CD11b+CD172a+) significantly reduced.

In a recent report, Xia et al. (2021) studied the heat stress induced mucosal barrier dysfunction in boars. The authors studied the impact of short-term heat stress on a number of variables which also included oxidation status and cytokine levels in pigs. Based on this experiment, heat stress was proved to significantly reduce serum IL-8, IL-12, IFNγ, and also the antioxidant activity which were reflected via increased malondialdehyde (MDA) and glutathione (GSH) content in addition with lower superoxide dismutase (SOD). Thus from the animal welfare point of view, the impact of climate change on the immune status in animals should be considered to be of alarming effect this can cause severe distress to the animal. In worst cases extreme heat stress in association with compromised health status of the animal may lead to increased mortality.

1.3.3 Health and Life Expectancy

Health status of animal is considered as one of the vital welfare variable. Impaired health status in animals, especially due to climate change, not only reflects the economic losses incurred but also the quantum of pain, discomfort or distress evinced by them. In addition to the direct impact on immune response, the varying environmental conditions lead to the emergence, re-emergence and rapid spread of a number of deadly diseases. Global climate change has also resulted in huge modifications on the micro and macroclimate of host and parasite in addition to the significant alteration in host-microbe interaction. High temperatures along with high relative humidity are ideal for the multiplication and survival of microbes, parasites and also disease transmitting animal vectors. Moreover, the rapid surge in livestock diseases during the warmer seasons further substantiates this concept of climate change on livestock diseases.

As mentioned earlier, animals exposed to increasing and prolonged heat load exhibit increased standing behavior. Though the primary purpose of this response by animals is for heat abatement and cooling body surfaces, prolonged standing time can predispose the animal to lameness. However, there are no direct studies conducted to link heat stress and lameness in animals (Polsky and von Keyserlingk 2017). Apart from the direct and indirect impact of climate change on immune response and diseases in livestock, there are also reports on association of some climatic adversities with certain diseases. In eastern Africa, the outbreak of Rift Valley Fever (RVF) has been often linked with El Niño. It may be noted that this disease if of major concern for having serious impacts on livestock morbidity and mortality.

Although the literature assessing the effect of climate change on livestock disease is limited, the postulated impact on the health status in animals should not be overlooked. This is of huge relevance from production, economic, animal welfare and public health point of view. The pain and suffering undergone by animals due to their deteriorating health status is grave. Moreover, their prolonged treatment also impairs the future productivity of the animal thereby reducing its life expectancy. Furthermore, most of the deadly diseases in livestock are zoonotic thereby risking their spread among the human population. Therefore, climate change and its impact on animal production, health and welfare are of great relevance that needs to be addressed efficiently to sustain animal production in the changing climate scenario.

Learning Outcomes

- Climate change deleterious impact on livestock production and welfare is primarily mediated by individual or combination of climate variables such as temperature, humidity, rainfall, wind velocity, solar radiation etc.

(continued)

- Climate change significantly reduces the economic traits like milk yield, milk composition, meat yield, body weight, body condition, egg production in livestock and poultry.
- The direct impact of climate change depends on its intensity and duration and affects the health and welfare of livestock by suppressing the immune system.

1.4 Conclusions

Livestock needs to be the priority focus in agriculture sector due to their high climate resilience and are tipped to be playing a significant role in meeting future food demands of growing human population. Although considered climate resilient, still livestock production is hampered in order to facilitate adaptation in farm animals. Thus the milk, meat, reproduction and immune system functions are compromised to support vital life sustaining activities of farm animals in the changing climate. Several welfare indicators covering farm animal behavior, physiology, hormones, metabolites, immune and health related variables must be considered to assess the animals' adaptive potential.

1.5 Future Perspectives

A clear region specific database on the various impacts of climate change on farm animal production needs to be developed. This will help the policy makers to identify the breeds with minimal impact for dissemination to the farmers. Although the various climate change associated environmental stressors impact was fairly established in farm animals, still only predominantly heat stress impacts were known in depth. Information particularly on assessing the impacts of climate change associated water stress on livestock production and welfare have not been fully explored. Such an effort would be very vital given the significance of water stress in the changing climate scenario. Likewise the information pertaining to climate change associated disease occurrences in livestock have not been fully established. Therefore, establishing the impacts of climate change associated water stress and sudden disease outbreaks on the performance and welfare of farm animals is the need of hour. More intensified research efforts are also needed to identify more quantifiable welfare indicators related to animal health. In addition, efforts are also equally needed to develop more cost effective individual-level sensors (e.g., accelerometers, GPS, and RFID) and automated monitoring systems for group level animal monitoring (e.g., cameras) needs to be developed to assess the welfare of grazing animals.

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Effects of Heat Stress and Climate Change Induced Bushfires on Beef Meat Quality

2

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Abstract

Heat stress is considered a very stressful event for livestock, and it has detrimental consequences for not only animal health and productivity but also product quality. During bushfires, which are increasingly prevalent under climate change, animals can be exposed to extreme heat stress events. Ruminants are very prone to heat stress because of increased metabolic rate. Under the influence of chronic heat stress, ruminants exhibit a reduction of muscle glycogen concentration, which leads to increased, ultimate pH and WHC, producing the quality defect of dark-cutting meat. In addition, heat stress causes extended protein and lipid oxidation, oxidative stress and shelf life reduction of meat. A case study is presented on the effects of the devastating bushfires in Australia in 2019–2020 on the quality of 450,000 beef carcases. This chapter focuses on the effects of bushfire and heat stress on the ruminants and the consecutive changes produced

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V. Sejian et al. (eds.), *Climate Change and Livestock Production: Recent Advances and Future Perspectives*, https://doi.org/10.1007/978-981-16-9836-1_2

on the quality of the meat. Discussion on knowledge gaps, and future perspectives are also provided.

Keywords

Beef · Bushfires · Cattle · Dark-cutting · Heat stress · Meat quality · MSA · PSE

Abbreviations

$	Dollar
%	Percentage
°C	Degree Celsius
ANS	Autonomic nervous system
AUS-MEAT	Authority for the uniform specification of meat and livestock
cAMP	Cyclic adenosine mono phosphate
cm^2	Centimeter square
h	Hour
HCO_3^-	Bicarbonate
HGP	Hormonal growth promotant
HSP	Heat shock protein
kg	Kilogram
L	Lightness
MLA	Meat and Livestock Australia
MSA	Meat Standards Australia
NSW	New South Wales
pH	Potential of hydrogen
PSE	Pale, soft and exudative
RH	Relative humidity
ROS	Reactive oxygen species
US	Unites States
WBSF	Warner Bratzler shear force
WHC	Water holding capacity
β	Beta

2.1 Introduction

The economic losses incurred by heat stress annually the U.S. beef industry is estimated to be about 369 million U.S. dollars which quantifies the hazardous effect of heat stress on a country's economy (St-Pierre et al. 2003). Heat stress is considered one of the most expensive and stressful events affecting the productivity, health and meat quality of the animal. The economic losses are associated with decreased fertility, reduced rate of growth, inappropriate carcase composition and quality, decreased carcase weights, leading to a surge in animal welfare issues and very high veterinary costs. Negative influences of heat stress on the economy have resulted in the developing imperative to address climate changing scenarios. The adverse effects of heat stress on animals' health and productivity are likely to continue in the upcoming years if the selection is more targeted towards improving the traits related to production. It is to be noted that these production traits are negatively correlated with traits associated with adaptability to climate change and thermo-tolerance.

From the perspective of increased metabolic rate, higher growth rate, improved production of basal metabolic heat and increased productivity; ruminants are highly susceptible to the negative influences of heat stress. The heat produced during ruminal fermentation is the major risk factor threatening the thermoregulation capacity of ruminants. (Kadzere et al. 2002; Tajima et al. 2007). Pale, soft and exudative meat (PSE), a condition characterised by decreased ultimate pH and reduced water holding capacity (WHC), was commonly found in carcases of poultry and pigs, (Kim et al. 2014; Warner et al. 2014). The reason for this condition is stress or struggling of animals at slaughter, exacerbated by any acute heat stress, leading to acceleration in the rate of muscle glycogenolysis and increased concentration of lactic acid, rapid reduction in muscle pH, during early phases post-portem when the carcase remains hot (Owens et al. 2009). In contrast to the PSE condition, animals that are subjected to chronic heat stress had significantly less glycogen reserves and subsequent reduction in lactic acid production, leading to increased, ultimate pH and WHC producing dark-cutting meat in ruminants (Adzitey and Nurul 2011; Gregory 2010; Kadim et al. 2008; Mitlöhner et al. 2002). Moreover, during hot climatic conditions, increased protein and lipid oxidation and reduced shelf life of meat were also observed (Mujahid et al. 2007; Wang et al. 2009).

Bushfires have become increasingly prevalent with climate change in Australia and overseas (Visner et al. 2021). Adverse health outcomes for cattle from bushfires can include direct contact with fire, exposure to radiant heat, and inhalation of bushfire smoke (Hillman et al. 2021). The potential effects of bushfires on meat and carcase quality are diverse and multi-faceted, including those that may impact carcase weight, condition of the animal and hence fat score, and marbling, meat pH, and colour. These outcomes for meat quality from bushfires are likely caused by; (i) heat stress from the radiant heat of the bushfire, (ii) reduced nutrient intake due to heat stress and lack of feed/pasture availability, and (iii) impact of stress associated with an unfamiliar environment, transport and more frequent movement of stock. The effects of bushfires on carcase and meat quality will result in income loss for cattle producers and have implications for animal welfare and productivity.

This chapter discusses the consequences of heat stress and bushfires for beef meat quality. Data on bushfire effects on beef quality are lacking, and a case study is presented on recent data which utilised animal and carcase data from the recent bushfires in Australia. Gaps in the literature are identified, and directions for future research are suggested. Knowledge gaps, scarcity in the literature and future endeavours were also discussed in this chapter.

2.2 Physiologic and Metabolic Consequences of Heat Stress

Response to heat stress is primarily mediated via the activation of the autonomic nervous system (ANS) by catecholamines (noradrenaline and adrenaline), which leads to increased body temperature, heart rate and respiratory rate, redirection of blood supply to the skin from viscera for the process of thermoregulation, increased utilisation of energy from body stores (Minton 1994) by muscle glycogenolysis acceleration and energy reserve suppression (Afsal et al. 2018; Gregory 2010). Muscle glycogenolysis acceleration is mediated via cyclic adenosine monophosphate (cAMP) by initiating cascades of reaction in the muscle produced by the action of catecholamines on β_2-receptors (muscle). This process, in turn, leads to glycogenesis inhibition and glycogenolysis activation by inhibiting glycogen synthase (Roach 1990) and activating glycogen phosphorylase, respectively (Franch et al. 1999).

As an adaptive mechanism to tackle the rise in metabolic heat production, animals reduce their feed intake under high environmental conditions, which is considered the major impact of heat stress on livestock (Kouba et al. 2001; Odongo et al. 2006; Russell 2007). This feed intake reduction explains only 50% of the animals' physiological and metabolic responses under heat stress, the rest of which can be explained by the hormonal profile and energy partition variations (Baumgard and Rhoads 2007). In beef cattle, the feed intake reduction starts at two combinations of temperature and relative humidity (R.H.), one occurs at an ambient temperature of about 27 °C, and $>$ 80% of R.H. and the other happens at an ambient temperature of about 30 °C and $<$ 80% of R.H. (Bernabucci et al. 2010).

Also, fasting of steers for about 7 days was found to decrease the glycogen concentration in muscle by about 30%. It is always important to provide adequate nutrition to the cattle before sending them for slaughter to prevent the incidence of (Knee et al. 2007; Pethick et al. 2005) dark-cutting meat, keeping in mind the fact (Warner et al. 2006) that there is always a reduction in glycogen reserve of animals sent for slaughter from the farm.

Providing ad-libitum water for heat-stressed animals is of paramount importance to avoid dehydration and negative influences of heat stress. Usually, it is found that there is a two-fold rise in the requirement of water for the animals experiencing heat stress (Beede and Collier 1986), which is considered to be a fundamental mechanism of thermoregulation to tackle evaporation of water through sweating (Marai et al. 2007) and panting. This is also proved to be a direct mechanism of cooling the reticulo-rumen to decrease body temperature (Bewley et al. 2008). If the availability of ad-libitum water to the animals is neglected under heat stress, this can cause severe dehydration and enhance the adrenergic mode of responses to heat stress (Matthews and Parrott 1991).

Increased respiratory rate under heat stress leads to respiratory alkalosis (Cottrell et al. 2015; Odongo et al. 2006; West 2003). The body tries to compensate for this situation via the urinary system by increasing the HCO_3^- excretion (Schneider et al. 1988), leading to metabolic acidosis. In this condition, the body's metabolic dependency shifts towards anaerobic conditions generating energy through the production of lactate from pyruvate, similar to the early stages of post-mortem metabolism. As mentioned above, this results in PSE-like conditions (Wang et al. 2009; Gholamreza et al. 2019) in meat.

Furthermore, there is also a surge in generation of reactive oxygen species (ROS) and reduction in antioxidant capacity of the animal under heat stress, both of the described conditions inducing oxidative stress to the animal (Chauhan et al. 2014a; Liu et al. 2016; Mujahid et al. 2007; Mujahid et al. 2006; Mujahid et al. 2005; Shakeri et al. 2018). This induced oxidative stress and acidosis in the tissue further produces much more adverse effects such as a free radical-mediated chain reaction, cytotoxicity, enhancement in lipid and protein oxidation, impaired health of animals, all of which cause a reduction in the shelf life of the meat (Celi and Gabai 2015; Chauhan et al. 2014b; Imik et al. 2012) and negative impairment of the meat quality.

2.3 Effect of Heat Stress on Beef Meat Quality

Heat stress is associated with dark-cutting in beef cattle (Gregory 2010). Pre-slaughter stress depletes muscle glycogen reserves, resulting in a high pH (>5.70 in Australia) that causes dark-cutting meat (AUS-MEAT 2005), and dark colour of meat occurs mainly due to reduced light scattering in the muscle and predominance of purple deoxymyoglobin in the meat surface. There is a multitude of hypotheses to explain the mechanisms behind the dark colour of meat, and the main ones are as follows. Mitochondria can thrive at high pH and increase their proportional oxygen-consumption activity; as a result, deoxymyoglobin (purplish-red) predominates, resulting in black meat (Faustman 1994; Suman and Joseph 2013). Additionally, the high pH prevents post-mortem myofibril and muscle cell shrinkage, minimising the meat's light scattering capabilities measured by low lightness (L*) values (Hughes et al. 2018). Finally, at high pH levels in meat, muscle proteins above their isoelectric point bind more water, resulting in densely packed myofibrils, and light scattering will be reduced (Abril et al. 2001).

Various reports suggest ruminants exhibit more dark-cutting during warm weather than during cold weather. (Hughes et al. 2018; Kadim et al. 2004; Kadim et al. 2008; McPhail et al. 2014; Węglarz 2010). Dark-cutting rates are higher in the northern hemisphere between September and October because of the more frequent occurrence of cold nights and hot days caused by unexpected weather changes and temperature differentials, such as those that occur during late spring and fall break (Boykin et al. 2017).

The increased incidence of dark-cutting in the summer can be attributed to a combination of poor pasture quality (Knee et al. 2004) and reduced feed intake. Heat stress affects feed intake, and liver glycogen cannot generally maintain energy homeostasis in ruminants that rely primarily on volatile fatty acids as glucose precursors; animals must rely on muscle glycogen as a glucose source during heat stress (Gardner et al. 2014). Sheep exposed to handling and transport stress had higher rectal temperatures associated with lower glycogen levels during slaughter (Pighin et al. 2014). This suggests a relationship between increased body temperature, glycolytic stress response, and maybe elevated muscle pH and dark-coloured meat.

Feedlot cattle are the most susceptible to heat stress among ruminants. They are frequently exposed to radiating surfaces, consume a lot of high-energy feed, and have few opportunities to seek out shade, water, or ventilation for cooling purposes (Gaughan et al. 1996; Holt et al. 2004; Mader et al. 1997; Renaudeau et al. 2012). Pre-slaughter body temperatures in grain-fed cattle are higher than in grass-fed cattle (Jacob et al. 2014), which is related to an earlier and stronger rigour mortis, resulting in tougher meat (Warner et al. 2014). Additionally, ruminants fed grains that ferment rapidly are more susceptible to heat stress (Gonzalez-Rivas et al. 2016; Gonzalez-Rivas et al. 2017; Mader et al. 1997). Therefore, it is plausible to hypothesise that meat from feedlot cattle will be more likely to exhibit poor quality due to heat stress. Shade may be advantageous in feedlot conditions since muscle pH was significantly higher in the loin of feedlot steers kept in the shade during the summer at 1 h (5.97 v. 6.03, $P = 0.014$) and 2 h (5.97 v. 6.03, $P = 0.043$) post-slaughter. However, the shade had no effect on hardness, texture, or drip loss of the meat (DiGiacomo et al. 2014).

A higher percentage (59%) of carcases classified as dark-cutting (here defined as an ultimate pH > 6.0) were found in cattle slaughtered under heat stress conditions (>30 °C) than animals slaughtered during the cold season. *Longissimus thoracis* muscles from hot-boned beef were found to have a higher pH (6.24 vs. 5.54), lower WBSF (10.1 vs. 15.6 kg/cm^2), and higher cooking loss (19.8 vs. 26.0%) when processed during the hot season and removed within 60 min post mortem, compared to those collected during the cool season (Kadim et al. 2004). For Omani beef that was harvested in the hot season, the *longissimus thoracis* muscles had darker, less red and less yellow meat than those collected in the cold season based on L* (31.45 vs. 35.58), a* (18.53 vs. 23.19) and b* (4.16 vs. 6.40) measurements made 48 h post-mortem (Kadim et al. 2004).

Holstein-Friesian cross meat produced in winter exhibited a brighter red colour and higher values of a*, b*, C and H values (redness, yelllowness, chroma, hue respectively) than meat produced in summer; nevertheless, in summer, there was a 30% greater frequency of meat with an ultimate pH > 5.8 measured in the *longissimus thoracis* at 48 h post-mortem (Węglarz 2010). A decreased incidence of dark-cutting was seen in West Texas feedlot heifers housed in shaded pens during summer relative to heifers in pens with no shade (8.3 vs. 19.8%), but no variations in hot carcase weight, marbling score, or *longissimus muscle* area were observed between the two groups (Mitlöhner et al. 2002). In contrast, shaded heifers in a prior trial had heavier hot carcases (+16 kg) and thicker fat than unshaded cattle; however, they found no change in meat quality or carcase yield (Mitlöhner et al. 2001). Animals under shade showed reduced aggressive behaviour, which is in line with earlier studies suggesting that heat stress can exacerbate bullying behaviour by depleting glycogen reserves, as reported by the authors (Kreikemeier et al. 1998). These research established that providing shade to beef cattle effectively mitigates the detrimental effects of heat stress on performance and meat quality.

In Korea, that Hanwoo beef cattle of different sexes slaughtered in summer had significantly smaller ribeye areas and higher marbling scores than those slaughtered in other seasons, but the maturity scores were higher (Panjono et al. 2009). Hanwoo cattle were used in another experiment, and it was found that *longissimus thoracis* from cattle slaughtered in summer had a higher pH at 48 h post-mortem than from cattle slaughtered in winter (5.46 vs. 5.36). This difference was attributed to the high temperature and humidity of the Korean summer (Panjono et al. 2011).

2.4 Impact of Bushfires on Beef Meat Quality—A Case Study from Australia

In Australia, the 2019/2020 summer period involved widespread and devastating bushfires in southeast Australia. Hillman et al. (2021) investigated the spatiotemporal relationships between exposure to bushfire and the Meat Standards Australia (MSA) carcase index of 400,00 beef carcases. The study cattle population was slaughtered and graded under the MSA program over the period November 2018–July 2020, and cattle included in th study had a property of origin within 50 km of a bushfire and the bushfire occurred between 2 and 180 days prior to slaughter. The MSA index is a quantitative indicator of the expected eating quality and potential value of a beef carcase. MSA grading data is calculated using only attributes influenced by pre-slaughter production and reflects the impact on beef eating quality of management, environmental and genetic differences between cattle at the point of slaughter (MSA 2021a). Geospatial data on the locations of all fires and farm properties were used to determine the distance of cattle from the fire prior to despatch for slaughter and other data on the fires. Other animals and carcase data were also collected. Data from the 'Long Paddock' database was used to estimate pasture biomass and pasture growth (Stone et al. 2019) for the two months prior to slaughter. Consistent trends were observed in the MSA index of beef carcases, with lower MSA index scores seen in cattle slaughtered after being in close proximity to fire (Hillman et al. 2021). Hence cattle closest to the fire produced carcases of lower predicted eating quality and lower value, relative to cattle up to 50 km from the fire.

A subsequent study used MSA grading data from additional beef processing plants in NSW and Victoria and utilised 450,000 carcases from cattle slaughtered over the bushfire period (Warner et al. unpublished results). Modelling of loin pH and incidence of dark-cutting (pH at grading >5.70) was conducted using all MSA carcase grading data, as well as fire and pasture variables referred to above. And step-wise regression was used to determine a parsimonious model. The number of continuous days of fire within 180 km of a property, the closest distance to a bushfire, the time since the bushfire, and interactions with animal factors significantly affected the ultimate pH of the loin muscle as well as the incidence of dark-cutting meat. The elevated loin pH and increased risk of dark-cutting caused by bushfires were exacerbated in grass-fed cattle (relative to grain-fed), steers (relative to heifers/cows), younger cattle (identified by ossification score at grading; MSA 2021b), lower *Bos indicus* content (identified by hump height at grading, mm; MSA 2021b) and cattle treated with hormonal growth promoters (HGPs, relative to non-treatment with HGPs; MSA 2021b). The results will be used to create a better understanding of what bushfires do to beef cattle and how to prepare and respond, which will be an invaluable tool for producers living in high bushfire areas of Australia (MLA 2021).

Learning Outcomes

- Heat Stress can negatively influence beef production and meat quality and this is attributed to the reduction in muscle glycogen and protein concentration and depletion of fat reserves.
- The harmful consequences of heat stress on meat quality and production is likely to continue with the current focus in breeding programs on production instead of climate adaptatation or thermo-tolerance.
- Provision of high-quality feeds to cattle subsequent to heat stress and bushfires will assist in ensuring quality carcases and meat.
- Bushfire remains a serious threat to livestock and to the quality and economic value of carcases under the current climate changing scenario. Therefore, identifying the risk factors and developing nutritional and genetic strategies for ameliorating bushfire associated heat stress gains significance.

2.5 Summary and Future Research

It is evident that cattle suffering under hot and humid conditions are associated with reduced live weight and carcase weight and also the production of dark-cutting meat. Under the acute and chronic influence of heat stress, the physiological and metabolic responses are proven to have a profound association with meat quality by reducing the protein concentration and glycogen reserve of meat and depletion and redirection of fat reserves. These conditions also induce cellular damage, oxidative stress and increased expression of HSPs, together hindering meat quality and producing deterioration of the meat.

Climate change has also resulted in increased bushfire occurrence, and negative consequences of bushfires for beef meat quality have recently been demonstrated. Under the current trends of global warming, unfortunately, the adverse effects of heat stress are expected to continue if the selection for traits associated with meat production is prioritised over the traits associated with adaptability to climate change and thermo-tolerance.

The development of nutritional and genetic strategies for ameliorating heat stress, particularly in the period following a bushfire event, is important for the future production of quality beef. Nutritional strategies are needed prior to or during a heat stress event and in the months following, particularly where bushfires are involved. Following the exposure of cattle to a bushfire event, in order to ensure muscle glycogen recovery and reduced occurrence of high pH dark-cutting beef carcases, producers should consider removing HGPs from beef production and providing high energy grain-based diets, particularly for older cattle. In the longer term, in bushfire prone areas or where heat stress events are increasingly common, heat-tolerant beef breeds such as zebu, brahman, droughmaster and other *Bos indicus* based breeds are recommended.

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Part II

Heat Stress Impact on Adaptive Responses in Livestock

Adaptation of Beef Cattle to Heat Stress Challenges

3

J. B. Gaughan, A. M. Lees, and J. C. Lees

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Abstract

The demand for animal protein is increasing with much of this demand in the tropics and sub-tropics and beef consumption, which represent approximately 25% of global meat consumption, is expected to increase from 54 to 118 million tonnes by 2050. Although heat stress in beef cattle is an increasing challenge in many parts of the world there is sustained growth in the number of tropical cattle, which represent more than 50% of all cattle worldwide. Beef production in the tropics and sub-tropics is characterised by climate variability (heat and rainfall), nutritional limitations, parasites, disease, and poor reproductive performance. If

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V. Sejian et al. (eds.), *Climate Change and Livestock Production: Recent Advances and Future Perspectives*, https://doi.org/10.1007/978-981-16-9836-1_3

we are to meet the challenges of increasing animal protein production in the tropics and sub-tropics, we will need to move away from traditional selection methods to more advanced molecular and large-scale genetic evaluations that consider the whole genome, not just a few specific traits.

This chapter will focus on what is required to improve the adaptive capacity of beef cattle in harsh environments, some of the mechanisms of adaptation, and the application, and challenges of selection and breeding strategies e.g., use of molecular genetic tools to 'speed up' adaptation to a changing climate.

Keywords

Adaptation · Beef cattle · Thermal challenge · Breeding · Selection

Abbreviations

%	Percentage
>	Greater than
ABVg	Australian genomic breeding value
DNA	Deoxyribonucleic acid
e.g.	Exempli gratia (for example)
GEBV	Genomic estimated breeding value
HT	Heat tolerance
N	Nitrogen

3.1 Introduction

The demand for animal protein is increasing with much of this demand in the tropics and sub-tropics (Cao and Li 2013; Ismail et al. 2020). Beef consumption, which represent approximately 25% of global meat consumption, is also expected to increase from 54 to 118 million tonnes by 2050 (Cooke et al. 2020b). Although heat stress in beef cattle is an increasing challenge in many parts of the world there is sustained growth in the number of tropical cattle, which represent more than 50% of all cattle worldwide (Barendse 2017) (Fig. 3.1). Tropical cattle are a very diverse category and include cattle that either arose in the tropics, are composites of tropical cattle and temperate cattle, or were temperate cattle that were translocated to the tropics and have been under natural or artificial selection for many generations (Barendse 2017). Cattle that have evolved in harsh environments have developed grazing patterns that allow them to be more nutritionally efficient especially with low-quality forages (Russell et al. 2012; Cooke et al. 2020a, b), they are resistant to internal and external parasites (Frisch and Vercoe 1984), and are quicker to respond to environmental stimuli (Lees et al. 2018). Another adaptation that allows cattle to grow and reproduce when ingesting low-protein diets is nitrogen (N) recycling to the rumen (Silva et al. 2019). This is an important physiological mechanism allowing

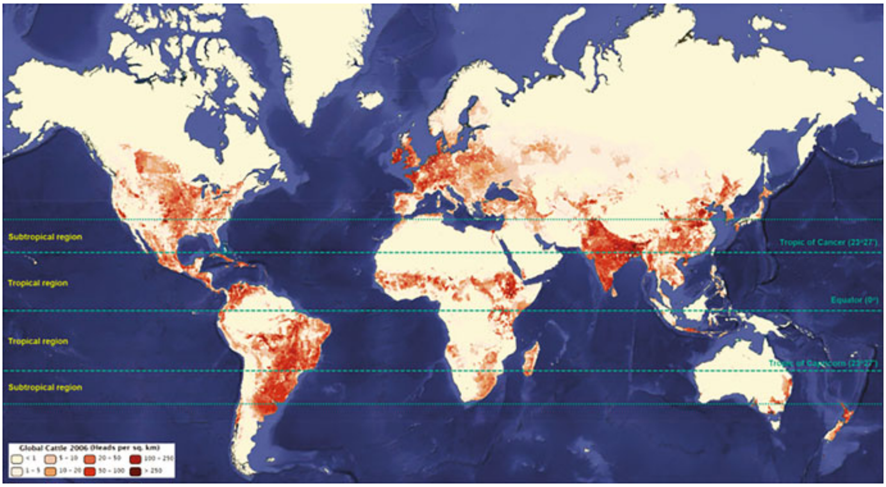

Fig. 3.1 Distribution of cattle around the world (Cooke et al. 2020a, b)

ruminants to grow efficiently when on low-protein diets (Silva et al. 2019). Selection for improved N recycling may be a useful addition to selection programs. Globally there are >800 cattle breeds, so selection for cattle that are heat tolerant is doable, but there are potentially associated costs, which will be discussed below.

Beef production in the tropics and sub-tropics is characterised by climate variability (heat and rainfall), nutritional limitations, parasites, disease, and poor reproductive performance (Gaughan and Cawdell-Smith 2015). Cattle are therefore exposed to multiple stressors such as droughts, floods, poor nutrition, and climate stress (which can be both cold and heat stress). Even though our focus here is on heat stress it should not be looked at in isolation. There is a need to move away from traditional selection methods to more advanced molecular and large-scale genetic evaluations that consider the whole genome, not just a few specific traits.

3.2 Adaptation in Cattle: For a Stressful Environment

One could argue that adaptation to, for example heat, occurs as natural selection in beef cattle in the tropics and sub-tropics. And while this is true, this adaptation comes at a cost (a trade-off) to other traits such as reproduction and growth (Frisch and Vercoe 1984). The trade-off is essentially production verses survivability. It is widely accepted that selection for heat tolerance, parasite resistance, and the ability to do well where nutrition is poor, generally has negative impacts (from our perspective not the animals) on fertility, growth, and milk production (Frisch and Vercoe 1984; Fordyce et al. 1993). Longevity of adapted cattle is another key factor that needs to select for (Cooke et al. 2020a). Our challenge then is to select animals that are adapted to the stressful environment and still retain adequate production performance. It is likely that simultaneous genetic improvement of productive and adaptive traits in tropically adapted breeds of beef cattle raised in tropical environments is feasible without major impacts on adaptive or production traits (Burrow 2012). However, further research is required to quantify the genetic antagonism between adaptation and production traits to evaluate the potential selection response (Renaudeau et al. 2012).

3.3 Mechanisms of Beef Cattle Adaptation to Heat

Animal adaptation is a function of a number of factors, essentially it is a function of Animal × Human × Resources. A large part of future adaptation will depend on how well we use the available resources i.e., land, feed, water, financial, animals, and people. A large part of this will be which genetic technologies we can afford to use. Before we get to that we need to decide: What do we want from our animals? And what adaptive traits should we be looking for? These traits could be any combination that improve the survivability, fertility, production (growth, milk, meat), product quality or animals that support economically and financially sustainable systems. The needs will vary from location to location and will be influenced by operation size and individual limitations.

Key to our understanding of a breed or species ability to adapt, is to define the stressor(s), and define what we want the animals to adapt too (Gaughan et al. 2019). We need to consider the following. How long does it take for adaptation to occur? We should start breeding now for a predicted climate in 30 years' time, but what if the predictions are wrong? Can we breed animals (our current breeds and/or species) for the level of tolerance we think they may need? What do we select for? How do we select animals to use? Are controlled studies a good indicator of animal responses in the field?

Some proposed strategies have been to identify stress tolerant animals within non-tolerant breeds e.g., within the Angus breed, and the use existing already adapted animals. There are large differences in thermal tolerance within and between breeds, so the opportunity exists the use of molecular techniques to improve thermal tolerance of beef cattle. However, further research is required to quantify the genetic antagonism between adaptation and production traits to evaluate the potential selection response. With the development of molecular biotechnologies, new opportunities are available to characterize gene expression and identify key cellular responses to heat stress. These new tools will enable scientists to improve the accuracy and the efficiency of selection for heat tolerance. Epigenetic regulation of gene expression and thermal imprinting of the genome could also be an efficient method to improve thermal tolerance (Renaudeau et al. 2012). However, Monteiro et al. (2016) reported long-term negative epigenetic effects (lower birth weight, lower weaning weight and decreased performance through first lactation) when dairy calves were exposed to heat stress in utero. Later studies (Ouellet et al. 2020) have confirmed that heat stress in late gestation negatively impacts the dairy cows' productivity and health, that in utero heat stress alters mammary development of the dam and the foetus, and importantly that in utero heat stress programs a lower productivity phenotype in offspring. Furthermore, there is evidence that offspring from prepartum heat-stressed cows have compromised passive immunity and impaired cell-mediated immune function (Tao and Dahl 2013). It is not known at that time if similar responses would be seen in beef cattle, but it is likely.

Traits such as coat colour (Brown-Brandl et al. 2006; Fanta 2017; Dikmen et al. 2017), heat shock protein expression (Collier et al. 2008), metabolic changes (Collier et al. 2012; Baumgard and Rhoads Jr 2013; Aleena et al. 2018), maintenance of feed intake (Verma et al. 2000) and performance have all been promoted as selection options for improved heat tolerance. Genes affecting hair and skin colour, hair length and density have been identified in cattle (Collier et al. 2008). Heat tolerance in *Bos taurus* beef cattle with the 'slick hair gene' is well established (Olson et al. 2003). However, the incorporation of these traits across breeding programs has not had a large effect on improving performance of cattle in hot environments, which highlights the difficulties in selecting for heat tolerance in non-tolerant breeds.

We need to accept that productivity levels in the tropics will never match what has been achieved in temperate regions. Selection must be realistic and in sync with the available resources, the prevailing, and future climate. There are currently more questions and unknowns than available answers.

3.4 Constraints to Adaptation

Probably the biggest constraint to adaptation is the genetic capacity of the animal. Given time most species have capacity to meet the direct challenges of a changing climate. Indirect effects such as habitat changes and human activities are more difficult predict and breed for. All aspects of the environment are interlinked; therefore, we cannot attempt to make a change in one area without understanding the overall impact of the change on the affected species—including humans. A key factor in animal adaptation is human adaptation and willingness to change.

The ability of animals to adapt to climate change will largely rest with how we manage the change. The system is very complex!

3.5 Beef Breeding Programs to Improve Tolerance to Harsh Environments

The use of natural selection, and traditional selection methods as the means of adaption to heat stress is not logical. Given the rapid changes in climate that animals are being exposed too we need to have methods that allow for rapid genetic change. Especially considering the generation interval of these animals, we only have one or two generations to make substantive change.

Genetic improvement in animal populations through conventional improvement programs mainly involves the selection of males and females that, when mated, are expected to produce progeny that perform better than the average of the current generation (Khare and Khare 2017). Performance is not (or at least should not be) focused on a single trait but on a combination of multiple characteristics, or traits, most of which are quantitative in nature. Quantitative traits that are controlled by multiple genes (>100 to perhaps thousands) which are affected by environment (Khare and Khare 2017). Therefore, selection for traits that are strongly influenced by the environment and have low heritability e.g., fertility, are not only difficult but require genetic the assessment of large numbers of animals (Hayes et al. 2019; Khare and Khare 2017).

Traditionally the main criteria that has been used to identify individuals to be used for breeding are estimates of their breeding values for the trait or traits of interest. The breeding value of an individual is the sum of the additive effects of all loci that contribute to the trait (quantitative trait loci), deviated from the population mean (Falconer and MacKay 1996 cited by Khare and Khare 2017). The main source of information for the estimation of breeding values of selected individuals are extensive data bases of recorded phenotypes for the traits of interest, or for traits that are genetically correlated to the traits of interest. These data bases are largely focused on animals located in temperate regions and there is little information for breeds in the tropics (Khare and Khare 2017).

Some traits of interest are only able to be recorded late in life (longevity), only on one sex (milk yield), require animals to be sacrificed (meat quality), or require animals to be exposed to conditions that would hamper the ability to market or export their germplasm (disease resistance) (Dekkers 2012). These phenotyping constraints limit the amount of genetic progress that can be made through conventional selection and breeding method (Khare and Khare 2017).

3.6 Molecular Genetics the Way Forward?

Pollak (2005) outlined the numerous tools that can be applied to beef cattle breeding, including: (i) reproductive innovations that focus on manipulating the physical process of reproduction (e.g., artificial insemination, embryo transfer, cloning) or reproductive cells (e.g. semen sexing, nuclear transfer); (ii) statistical methodologies and computational algorithms designed to implement those methodologies; (iii) ‘mechanical’ inventions that provide mechanisms to capture new phenotypes, such as for the capture of individual feed consumption, images for indicating tenderness, and ultrasound measures of carcase attributes; and (iv) procedures that provide information on DNA, generally characterised as laboratory detection of DNA polymorphisms. While all of these have merit it is the use of molecular genetics that is showing the best promise for rapid genetic change.

Molecular genetics is the field of science that combines molecular biology and genetics. It is the study of the structure and function of genes, but at the molecular level (Fulton 2008). Molecular genetics is the study of the sequence of genes as well as the structure of the protein it codes for. Molecular genetics allows for the identification and mapping of genes and genetic polymorphisms i.e., whole genome sequence analysis. Molecular genetics techniques make it possible to identify genes that are involved in variety of traits, thereby allowing selection of animals on the basis of their genetic makeup. The use of molecular information in selection programs has the potential to increase productivity, enhance environmental adaptation and maintain genetic diversity. The use of molecular genetics technologies potentially offers a way to select breeding animal at an early age (even embryos); to select for a wide range of traits and to enhance reliability in predicting the mature phenotype of the individual (Khare and Khare 2017).

Molecular techniques have the potential for rapid changes in selection criteria for animals used in breeding programs based on their phenotypes. Molecular techniques offer the potential for selecting animals that are resistant to disease. Molecular techniques allow selection for complex traits such as reproductive efficiency, and feed efficiency in animals (Khare and Khare 2017; Mrode et al. 2019). The use of molecular genetics techniques should be used in association with conventional animal breeding tools (e.g., still need to assess for conformation faults) so to optimize the animal breeding program (Singh et al. 2014). Molecular techniques may allow the selection of animals and develop animal lines that are suited to particular environments, and rapid genetic change in the face of climate change.

Genomic selection is an attractive alternative to traditional breeding as it will allow young bulls (and heifers) to be selected on their heat tolerance genomic estimated breeding value (GEBV) as well as on other traits (Nguyen et al. 2016). The work of Nguyen et al. (2016) has resulted in the implementation plan of the Australian genomic breeding values (ABVg) for heat tolerance (HT) (HT ABVg) for Holstein and Jersey dairy cattle (Nguyen et al. 2017). However, the authors reported that there has been a deterioration in the genetic trend of HT, and to moderate the decline they suggested that the HT ABVg should be included in a multi-trait economic index with other traits that contribute to farm profit (Nguyen et al. 2017). This highlights that there is still a need for traditional multi-trait selection.

The use of this technology is limited in many because most of the production occurs in small holder systems which are characterized by small herd sizes, lack of performance, and pedigree recording and therefore, the non-existence of conventional genetic evaluation systems (Kosgey and Okeyo 2007; Nguyen et al. 2016; Mrode et al. 2019). Therefore, there is a need for interventions that will allow these societies to reap the benefits of the new genetic revolution.

3.7 Advantages of Molecular Approach Over Traditional Approach

The following summary of the advantages of molecular genetics over traditional breeding has been adapted from Khare and Khare (2017)[1] and Barendse (2017).[2]

- Genetic improvement of tropical cattle must move beyond the contemplation of breeds and breed characteristics.[2]
- The use of genomic breeding values (GEBV) in tropical cattle will require improvements in methods of genetic prediction for crossbreed cattle.[2]
- Assuming no genotyping errors, molecular genetic information is not affected by environmental effects and therefore has heritability equal to one.[1]
- Molecular genetic information can be available at an early age, in principle at the embryo stage, thereby allowing early selection and reduction of generation intervals.[2]
- Molecular genetic information can be obtained on all selection candidates, which is especially beneficial for sex-limited traits, traits that are expensive or difficult to record, or traits that require slaughter of the animal (carcase traits).[1]
- Molecular genetic information enhances reliability in predicting the mature phenotype of the individual and allows selection for a wide range of traits.[1]
- High-level genome sequence coverage of adapted breeds needs to be obtained before the animals become rare, because there is the possibility of discovering unique or unusual genes affecting adaptation to tropical areas.[2]
- DNA tests for GEBV have not reached commercial application for tropical cattle owing to a lack of predictive accuracy and infrastructure to implement GEBV.[2]

However, there are still limitation in applying the technology in the field in many locations. There is an urgent need to develop programs that will enhance genetic improvement across both large-scale and small-scale beef production.

Learning Outcomes

- Beef consumption represents about 25% of global meat consumption and is expected to increase from 54 to 118 million tonnes by 2050.
- Traits to be targeted in breeding program for climate resilience should be a combination that improve the survivability, fertility, production (growth, milk, meat), product quality or animals that support economically and financially sustainable systems.
- Genomic selection is an attractive alternative to traditional breeding as it will allow young bulls (and heifers) to be selected on their heat tolerance genomic estimated breeding value (GEBV) as well as on other traits.

3.8 Conclusions

Livestock which are adapted to heat stress whilst still maintaining adequate productivity (growth, fertility etc.) is a challenge. Breeding strategies that will lead to rapid genetic change are required to meet the challenge of rapid climate change. The development of genomic breeding values is an important step forward but needs to be considered as a tool alongside traditional breeding and selection strategies. The use of molecular genetics will play a major role in the selection of heat tolerant animals however the inability to collect sufficient performance and pedigree data in many regions will limit the use of tools such as GEBV for some time.

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Behavioural Responses of Domestic Animals for Adapting to Thermal Stress

4

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V. Sejian et al. (eds.), *Climate Change and Livestock Production: Recent Advances and Future Perspectives*, https://doi.org/10.1007/978-981-16-9836-1_4

Abstract

Animals use behaviour to alleviate the feelings of hot and cold, by avoiding situations that arouse those feelings. That is the basis of behavioural thermoregulation. By employing behavioural thermoregulation, domestic animals can manipulate heat exchanges between body surface and surrounding environment, thus reducing demands for evaporative water loss and heat production in order to regulate body temperature. Availability of buffered microclimates and possibility for animals to employ behavioural thermoregulation is of utmost importance for those that have a high surface area to volume ratio. By observing the animal's behaviour, farmers can easily predict how animals are coping with thermal challenges and to get insights about their preferences. This chapter briefly discusses the importance of behavioural thermoregulation under the context of domestic animals. Specifically, (1) the association between body size and relevance of thermoregulatory behaviour; (2) evidences on how shade or shelter-seeking behaviour and body orientation adjustments helps neonates and adult animals to save water and energy; and ultimately (3) to prospect future studies involving behavioural thermoregulation of free-ranging livestock, particularly to better predict how they use some kind of behavioural plasticity to buffer direct and indirect impact of climate change.

Keywords

Animal behaviour · Climate change · Heat stress · Thermoregulation · Thermoneutral zone

Abbreviations

%	Percentage
>	Greater than
°C	Degree Celsius
e.g.	Exempli gratia
g	Gram
h	Hours
i.e.	id est (that is)
LCT	Lower critical temperature
m^{-2}	Square meter
SSH	Source of supplemental heat
TRM	Mean radiant temperature
T_s	Surface temperature
UCT	Upper critical temperature
W m^{-2}	Watt per square metre

4.1 Introduction

The voluntary changes in behaviour are the most powerful mechanism for animals to regulate body temperature, mostly because it requires lower energetic costs and can be sustained longer than autonomic responses (e.g., panting, sweating, or non-shivering thermogenesis) (Bicego et al. 2007). The behavioural thermoregulation can anticipate changes in core body temperature, and therefore decreases the demand for evaporative cooling or heat production mechanisms. This anticipatory control is due to a well designed peripheral-neural network that involves early detection of temperature changes in the skin, integration with the central nervous system through the preoptic area in hypothalamus, thus evoking voluntary behavioural changes in order to conserve body heat or enhance heat dissipation.

By altering the behaviour, domestic animals manipulate heat exchanges between body surface and their surrounding environment, which enhances their capacity to regulate body temperature within a thermal zone of low thermoregulatory effort (Fig. 4.1), namely thermoneutral zone. When an animal is outside of its thermoneutral zone, costs of thermal homeostasis in terms of water use to cope with heat stress or chemical energy use to cope with cold stress increases (Fig. 4.1), and consequently less energy is available for animal production. The body size is one the most important feature that determines the width of the thermoneutral zone, as well as, environmental threshold to employ the thermoregulatory behaviour. For instance, when exposed to high levels of solar radiation, due to a higher

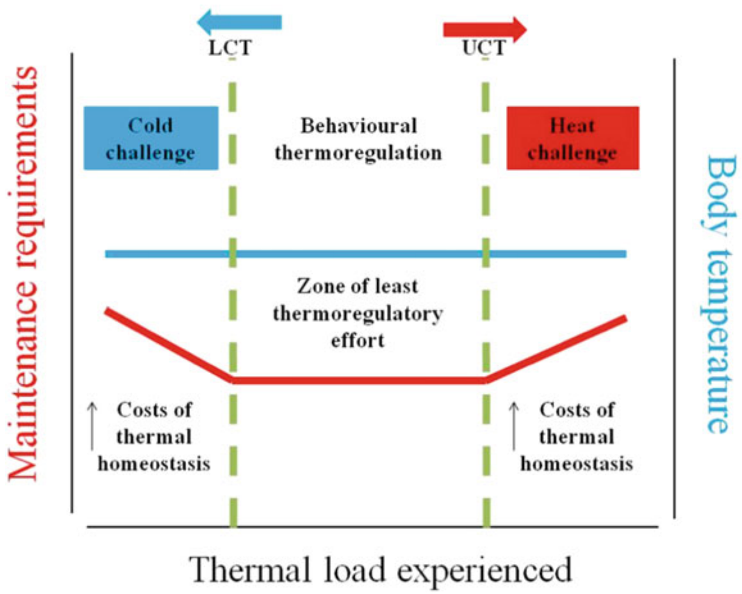

Fig. 4.1 Simple illustration of the thermal load thresholds delimiting zone of least thermoregulatory effort. *LCT* lower critical temperature, *UCT* upper critical temperature

mass-specific rate of radiant heat gain and heat loss, newborn lambs are likely to employ shade-seeking behaviour earlier than their mothers. In some cases, there must be a trade-off between the thermoregulatory behaviour and there will be a strong motivation of newborns to be in close association with mothers, particularly if the shade or shelter resources are placed in large paddocks.

By observing the behaviour of animals, farmers can immediately predict how they are coping with the thermal challenges and to get good insights about their preferences. These insights may help farmers to design more sustainable environmental management strategies in order to alleviate impacts of thermal stress on animal welfare. For instance, dairy cows and sheep kept in open field will prefer to graze at times with solar irradiance levels below 650 W m^{-2} (Oliveira et al. 2014). Sheep will experience lower radiant heat load when using shades of photovoltaic panels than the shade of a shade-cloth structure that blocks 90% of solar radiation, thereby explaining their preference to use shade provided by the photovoltaic panels (Maia et al. 2020). Here in this chapter we briefly discuss about the following aspects involving behavioural thermoregulation under the context of domestic animals: (1) association between body size and relevance of thermoregulatory behaviour; (2) evidences on how shade or shelter-seeking behaviour and body orientation helps neonates and adult animals to save water and energy; and ultimately (3) to prospect future studies involving behavioural thermoregulation of free-ranging livestock, particularly to better understand how they employ some kind of plastic behaviour to buffer direct and indirect impact of climate change.

4.2 Body Size, Heat Exchanges and Importance of Thermoregulatory Behavior

Several studies have been postulated that small body sized animals have advantage over the larger ones to cope with high heat load, by claiming that a larger body surface area to volume ratio makes them efficient heat dissipaters in warm climates. Yes, they are, as rates of heat transfer scales against body mass with exponent −0.55. However, lets rethink about this statement by considering drivers governing heat exchanges between the animals and its surrounding environment, e.g., physical requirements for body heat dissipation is that the surface temperature > operative temperature (i.e., equivalent temperature for heat exchanges by convection and long-wave radiation), while the opposite represents requirements for body heat gain.

Free-ranging animals grazing in arid and semi-arid conditions normally experience operative temperature greater than their body surface temperature during most part of daytime, which favors heat influx from the surrounding environment. Under such circumstances, due to a greater mass-specific dry heat gain, smaller animals would gain environmental heat more rapidly, per kilogram of body mass, than the larger animals. Naturally adapted small body sized goats and sheep, when exposed to levels of solar irradiance above 800 W m^{-2} and operative temperature above to 40 °C, can absorb near to 350 W m^{-2} of radiant heat (Maia et al. 2015). This heat load can represent almost ten times the amount of their heat produced by metabolism. To maintain their thermal equilibrium, goats substantially increase the rate of evaporative water loss, particularly through cutaneous surface. Again, due to a greater mass-specific rate of evaporation, more rapid dehydration is expected for smaller animals than larger ones, particularly if they do not have opportunity to replace fluids or to seek for more buffered microclimate.

While high levels of solar radiation and air temperatures of arid and semi-arid areas result in high rates of heat influx on animals during daytime, direction of heat exchange between the environment and animal is usually reversed at night (Fuller et al. 2021). The low operative temperature of clear night skies can result in substantial amount of radiant and convective heat loss (Mitchell et al. 2018), particularly for small body sized animals due to its high mass-specific radiant heat loss. Small body sized sheep living in semi-arid regions of Brazil will have difficulty in conserving body heat at night, with dry heat loss alone having the potential to double the metabolic heat production (Fonsêca et al. 2019).

We may predict therefore that smaller animals are more susceptible to thermal challenges than the larger ones. Indeed, it is, unless they have the opportunity to find more buffered microclimate. Historically, larger animals were more vulnerable to other past significant changes on earth climate (Mitchell et al. 2018), and that vulnerability of larger animals is probably related to factors such as their inability to easily access more buffered microclimates, and on the other hand, may explain the advantage of small body sized animals.

4.3 The Role of Behavioural Thermoregulation: Precocial Domestic Neonates

Although precocious neonates (e.g., newborn lambs, goats, calves) born with a well-developed thermoregulatory system that help them to maintain body temperature within a narrow range, high rates of neonatal mortality due to thermal stress are still a prevalent problem to animal production around the world. Apart from the risk factors to neonatal mortality such as poor maternal care, birth trauma, and infectious diseases, maintenance of body temperature can be challenging for the survival of newborns, mostly due to their poor insulation, small body size and consequent high body surface area to volume ratio.

Depending on the level of cold exposure after birth, the brown adipose tissue reserves and colostrum intake, which are the main sources of heat production, may not be sufficient to offset the rates of heat loss of newborn lambs to the environment. Rates of heat loss can be decreased if they have opportunity to employ thermoregulatory behaviour, by seeking a more buffered microclimate, e.g., a well designed shelter can reduce heat loss by convection and radiation over the body surface of newborns. Additionally, if supplementary heat is provided by using heat lamps, the employment of thermoregulatory behaviour can also enhance radiant heat gain to newborns. On the other hand, neonates delivered under an equatorial semi-arid condition can also face high levels of radiant heat load over the year, e.g., levels of impinging solar radiation up to 1000 W m^{-2} and mean radiant temperatures up to

Fig. 4.2 Black Santa Ines newborn lamb exposed to direct solar radiation in an equatorial semi-arid region. Thermal image taken by using an infrared thermal camera (Fluke—Model TiX500 9 Hz; range: range: −20 °C and +650 °C; accuracy: ±0.1 °C/1 °C; emissivity: ε = 0.98, Everett, WA, USA) at a distance of 1.0 m from the animal. Meteorological conditions at time which image was taken: T_a: 35 °C; R_H: 35.3%; R_S: 900 W m^2; W_S: 3.7 m s^{-1}. (Photo by J.D.C.S.)

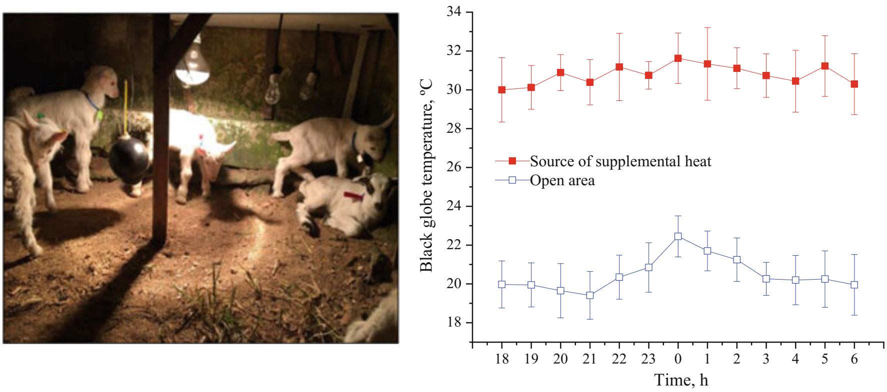

Fig. 4.3 Newborn goats within the source of supplemental heat (SSH) and thermal load experienced (mean ± SEM) by them within the SSH and in open area. (Photo by V.F.C.F.)

60 °C (Fig. 4.2). Under such circumstances, autonomic responses such as panting and sweating may not be sufficient to offset the rates of heat gain of newborns from the environment. The shade-seeking behaviour may therefore play an important role to decrease heat load influx on neonates.

In maternity corral pens, we observed that newborn goats delivered under tropical conditions voluntarily accessed a private source of supplemental heat (SSH) over nocturnal periods, which efficiently allowed them to conserve body heat, and to escape from low levels of radiant temperatures in the open environment. When the newborns were within the SSH (Fig. 4.3), they were benefited with a lower thermal gradient between its surface temperature and the mean radiant temperature (mean T_s = 30 °C vs. mean TRM_{SSH} = 32 °C), than if they were experiencing radiant temperature of the open environment (mean $T_s = 28$ °C vs. mean TRM = 20 °C). Over the daytime, newborn goats also employed shade-seeking behaviour, which benefited them to avoid high levels of impinging solar radiation. Similarly, our recent study with newborn lambs from two hair coat sheep breeds also showed that behavioural thermoregulation as shade-seeking appeared to play an important role in order to defend the body rectal temperature to rise.

4.4 Role of Behavioural Thermoregulation: Shade-Seeking Behaviour and Body Orientation Adjustments for Livestock Grazing in Equatorial Semi-Arid Regions

Grazing cows under tropical conditions may face incoming solar radiation up to 1000 W m^{-2}, which have potential to absorb as much as 640 W m^{-2} of thermal energy. If shade is not available, how a dairy cow would manage this amount of radiant heat gain? By modeling a thermal balance, requirements for evaporative water loss may be close to 900 g h^{-1} to maintain their thermal equilibrium. High levels of solar radiation also impose challenge for sheep naturally adapted to hot conditions. We investigated the impact of solar radiation on the thermal equilibrium of hair-coat sheep raised under natural conditions in an equatorial semi-arid region of Brazil. When exposed to levels of solar radiation near to 850 W m^{-2}, sheep had potential to absorb up to 350 W m^{-2} of thermal energy by long and short wave solar radiation. Under such circumstances, by accounting for heat generated through metabolism (45 W m^{-2}), absorbed from thermal radiation (350 W m^{-2}), minus that eliminated through the respiratory evaporation (20 W m^{-2}), and then solving for the evaporative requirements to maintain their thermal equilibrium, sheep may need to evaporate up to 500 g m^{-2} h^{-1} of sweat through the skin surface to offset the accumulated heat.

Livestock that grazes unprotected against solar radiation will therefore need a large amount of water to sustain high level of evaporative water loss (Mitchell et al. 2018), an increasingly limited natural resource, particularly for livestock species living in arid and semi-arid regions. However, they can behaviorally manipulate their thermal equilibrium by seeking shade (Fig. 4.4), thus reducing sensible heat gain and requirements for evaporative cooling.

The solar radiation is the most important variable for predicting shade use by livestock in the tropical environment (Oliveira et al. 2019). The critical level of solar radiation that motivates dairy cows to stop grazing and seek shade is in the interval between 500 and 700 W m^{-2} (Oliveira et al. 2014). In equatorial semi-arid regions, this level of solar radiation is normally recorded after 08:30 h and prior to 16:00 h, suggesting that cows under pasture based systems should have access to paddocks in the early hours of day, when the solar radiation is well tolerated, and consequently cows can devote more time in grazing. Moreover, farmers may implement nocturnal grazing to complement hours of grazing. Figure 4.5 illustrates how the understanding of shade requirements of domestic animals gives important insights on how to

Fig. 4.4 Shade-seeking behaviour of hair coat sheep during the hottest hours of the day in an equatorial semi-arid region. (Photo by V.F.C.F)

employ environmental management strategies to improve the thermal comfort and welfare of grazing dairy cows.

4.4.1 Body Orientation

When shade is not available, changes in body orientation allow animals to manipulate the radiant heat gain. This subject is relatively well investigated with animals living in high latitudes. For instance, in cold days animals may increase absorption of radiant heat by exposing body long axis perpendicular to the incoming solar radiation. Oppositely, in hot days animals can reduce surface area exposed to solar radiation by orienting long axis parallel to the sun's rays, thus minimizing heat load. However, studies evaluating changes in body orientation of livestock grazing in low latitude regions are scarce. When compared to subtropical and temperate latitudes, the incoming radiation reaches the surfaces of equatorial regions at a highly elevated angle in relation to horizon over the year. At high solar elevation, we expected that the benefits in terms of reducing heat load when animals orient their body axis parallel to incoming radiation may be reduced. Preliminary investigation performed by our team shows that the body axis orientation seems not to be employed by black and white hair coat sheep as thermoregulatory strategy when exposed to solar radiation in an equatorial semi-arid environment. More studies however are needed to better understand the role of adjustments in body orientation on heat balance of livestock living in arid and semi-arid equatorial regions.

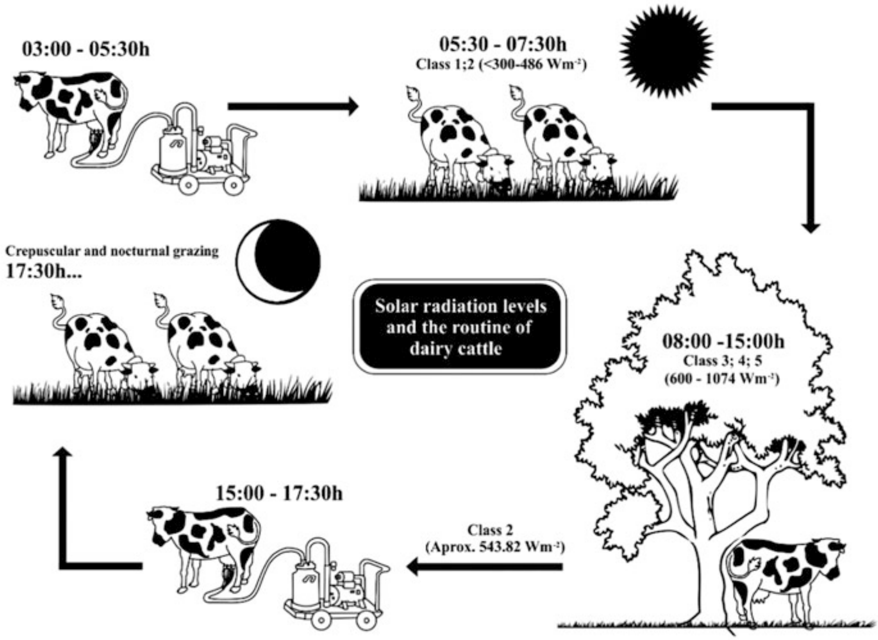

Fig. 4.5 Proposed daily routine adaptation for dairy cows raised in equatorial semi-arid regions, according to the level of solar radiation tolerated by cows (Oliveira et al. 2014). Levels of solar radiation (RS, $W\ m^{-2}$) recorded in the study were divided into five classes: Class 1 ($RS < 300\ W\ m^{-2}$), class 2 ($300\ W\ m^{-2} < RS \leq 500\ W\ m^{-2}$), class 3 ($500\ W\ m^{-2} < RS \leq 700\ W\ m^{-2}$), class 4 ($RS \leq 900\ W\ m^{-2}$), class 5 ($RS > 900\ W\ m^{-2}$)

Learning Outcomes

- Behavioural thermoregulatory mechanism is one of the primary means by which the farm animals cope with thermal stress.
- When compared with large body sized animals, smaller livestock have better opportunities to seek more buffered microclimates to cope with extremes in weather.
- Shade-seeking is one of the important behavioural thermoregulatory mechanisms exhibited by neonates to maintain body temperature during heat stress exposure.
- Understanding the extent of behavioural plasticity exhibited by livestock is considered a most promising strategy to identify climate resilient breeds.

4.5 Future Studies: Behavioural Plasticity of Free-Ranging Livestock

Naturally adapted livestock living in arid and semi-arid regions are facing an increasing heat load, in addition to food and water shortages, as consequences of the direct and indirect impacts of climate change. This is occurring at a faster and more intense rate than the time required for genetic adaptation, leaving only the expression of phenotypic plasticity of small ruminants to adjust to the new environmental conditions. The compound effects of thermal stress, and lack of food and water, may impair the ability of dry land ruminants to sustain the high rates of evaporative water loss and heat production in order to maintain their core body temperature within a narrow range. Recent findings in wildlife have shown that these animals employ a plastic behaviour by changing daily activity, grazing patterns, and microclimate selection to cope with seasonal changes in heat load, food and water availability. Whether this can be confirmed for farm livestock raised exclusively in various arid and semi-arid biotopes around the world, still needs to be investigated. The use of miniaturized animal-implantable devices for logging data recording the movement and microclimate selection over the extended periods of time will therefore elucidate if they use some type of behavioural plasticity in response to heat load, and lack of food and water. Ultimately, understanding the extent of this behavioural plasticity will help us to identify the resilient breeds of livestock, and better predict their potential to adapt to the rapid changes in climate.

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Heat Stress Associated Changes in the Immune System Related Responses in Sheep

5

Mariangela Caroprese, Maria Giovanna Ciliberti, Marzia Albenzio, and Agostino Sevi

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Abstract

Very few studies have dealt with sheep under heat stress, with their physiological mechanisms of adaptation and, above all, with alterations on sheep immunological responses. This chapter will focus on the complex network of mechanisms activated by heat stress as affecting immune responses in sheep. In particular, heat stress will be discussed as a particular typology of stress response, with the description of the changes induced by heat stress on both innate and adaptive immunity, as well as on cellular functioning. Finally, the study of innovative indicators of cell-to-cell communications will be delineated as future perspectives for the fully understanding of the changes in sheep immune responses as induced by heat stress.

Keywords

Cytokines · Heat stress · Immune responses · Sheep · Stress response

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V. Sejian et al. (eds.), *Climate Change and Livestock Production: Recent Advances and Future Perspectives*, https://doi.org/10.1007/978-981-16-9836-1_5

Abbreviations

%	Percentage
°C	Degree Celsius
ATP	Adenosine triphosphate
B cells	B lymphocytes
CD	Cluster of differentiation
cDNA	Complementary deoxyribonucleic acid
CLR	C-type lectin receptor
COX-2	Cyclooygenase-2
DAMP	Danger associated molecular pattern
DC	Dendritic cell
ECM	Extracellular matrix
Fig	Figure
h	Hour
HMGB1	High mobility group box protein 1
HPA	Hypothalamic-pituitary-adrenal
HS	Heat stress
HSP	Heat shock protein
IFN	Interferon
IL	Interleukin
iNOS	Inducible nitric oxide synthase
miRNA	Micro RNA
mL	Millilitre
mRNA	messenger ribonucleic acid
NF-κB	Nuclear factor kappa B
ng	Nanogram
NK	Natural killer
NLR	Nucleotide-binding domain and leucine-rich repeat containing receptor
NO	Nitric oxide
PAMP	Pathogen-associated molecular pattern
PBMC	Peripheral blood mononuclear cell
PGE_2	Prostaglandin E_2
PHA	Phytohemagglutinin
PRR	Pattern recognition receptor
RIG-I	Retinoic-acid inducible gene I
RLR	Retinoic-acid inducible gene I like receptor
ROS	Reactive oxygen species
T cells	T Lymphocytes
T_3	Triiodothyronine
T_4	Thyroxine
TCR	T cell express cell surface receptor
Th cells	T helper cells
TLR	Toll-like receptor

TNF	Tumour necrosis factor
α	Alpha
β	Beta
γ	Gamma
δ	Delta

5.1 Introduction

The alterations of immune responses described during and after heat stress exposure of livestock are only one typology of the complex system of responses activated to restore body homeostasis after a stressor. The immune responses are part of the ancestral and conserved wider response system named stress response, which has the aim to preserve life and to help coping with aversive situations. Based on this, defense and adaptation mechanisms activated by livestock define their success to survive in hostile environments. Heat stress response is a well characterized type of stress responses which affects even sheep that are considered as one of the most resistant species to climatic extremes, especially to high ambient temperatures. The compensatory physiological, endocrine, and behavioral mechanisms activated by sheep with the intention to survive, and to cope with high environmental conditions are numerous. After the stimulation of temperature receptors into the hypothalamus, sheep increase their respiration rate, rectal temperature, and exhibit an alteration of protein and energy metabolism, of mineral balance, enzymatic reactions, and hormonal secretions (Sevi and Caroprese 2012). The first responses including increased water intake, sweating and respiration rates, are considered homeostatic mechanisms (Horowitz 2002). If the exposure to thermal extremes is prolonged, heat acclimation is achieved by an acclimatory homeostasis (Horowitz 2002). It has been proposed the definition of acclimation as the altered expression of pre-existing mechanisms driven by the endocrine system in order to sustain animal welfare and health regardless of environmental challenges. Based on this, acclimation should be considered a homeorhetic mechanism (Collier et al. 2005) because it alters the set-points of homeostatic-related systems such as the carbohydrate metabolism rather than acclimatory homeostasis (Horowitz 2002). However, the control of heat stress response is characterized by a dynamic orchestration of physiological processes that simultaneously influences multiple tissues and systems, which results in an overall coordinated response, and it is mediated by altered responses to homeostatic signals. The changes in immune responses observed in sheep under heat stress are therefore the results of alterations in a number of tissues and systems to help the animals to cope with heat load.

5.2 Sheep Endocrine Responses to Heat Stress

Dairy animals under heat stress often display a depression of immune system (Caroprese et al. 2012), which increases their susceptibility to diseases and sometimes lead them to death. Such a depression stems from alterations in particular endocrine secretions, namely those from the pituitary (prolactin), adrenal (cortisol) and thyroid glands (Triiodothyronine-T3 and Thyroxine-T4). Prolactin is a multifunctional hormone involved in a variety of biological functions related mainly to sustain galactopoiesis and lactogenesis; beside these actions prolactin exerts a thermoregulatory role in small ruminants. In sheep, a significant association between heat stress and increased prolactin mRNA expression in sweat glands was found, thus leading to the hypothesis that prolactin can contribute to increase sweat gland activity in order to reduce heat load (Choy et al. 1997). Based on previous statements the increase in prolactin secretions during heat stress seems to improve the ability of small ruminants to adapt to heat stress. The increase of plasma cortisol secretions in sheep under heat stress is caused by the activation of hypothalamic-pituitary-adrenal (HPA) axis with the aim of rising the circulating glucose. The cortisol secretion during heat stress has the role of stimulating gluconeogenesis by the conversion of non-carbohydrate molecules into glycogen in the liver to increase blood glucose levels. The increase in circulating glucose during heat stress is essential for allowing ruminants to cope with heat stress; therefore, similarly insulin has a crucial role in post absorptive nutrient partitioning subsequent to heat stress. In dairy cows under heat stress, basal insulin concentrations gradually increase, stimulating glucose uptake via glucose transporter type 4 in muscles and adipose tissue, and being responsible for the heat-induced hypoglycemia frequently reported. Insulin is also a potent regulator of lipid metabolism as a potent antilipolytic hormone, and this may explain why heat-stressed cows do not mobilize adipose tissue triglycerides (Baumgard and Rhoads Jr 2013). On the contrary, recent studies found that both in dairy ewes (Mehaba et al. 2021), and in dairy goats (Salama et al. 2014) no increase in the level of insulin during heat stress is observed. Nevertheless, some metabolic adaptations allowed dairy ewes to spare glucose and to avoid reductions in milk yield (Mehaba et al. 2021). Ewes under heat stress have higher blood glucose levels after glucose tolerance test than ewes under thermoneutral conditions implying the activation of both insulin- and non-insulin–mediated pathways. Further, adipose tissue of heat stressed ewes became more resistant to the lipolytic signals. Finally, in sheep under heat stress beside the activation of the pituitary gland and HPA axis, the thyroid gland shows a reduced functioning, with a consequent decrease of the secretion of thyroid hormones, triiodothyronine (T3), and thyroxine (T4) in the attempt to thermoregulate. Such a complex and orchestrated alterations observed during heat stress in ruminants are associated with and are the cause of evident changes in immune responses and diseases susceptibility, and relevant events occurring at cellular level.

5.3 Sheep Immune Responses Functioning

An understanding of immune system structure and function in sheep is of great importance to provide information in relation to the changes induced by stressful events on immune functioning. Innate compartment of immune system consists of physical barriers, soluble factors, and cells among which the most important are macrophages and neutrophils; cells play an important role in innate immunity by producing nitric oxide (NO), synthesized by inducible nitric oxide synthase (iNOS), and prostaglandin E_2 (PGE_2) synthesized by cyclooxygenase (COX-2), that are some of the inflammatory mediators. The cells of the innate immune systems, such as dendritic cells (DCs), macrophages, neutrophils, natural killer (NK) cells and γδ T lymphocytes, can recognize common structures shared by the vast majority of microorganisms called pathogen-associated molecular patterns (PAMPs). PAMPs are recognized through binding to receptors of the innate immune system named the pattern recognition receptors (PRRs). Several classes of PRRs have been described, including the Toll-like receptors (TLRs), the retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs), the nucleotide-binding domain and leucine-rich repeat containing receptors (NLRs), the C-type lectin receptors (CLRs), and others. Each TLR is an alert signal for the immune system able to activate the induction of proinflammatory mediators as a result of the activation of innate immune system, and to initiate the adaptive immune responses. Full-length cDNA sequences for all ten TLR genes of the domestic sheep have been found, showing that sheep TLR sequences share high similarity to cattle, pig, human and mouse genes. The cells of adaptive immunity are B lymphocytes (B-cells) and T lymphocytes (T-cells). B-cells are responsible for the secretion of specific antibodies, which enable phagocytes to recognize microorganisms and destroy them by binding to antigenic sites on both extracellular microorganisms and toxins. T-cells are the effector of cell-mediated immune responses of adaptive immunity, are able to recognize and fight against intracellular viruses and microorganisms. The T-cells express cell surface receptors (TCRs) consisting of α and β chains in sheep. Among the α/β T-cells there two different sub-lineages; the first sub-lineage expresses co-receptor molecule CD4 (cluster of differentiation), consists of cells known as T-helper cells (Th cells), and can neutralize intracellular microorganisms, fungi, and parasites by interacting with other immune cells, and activating an antigen-specific response; the second sub-lineage expresses co-receptor molecules T-CD8, consists of cells known as T cytotoxic cells, and can recognize and neutralize viral infected cells. Th-cells are further divided into two different subsets: T helper cells type 1 (Th1 cells) and T helper cells type 2 (Th2 cells). Another group of T-cells is characterized by γ and δ chains and expresses functions of both innate and adaptive immune cells. Sheep display a large number of γ/δ T-cells in the blood. In young cattle and sheep, up to 60% of PBMC can be γδ T cells, supporting the notion that they are important for immunity early in life. TCR genes of both γ and δ chains are well characterized in cattle, sheep, and in goats. The complex communications between the different cells of the immune system, as well as the interactions between the immune system and the stress responses has phylogenetically evolved in a diversified and complicated system of cytokines. Cytokines are also considered responsible for the complex cross-talk between brain and immune system. Both cell-mediated and humoral responses, as well as innate immunity, are activated and regulated by proinflammatory cytokines, such as IL-6 and IL-1β. Interleukin-6 stimulates hepatocytes, B cells, and cytotoxic T cells; IL-1β promotes leukocyte accumulation in inflamed sites by inducing adhesion receptors on vascular endothelium. The regulation of transcription of DNA, cytokine production, and cell survival is mainly attributed to nuclear factor kappa B (NF-κB), a protein complex that controls intracellular signal transduction pathways. Besides the pivotal role in controlling the host response to stress and infection, cytokines can also have a negative role in provoking tissues damages when produced in uncontrolled way. In order to avoid this event, the cytokine secretion is tightly regulated.

5.4 Sheep Immune Responses to Heat Stress

The effect of stressors on the immune system is either a suppressive or a stimulatory effect according to a number of factors such as duration and intensity of stressors, basal animal health status, and also the type of indicators of immune response measured (Ciliberti et al. 2017). When the animals undergo a stressor, a number of endogenous danger signals, molecules that are actively secreted by cells in danger, known as danger-associated molecular patterns or DAMPs, are recognized by the PRR of the innate immune system. Recognized DAMPs are nucleic acids, ATP, uric acid, heat-shock proteins (HSP), mitochondrial danger signals, high mobility group box protein 1 (HMGB1), degradation products of the extracellular matrix (ECM). The cytokines, such as IL-1β, TNF-α, and the interferons, are considered secondary endogenous danger signals which, produced by activated immune cells upon PRR triggering, induce the stimulation of innate and adaptive immunity. The pro-inflammatory cytokines activate and sustain the signaling pathway and the effector functions of the classic PRRs, such as the activation of the transcription factor NFkB, and the induction of proinflammatory processes, respectively. This type of reaction of the immune system to non-infectious agents is called "sterile inflammation," in which the innate immune system is activated by signals that do not come from microorganisms but can stem from stressors of different nature. The biology of stress, however, is extremely complex and even more complicated is the number and type of the interactions between the neuroendocrine and immune system; the immune system is affected by the stress response and, in turn, it affects the stress response. In particular, heat stress response is a particular type of stress response during which the described alterations of endocrine secretions have a typical suppressive effect on immune responses through the alterations of cytokine secretions. Conversely, the complex network of cytokines secreted exerts a crucial regulatory role rules on the stress responses. Prolactin during heat stress can reduce immune responses in PRL-signaling pathway genes associated with differential cytokine secretion; particularly, prolactin reduces expression of TNF-α, and lymphocyte proliferation (Do Amaral et al. 2010). The cortisol secretion affects the

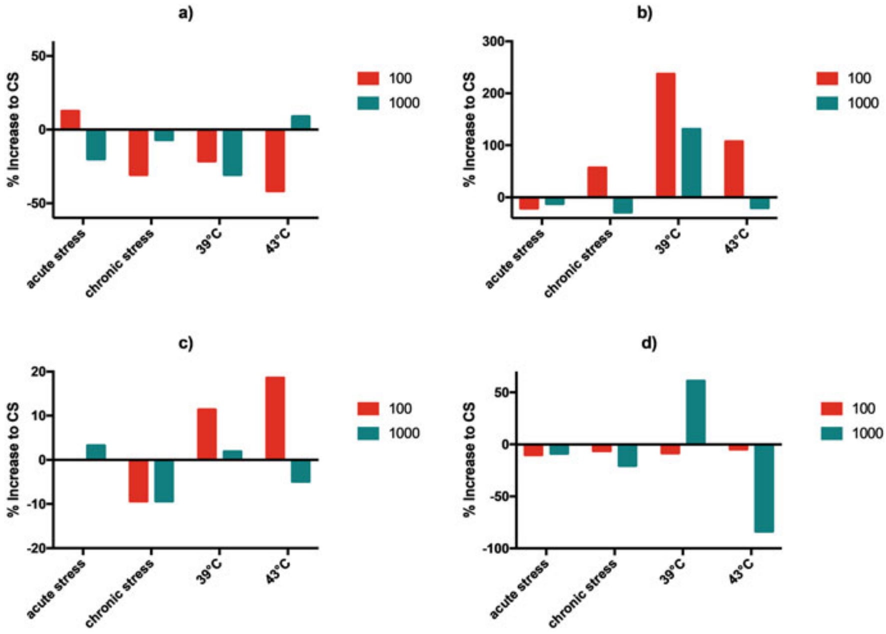

Fig. 5.1 Percentage increases calculated to stimulated PBMC (CS) of (**a**) cell proliferation, (**b**) IL-6, (**c**) IL-1beta, and (**d**) IL-10 levels in stimulated PBMC treated with 100 ng/mL of cortisol (100) and stimulated PBMC treated with 1000 ng/mL of cortisol (1000) after acute stress (24 h), chronic stress (96 h), normothermia (39 °C) and hyperthermia (43 °C) in vitro challenge. Stimulated PBMC were treated with Phytohemagglutinin (PHA)

expression of a number of genes related to innate immune responses by a strong and fast activity of down-regulation; especially, it can inhibit cytokine induced transcription factors (NF-κB). However, it has been suggested that the reduction in cytokine production following the down-regulation of NF-κB can lead to a reduction of the glucocorticoid receptor, thus limiting the cell sensitivity to glucocorticoids and allowing again the translation of NFkB1 (Sgorlon et al. 2012). The cross-talk between glucocorticoids and cytokines is bidirectional; via negative feedback, pro-inflammatory cytokines, such as IL-1, IL-6 and TNF-α, can increase the HPA axis sensitivity to later stress challenges. However, the precise mechanisms of glucocorticoids action on cytokine production and cell proliferation during stress, and in particular during heat stress, are not fully explained. A number of factors can influence the possible effects of cortisol on immune cell proliferation and cytokine production, such as the magnitude of the stressor, and its duration (Ciliberti et al. 2017). Physiological levels of cortisol after a stressor can exert both immunostimulatory and immunosuppressive effects in vitro depending on the duration of stress (Fig. 5.1). In contrast, levels of cortisol higher than physiological levels exert immunosuppressive effects after both an acute stress and a chronic stressor (Ciliberti et al. 2017). During heat stress, physiological levels of cortisol after a stressor decrease by 50% cell proliferation of immune cells when in presence of an antigen (Caroprese et al. 2018). In addition, cortisol is able to regulate cytokine production by sustaining pro-inflammatory cytokines levels even if during heat stress cortisol drives the reduction of IL6 levels, as reported in Fig. 5.1 (Caroprese et al. 2018). The described effects of cortisol on immune cell proliferation and cytokine production are particularly apparent in the presence of an immune challenge highlighting that hyperthermia has a relevant inhibitory effect on immune responses with a crucial reduction of immune competence. Such results from in vitro study are confirmed by in vivo studies; in sheep under heat stress adaptive responses are impaired as suggested by a decrease in cellular immune response after the intradermal injection of mitogens (Sevi and Caroprese 2012; Caroprese et al. 2012).

The suppression of cell-mediated immunity during hyperthermia has been attributed to a down regulation of Th1 cytokines in favour of the secretion of Th2 cytokines. Environmental conditions can influence the levels of Th1 (IFN-γ, and IL-12), or Th2 cytokines (IL-10, IL-4, IL-13) secretion. In sheep, a reduction of both IL-4 and IL-13 was measured during heat stress, also associated to a reduction of cell-mediated immune response (Caroprese et al. 2014). It is stated that when the Th2 responses diminish, mainly because of the reduction of IL-4 and IL-13 production, concomitantly also the Th1 response appear inappropriate. Beside the reduction of adaptive responses, heat stress can suppress also innate immune responses, as demonstrated by the reduced reactivity in terms of phagocytosis and oxidative burst measured as reactive oxygen species (ROS) production found in polymorphonuclear cells (Lecchi et al. 2016). During the hot humid season associated with high cortisol levels, milk neutrophils exhibit a reduction in phagocytic activity. The alterations in adaptive and innate immune responses are, however, the result of a coordinated system which modulates gene expression at cellular level in order to minimizing the negative effects of heat stress on cellular functions and across a variety of cells and tissues through the changes in endocrine secretions (Collier et al. 2008). The principal response to heat stress at cellular level is the heat shock cellular response, with the induction of HSPs, which develops rapidly, and shows the induced alterations in 24 h. The expression of HSPs has a central role in cytoprotection, and is the cellular response to the increase of oxidative stress, and of alterations in protein synthesis, structure and functions. The cellular response to stress is also modulated by the alterations of hormonal secretions induced by heat stress. Prolactin, insulin, and catecholamines can contribute to the enhancement of HSP expression and proteins activity (Collier et al. 2008). Glucocorticoids, when enter the cytoplasm, cause the release of preformed cytoplasmic HSP70 and 90, which are bound to the glucocorticoid receptor. The hormone-receptor complex then moves into the nucleus and enhances expression of HSP genes. The preformed HSP70 and 90 represent an instant pool of HSP to protect against protein denaturation and the first line of defense against HS because the proteins are already in the cytoplasm and do not require synthesis (Collier et al. 2008). Prolactin also modifies HSP gene expression although the mechanism is not well known, but it seems to prolactin also upregulate HSP 70 gene expression unknown (Collier et al. 2008). A crosstalk between the endocrine secretions, HSPs, and the immune system is established with HSPs playing both a stimulatory and regulatory role. HSPs are considered activators of the innate immune system capable of inducing the production of pro-inflammatory cytokines but also to support immune homeostasis and to have a dampening effect on immune activation. Intracellular HSPs can activate Treg cells and inhibit immunity and inflammation. Extracellular HSP70 can exert an anti-inflammatory response on innate immune responses by binding to cell surface receptors and inducing IL-10 production.

Learning Outcomes

- Heat stress response causes alterations in endocrine secretions which have a typical suppressive effect on immune responses through the alterations of cytokine secretions.
- A crosstalk between the endocrine secretions, HSPs, and the immune system is established during heat stress with HSPs playing both a stimulatory and regulatory role.
- Differed types of miRNA can regulate cellular responses and homeostasis depending on the types of stressors, suggesting a significant role of miRNA in thermal stress responses in animals.

5.5 Future Perspective

Heat stress affects immune responses of sheep by reducing the animals' ability to promptly cope with immunological challenges, thus being the main cause of increased diseases susceptibility and even death during hot season. Stress responses in general, and particularly heat stress responses, are able to perturb multiple molecular pathways in the organism. At the same time, the molecular responses try to restore functional and structural homeostasis in stressed cells and tissues. In the recent years, micro molecules of small non-coding RNA (miRNAs) have been found to control gene expression at the post-transcriptional level, being considered as novel regulators in the mammalian stress response. It seems that differed types of miRNA can regulate cellular responses and homeostasis according to different types of stressors, suggesting a significant role of miRNA in thermal stress responses in animals. Recently, it appears that during heat stress the role of several kinds of miRNA can play regulatory roles by targeting genes that are related to the stress responses in livestock, therefore controlling the synthesis of heat shock proteins (HSPs), and the expression of genes responsible for the regulation of immune responses, apoptosis, and inflammation-related molecules. The study of miRNA profiling in sheep under heat stress in the next future could contribute to elucidate the complex network of interactions at cellular level responsible for the fully understanding of immune response to heat stress.

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6 Climate Change and Livestock Production: Significance of Studying Multiple Stressors Impact in Cattle in the Changing Climate Scenario

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Abstract

Climate change directly impacts food production system stability, domestic or through the global food market. This threat necessitates the identification of various measures to sustain agricultural production to meet the growing population's demand. Among agricultural sectors, livestock has the potential to fortify resilience to climate change, as it tends to be more resilient than crop-based systems. Therefore, in light of the changing climate, it may benefit from investing

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V. Sejian et al. (eds.), *Climate Change and Livestock Production: Recent Advances and Future Perspectives*, https://doi.org/10.1007/978-981-16-9836-1_6

in the cattle sector to increase its production capacity for the future. Climate change does not contribute only to the usual suspect, heat stress rather it contributes to multiple environmental stresses. Unfortunately, predominant research efforts are oriented towards tackling heat stress alone may not provide rich dividends in the future as there are several other associated stresses emerge in the ever changing climate. In tropical countries, especially where the predominantly extensive rearing system is being practised, it is vital to quantify multiple stressors' cumulative impact on animals rather than just concentrating on heat stress. Milk and meat production will play a huge role in feeding the growing human population of 9.6 billion by 2050. Therefore sustaining the milk and meat production from the cattle forms a significant component for future policies to ensure food security especially in the developing part of the world. This warrens more research, particularly in quantifying the multiple environmental stressors on dairy and beef cattle production, which could be the way forward approach to revisit the amelioration strategies that could play a significant role in sustainable livestock production in the changing climate.

Keywords

Climate change · Cattle · Food security · Livestock · Multiple stress

Abbreviations

%	Percentage
°C	Degree Celsius
ACOT7	Acyl-CoA thioesterase 7
ATF4	Activating trasncription factor 4
CDC42	Cell division cycle 42
CFTR	Cystic fibrosis transmembrane conductance regulator
CO_2	Carbon dioxide
CPT1C	Carnitine palmitoyltransferase1C
CRYAB	Crystallin ALPHA B
DM	Dry matter
DMI	Dry matter intake
DYRK1A	Dual specificity tyrosine phosphorylation regulated kinase 1A
Fig	Figure
GH	Growth hormone
GPX	Glutathione peroxidases
GSTM	Glutathione S-transferase mu
HPA	Hypothalamic-pituitary-adrenal
HSE	Heat shock elements
HSF	Heat shock factors
HSP	Heat shock proteins
ID2	Inhibitor of DNA binding 2
IGF-1	Insulin-like growth factor-1

IGFBP	Insulin-like growth factor binding proteins
IPCC	Intergovernmental Panel on Climate Change
ITPR2	Inositol 1,4,5-Trisphosphate receptor type 2
MANF	Mesencephalic astrocyte derived neurotrophic factor
min	Minute
MLPH	Melanophilin
NDF	Neutral detergent fibre
NEFA	Non-esterified fatty acids
pH	Potential of hydrogen
PI3-kinase	Phosphoinositide 3-kinases
PIK3C2G	Phosphatidylinositol-4-phosphate 3-kinase catalytic subunit type 2 gamma
PRKAB2	5′-AMP-activated protein kinase subunit β-2
RAC1	Ras-related C3 botulinum toxin substrate 1
RAP	Receptor associated protein
SAM	Sympathetic adrenal medullary
SLC23A1	Solute carrier 23 member 1
SOD	Superoxide dismutase
STAT-5	Signal tranducer and activator of transcription 5
T_3	Triiodothyronine
T_4	Thyroxine
THI	Temperature humidity index
TNZ	Thermo neutral zone
US	United States
ZNF516	Zinc finger protein 516

6.1 Introduction

The human population is expected to stretch from 7.2 to 9.6 billion by 2050; accordingly, agricultural systems worldwide will have to make available extra food to feed this increasing population. Livestock and crop systems are the central pillars of an agricultural system. If we peek into livestock systems alone, 17% of global kilocalorie consumption and 33% of global protein consumption is through livestock products, and it is considered a vital agricultural commodity for global food security. Growing demand for livestock products, particularly its rapid growth in developing countries, have stated it as a “livestock revolution”. Despite this, the current expansion is insufficient to meet the growing demand for livestock products, especially in the wake of the diminishing water and land resources caused by the entrenched rogue known as *climate change*.

Several stresses other than heat stress constrain livestock production in the current climate change scenario. Animals, when exposed to single stress at a time, can counter efficiently without any fluctuations in the productive capabilities due to stored body reserves. However, if animals are exposed to multiple stressors, the cumulative effects of various stressors might harm the exposed animals. In this case, the animal's body reserves are not plentiful enough to efficiently counter multiple environmental stressors as the overall biological cost is inconsequential to the animal's welfare. Resulting in hampered adaptive capabilities, and the animals struggle to maintain normal homeothermy. With the present climate changing scenerio, the frequency and intensity of livestock exposure to abiotic and biotic stressors increased. Abiotic stressors such as heat and nutritional stress significantly impact livestock productivity (Sejian et al. 2015). According to the IPCC Fifth Assessment Report, the anticipated intensification in global average surface temperature by 2100 will be between 0.3 and 4.8 °C (IPCC 2013). The United States Livestock industry witnessed an annual economic loss of 1.63–2.36 billion US dollars because of heat stress, of which the dairy industry bears 50%. Latent impacts on livestock include fluctuations in production, quality and quantity of feed crop and forage, water accessibility, animal growth, and milk production. Increased Elevated temperature and dry conditions lead to discrepancies in water-soluble carbohydrates and nitrogen levels, affecting the quality of forage and feed crops. On the other hand, the increased temperature might increase lignin and cell wall components in plants, which reduces digestibility and degradation rate, leading to a reduction in nutrient availability for livestock. Hence, it is not only the heat stress that needs to be counteracted, but nutrition stress is also of great concern.

Livestock has the potential to reinforce climate change resilience, as it is generally more resilient than crop-based systems. A better understanding of how the industry is affected is required to augment livestock resilience to climate change. Multiple stresses affecting livestock proficiency are common due to climate change in the tropical environment. Most research focuses on a single stress response since stress responses are multidimensional and comprehensive; balanced multifactorial experiments are practically difficult to manage, evaluate, and interpret. Reports assessing the effects of multiple stresses in cattle are limited. Dietary nutrients wield their roles through numerous nutrient-metabolic and cell signalling pathways; our current information is still not deep enough to untie the complexities of dietary nutrients and animal genome expression. Research efforts are required to study physiological and genotypic traits responsible for growth, improve production ability, and identify different biological markers used in future breeding programs.

6.2 Concept of Multiple Stresses

Stress continuously moulds evolution on earth, and exposure to stress is the organism's daily fate. However, after years of research, understanding the concept of stress remains a mystery to humankind. Currently, there is an increasing appreciation within livestock stakeholders and industry that stress on animals is real, and then it must be addressed. With significance for animal health and well-being, reducing animal stress through better animal management measures and selective breeding should be our topmost priority. Nevertheless, efficient stress management

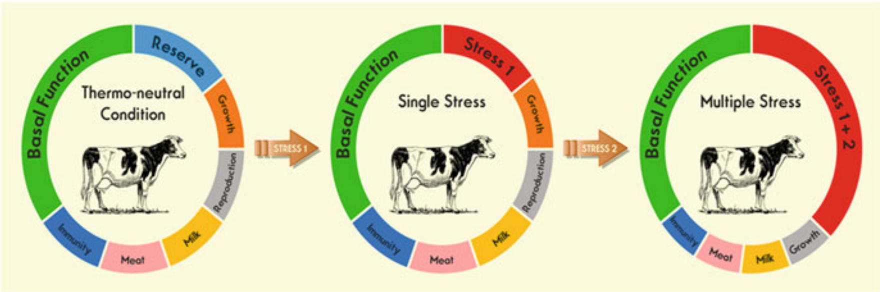

Fig. 6.1 Pictorial representation of summation effect of multiple stresses on productive functions in cattle. (Source: Modified from Sejian et al. (2012))

depends on identifying and computing the effects of several stressors and determining if the individual or combined stressors have different biological effects.

Animals have evolved mechanisms to manage short-term stressors. During the short-term exposure, the biological cost is minimal because adequate reserves of biological reserves exist to cope with the stressor and meet the impact of the stress without any disturbances on biological functions. If the animal gets challenged by multiple stressors, there will be insufficient biological reserves to satisfy the biological cost of the stress response; to counteract this, resources will be channelled from other biological functions. As shown in Fig. 6.1, when resources are sidetracked from productive functions, it leads to impairment of biological functions. For example, when multiple stresses deplete body reserves, metabolism shifts away from growth, the young animal no longer blooms, and growth is restricted. When energy is shifted from reproduction and its process, reproductive success is reduced. This metabolic maintenance behaviour of an animal's body rather than production will last until the animal restocks its resources/reserves sufficiently to re-establish normal functions.

Looking at the different climate change predictions, we can envision a future of immense struggle to adjust and adapt to new environmental challenges both by humans and livestock. Therefore, to secure food security, recognising animals with superior genetic traits that are economically beneficial and identifying the biomarkers to find a solution to animal productivity to climate change should be of utmost preference to policymakers and researchers, especially when animals get exposed to multiple stressors.

6.3 Effect of Multiple Stressors on Cattle

Livestock must maintain a steady body temperature to optimise production efficiency and maintain physiological limits within a particular environmental temperature range, known as the "Thermo neutral zone" (TNZ). Heat stress in cattle is the primary consequence of rising global temperature. Heat stress is manifested when there is an increase in the core body temperature of animals and the inability to dissipate the body heat temperature due to high ambient temperature combined with high humidity and slow air movement. The effects on livestock will vary by region, animal species, and production type, whether positive or negative. Changes in rainfall patterns, upsurge in temperatures, more regularity of extreme weather events, increased heatwave situations, and reduced water availability affects livestock productivity and hampers production systems globally, both directly and indirectly.

6.4 Physiological Changes

Livestock reared under tropical climates are subjected to various challenges. Along with heat stress, multiple stresses have a negative impact on livestock adaptation and productivity. Whenever animals are exposed to stress primary sign of identification by livestock holders is through observing changes in physiological parameters like respiration rate, rectal temperature, feed intake, water intake and heart/pulse rate. Multiple stresses negatively affect the dry matter intake (DMI), leading to the decreased growth rate in cattle. For example, heifers reared in heat stress conditions (38 °C) displayed reduced feed intake; further average daily gain was reduced compared to the calves in ambient temperature (17 °C). Speaking on a percentage basis, a reduction around 9% of dry matter intake and 22% average daily gain of prepubertal Holstein heifers maintained at 33 °C, while evaporative water loss, such as sweating, was higher in those heifers, which in turn caused a 23% increase in water intake compared to heifers maintained at 28 °C. Changes in fat-storing mechanisms (including reduced protein turnover and fat thickness) have also been noted in prepubertal heifers reared under Heat Stress conditions (33 °C). However, reports suggest that if animals are maintained under proper nutrition balance, the downfall of body weight due to heat stress can be avoided. Modifying the feeding time and providing proper shading can be a good strategy to achieve the above statement. If dissipation of metabolic heat production during digestion can be achieved during cooler parts of the day by feeding the animal around mid-afternoon, bodyweight in cattle can be maintained even during heat stress. Heat stress associated with economic losses is majorly from three factors: declined performance, increased mortality, and decreased reproduction. Reduced growth performance and production efficiency could be partially due to sacrificing a fraction of growth energy for cows heat regulation.

During thermal stress, numerous physiological modifications occur in dairy cows to reduce the external heat load or metabolic heat load production. Neurons act as temperature-sensitive sensors, which send information to the hypothalamus in response to numerous physiological and behavioural responses like fluctuations in blood flow, endocrine responses, acid-base chemistry, and physiological traits like increased respiration rate, pulse rate and rectal temperature. Respiration rate is directly proportional to Temperature Humidity Index in lactating dairy cows. If the respiration rate is more than 60 breaths/min is considered an indicator of heat stress in cattle. Various studies reported an increase in respiration and panting rate in cows exposed to heat stress. Increased respiration rate certifies that heat load on animals beyond the threshold is dissipated through boosted evaporative heat loss, leading to changes in blood acid-base chemistry. Enhanced respiration rate and panting result in more exhalation of CO_2 via pulmonary ventilation. Lower blood carbonic acid concentration and an imbalance in carbonic acid to bicarbonate ratio can lead to respiratory alkalosis in lactating cows. Therefore, the need to counteract a higher blood pH through increased urinary bicarbonate excretion and a loss of blood carbonic acid become the utmost priority during heat stress. Another mechanism through which dairy cattle counteract the heat load is through the thermoregulatory centre in the hypothalamus, which triggers thermolysis through evaporation, resulting in increased respiration rate in cattle.

Respiration rate and rectal temperature run parallelly in animals. Rectal temperature is an efficient indicator to assess stress in animals. Measuring rectal temperature is generally considered one of the best methods to assess the core body temperature, and any changes in rectal temperature reflect similar changes in core body temperature. The average rectal temperature of dairy cattle is around 38.5 °C and a threshold range of 39.4–39.7 °C. When cattle get exposed to heat stress and even combined stresses, the rectal temperature increases due to the reason, once the threshold temperature crosses, dairy cows initiate to store heat; eventually, rectal temperature escalates, heat loss through cutaneous evaporative upsurges and variation (in core body temperature) between cows is much significant than cows exposed to below 35 °C. This might be due to differences in sweat glands' number (and activity) and hair coat characteristics. Even heart rate increases gradually when cattle are subjected to heat stress. Increased heart rate helps control blood pressure due to elevated vasodilatation and increased blood flow due to heat stress. Peripheral tissues get the blood flow redistributed, ensuring that body heat is transferred from core body organs to the body's surface. There is a possibility that heart rate changes may serve as a cows method of temperature control to keep their heat balance.

Conversely, during nutrition stress, the thermoregulatory mechanism works differently. Due to limited feed intake, a drop in body temperature, respiration rate and pulse rate can be witnessed due to less metabolic rate, as Vant Hoff's law describes. Various reports suggest that during hot weather, cows fed with low fibre feeds (NDF, 30% of Dry matter) showed lower body temperature and respiratory rates compared to animals fed with high fibre feeds (NDF, 42% of DM). This confirms that diet intake has an applicable effect on metabolic heat production. Physiological traits like respiration rate, rectal temperature and heart rate increase during initial exposure then drops progressively with the days; this clearly explains the duration of heat stress and acclimation capability of cattle affects the intensity of the response. Once the animals get acclimatised to stimuli, it reduces the endogenous heat production and helps heat loss. This adaptive mechanism can be observed in zebu cattle. Genetic adaptation allows zebu cattle to have lesser rectal temperature than Bos taurus when subjected to comparable heat stress situations. If animal exposure is prolonged (and not lethal), by the definition of acclimation, changes in endocrine status lead to gradual homeostasis. However, reaching that equilibrium varies according to breed, age and sex; for example, it ranges from 9 to 14 days for Angus and Polled Hereford cattle to get acclimated to heat stress (Bernabucci et al. 2010).

6.5 Metabolic Changes

Any changes in the environment are acclimatised by differences in homeostatic responses, including altered endocrine status. In addition to temperature regulation, hormones are also implicated in the acclimatory reaction to environmental stresses. Thyroxine (T_4) and Triiodothyronine (T_3) hormones play a significant role in acclimation. Besides producing hormones, the thyroid gland has a significant role in maintaining the metabolic rate and homeostasis of protein and energy metabolism. Any changes in the concentration of thyroid hormones mirror the animal body's metabolic and nutrient status, and it positively correlates with growth gain. Studies suggest a decrease in the concentration of T_4 and T_3 in Holstein cows exposed to heat stress. Likewise, a reduction in T_3 concentration around 18.3, 16.0, 22.0 and 14.4% in cattle species like Alentejana, Frisian, Limousine and Mertolenga and T_4 concentrations around 15.3, 15.0, 23.8 and 21.7% in the same cattle species mentioned above, when exposed to heat stress were reported. This shows an evident decrease in T_3 and T_4 in the blood, which indicates that the pituitary thyroid axis is depressed during heat stress. Another face of this decrease in thyroid hormones might be due to increased glucocorticoid hormone concentration. Reports suggest an interaction between the thyroid gland and adrenaline, noradrenaline released in response to temperature changes in the environment (Johnson et al. 1988). As mentioned previously, feed intake and thyroid hormones status go side by side. When steers are subjected to a restricted diet, T_4 concentration was reduced significantly after 56 days, and T_3 reduced around 83 days. The data infers that feed restriction in steers regulates both thyroidal secretion and extrathyroidal T_3 production and even indicates T_4 in circulation is more closely linked with energy consumption than T_3. T_3 is the metabolic active thyroid hormone, whereas T_4 is the thyroid gland's main product and the most abundant iodothyronine in blood circulation. Hence, maintaining the concentrations of T_3, especially during feed restriction, could be metabolically necessary for growing steers.

During heat stress, the hypothalamic-pituitary-adrenal (HPA) and Sympathetic-adrenal-medullary (SAM) axis trigger to preserve homeostasis (Sejian et al. 2018). It results in plasma glucocorticoids' production, primarily cortisol, the primary stress hormone in ruminants, which provokes physiological modifications in animals to tolerate stress. In response to thermoreceptors in the skin, adrenocorticotropin release in the hypothalamus increases plasma glucocorticoids. Glucocorticoids help in vasodilatation and stimulate proteolysis and lipolysis by distributing energy to the animal, even during reduced intake. During acute stress, cortisol levels increase since glucocorticoid hormones have hyperglycemic action, enhancing glucose formation through the gluconeogenesis process in heat-stressed animals.

On the other hand, the story of chronic heat stress is the opposite of acute stress. Cortisol is thermogenic in animals; the reduction in the activity of adreno-corticoids during heat stress act as a thermoregulatory protective mechanism to prevent metabolic heat production during the thermal environment. This depicts the relation of the adrenal-thyroid axis with acute and chronic stress adaptation in animals. Interestingly, when cattle are fed limited or exposed to the restricted feeding regime, the cortisol concentrations were significantly higher in the fasted group of dairy cattle than the regular fed group (Chouzouris et al. 2019). Feed restriction is considered one of the potent stressors; animals adapt to stress by higher glucocorticoid production. The following reason can justify the above statement. The concentration of peripheral cortisol mainly depends less on the energy status than on the intensity of stress stimulus on animals; in this case, hunger was the significant stress on animals. Another study confirms that cows were fed only straw displayed higher metabolic stress and higher cortisol concentrations than cows fed with a silage-based diet, which suggests more profound physiological stress. Another angle of looking into the above statement is, Phenylalanine is enzymatically converted to Tyrosine due to glucagon, and Insulin inhibits it. Catabolism of Tyrosine is brought by cortisol and other glucocorticoids hormones. During feed restriction, there might be a more significant degradation of Phenylalanine and Tyrosine, which might be due to a decrease in Insulin and increase in plasma cortisol concentrations, which deviates their process of catabolism towards gluconeogenesis from these amino acids and thus reducing protein synthesis capability.

Non-Esterified fatty acids (NEFA) and glycerol are the hydrolysis products, unlike triacylglycerols, of stored triglycerides in animals' adipose tissue by enzyme hormone-sensitive lipase regulated by Insulin- and catecholamines. NEFA is a vital marker for evaluating metabolic adaptation in different livestock species. During heat stress, animal's Dry Matter Intake reduces to maintain the production of metabolic heat. As a result, the energy supply through feed reduces; the animal comes under a negative energy balance. Characteristic response of Negative energy balance is the reduction in plasma Insulin. Reduced insulin concentrations lead to adipose lipolysis and mobilisation of NEFA. Many studies reported decreased levels of NEFA when animals were exposed to heat stress, which might be due to augment glucose as fuel to counteract the energy deficiency, a novel strategy to reduce metabolic heat production. Decreased NEFA concentrations might be due to failure in registering shifts in post-absorptive energetic metabolism (although insufficient intake of nutrients) may confirm heat stress on animals directly (not mediated by feed intake) affects energetics. Higher plasma NEFA is a typical glucose-sparing mechanism that animals on a low nutrient intake implement to increase milk synthesis. During heat stress, many metabolic and physiological adaptations occur in animals to maintain homeorhetic hormones. The direct physiological effects of the growth hormone result from the triggering Growth hormone receptor, resulting in NEFA mobilisation and gluconeogenesis from adipose tissue by emphasising the lipolytic stimulus to β-adrenergic signals impeding insulin-mediated lipogenesis and glucose utilisation correspondingly. Contrastingly, in a study where 50% reduced feed and fed with straw cow group had the most significant increase in NEFA concentrations with >fourfold increases the first day of feed restriction and peaking a sevenfold change on the second day. During feed restriction, insulin levels decrease, final products of lipolysis, NEFA is used as a metabolic energy source to spare amino acids and glucose.

Growth hormone (GH) or somatotropin, a peptide hormone that triggers cell growth, reproduction and regeneration. GH is a single chain polypeptide containing 191-amino acids produced, stockpiled and secreted by specified somatotropic cells in the anterior pituitary gland. Studies reported a decrease in GH production in chronic heat stress Jersey cows' group (35 °C) compared to thermoneutral zone Jersey cows (18 °C). Few reports suggest when dairy cattle get exposed to THI more than 70, GH production declined, decrease in GH production might be due to countermeasure the metabolic heat in dairy cows. Chronic heat stress negatively impacts GH, followed by a negative energy balance resulting in decreased milk production and even characterised by a decrease in catecholamines and glucocorticoid levels. The change in endocrine status impacts the circulating levels of T_4 and T_3, reducing basic metabolic rate and finally, reduction in heat production.

When the animals are subjected to feed restriction, plasma GH concentrations will increase, linked with GH resistance. During feed restriction, as a consequence of reduced plasma levels of hormones such as T_3, T_4 and production of Growth hormone receptors and GH binding proteins in the plasma are decreased along with other hormones. The decreased attachment of GH with the target tissue, especially the liver will be reduced. Few studies reported an increase in serum growth hormone concentration in Holstein heifers during the restriction phase compared with the control group results. An increase in GH reflects the animals attempt to summon energy reserves from adipose tissue and thereby maintain homeostasis during negative energy balance. Growth hormone has a lipolytic effect on adipose tissue, especially during the feed restriction due to high GH concentration, adipose tissue mobilisation and increasing blood Non-esterified fatty acid concentration. Particularly during feed restriction, growth hormone secretion changes and even GH inhibiting factors from the hypothalamus, finally resulting in increased GH.

Insulin-like growth factor-1 (IGF-1), also named Somatomedin C, is a growth-promoting factor in many body tissues. IGF-1 has a resemblance to insulin molecular structure. The IGF circulate in the body monocovalently bound to greater molecular weight Insulin-like growth factor binding proteins (IGFBP). The liver is the primary source of IGF-1, however it is also produced in other tissues. IGF-1 mediates GH effects. Generally, GH binds to its specific receptors as a part of the somatotropic axis pathway on the hepatocyte surface. During summer, the concentration of IGF-1 was found to be decreased in few studies. Circulating IGF-1 in dairy cows helps regulate milk synthesis, and blood-borne IGF-1 production depends on GH response on the liver. Heat stress in cattle reduced the hepatic GH receptor abundance, and along with reduced GH signalling through STAT-5, hepatic IGF-1 mRNA was significantly lower in heat-stressed animals. The mechanism behind reducing GH receptors in hepatic tissue during heat stress is still unclear but may help modify the GH-dependent gluconeogenesis in dairy cows. Because of this, lower levels of insulin-like growth factor 1 (IGF-1) could also explain the decrease in milk synthesis and increase nutrients utilisation to maintain homeostasis in the liver and mammary tissues.

Linear decrease in plasma IGF-1 concentrations decreases as a result of lower GH-receptor availability during feed restriction. Several studies validate the above statement in growing cattle, under chronic feed restriction phase, IGF-1 concentration reduced. The number of GH receptors binding sites in the liver which are available is critically essential for the production of IGF-1. The reason being, during feed restriction, the liver will have refractory effects to the effects of Growth Hormone, resulting in lower IGF-1 concentration and conversely higher GH concentration production, due to negative feedback regulation of IGF-1 on hypothalamic growth production.

6.6 Molecular Changes During Heat and Nutrition Stress

When the animal gets exposed to high ambient temperature, body temperature surpasses the average specified for their thermoneutral zone. The total heat load exceeds the animal's heat dissipation capacity. An increase in the heat load increases the health problems and mortality rates, especially for high productive animals and even for intolerant or less resistant animals. Heat tolerance can be characterised as animals' ability to maintain their hereditary functional capacities in hot conditions. A heat-tolerant animal's physiological traits are wide skin area to weight ratio, protective eyes, pigmented skin, and light coloured or whitish body cover. Amelioration of climate change stress on animals can be addressed by understanding the basic cellular and molecular responses. Understanding the underlying process that may provide ways to select stress-tolerant animals and maintain the same animals in these changing environments is necessary.

Heat stress triggers cytotoxicity by altering the biological molecules, impairs cell functions, modifying metabolic reactions, inducing cell damage through free radicals, and triggering necrotic and apoptosis pathways. These abnormalities are responsible for significant transcription and protein synthesis changes known as the heat stress response. Heat shock factors (HSF) have been linked as an impactful first line of defence during cell temperature initiation. These factors coordinate cellular reactions against thermal stress and influence gene expression in many genes, including Heat Shock Proteins (HSP). The HSP dissociates from HSF1 monomers when stimulated by heat stress, which unfolds and binds to other HSF1 monomers to trimers before their nuclear translocation. In the nucleus, homotrimeric Heat shock factor1 binds to promoters containing heat shock elements (HSE) to trigger heat stress specified gene transcription. While HSF1 has predominantly been linked to HSP regulation, emerging reports now point to its involvement in regulating carbohydrate metabolism, molecules' transport, cytoskeleton, and ubiquitination, especially during heat stress. A study on lactating dairy cattle evaluated the hepatic tissue gene expression profile in response to an extended period of heat stress (Rhoads et al. 2005). The liver's vital role in whole-body metabolism (integration of both Exo and endogenous nutrients) prone to be affected by reduced feed intake and metabolism shifts due to heat stress and even suggested that cellular response to heat stress is a multi-tier process starting with HSF1 activation, preceded by enhanced expression of HSP, lower fatty acid metabolism, activation of stress response via endocrine system, and immune system activation all occurring simultaneously.

Many genes contribute to the heat tolerance capability in livestock; Taye et al. (2017) worked on different cattle breeds of Africa and identified reassuring signatures of genes for their tolerance capability towards heat stress. Researchers identified heat stress tolerance is directly associated with IGF-1 and HSF5 genes; SOD1, GPX7, GSTM2, GSTM4, SLC23A1, and SLC23A1 were positively selected as the genes related to the oxidative stress response. RAP17 and MLPH genes were found linked with coat colour, ITPR2 and CFTR genes were responsible for sweat glands development in native African cattle breeds which helped in more heat dissipation. Holstein calves' molecular response to thermal stress involves genes that may potentially breed Holstein cattle with superior thermotolerance capability. Increased HSP expression aimed at preventing protein aggregation and disintegration of misfolded proteins and many differentially expressed genes involved in fatty acid metabolism like ATF4 (activating transcription factor 4), which ensures fatty acid synthesis, CPT1C (carnitine palmitoyltransferase 1C) associated in fatty acid β-oxidation, ACOT7 (acyl-CoA thioesterase 7) engaged in long-chain hydrolation of fatty acids were up-regulated and CDC42 (cell division cycle 42), RAC1 (Ras-related C3 botulinum toxin substrate 1), and PRKAB2 (5′-AMP-activated protein kinase subunit β-2) which maintains vital enzymes participating in de-novo biogenesis of fatty acids were down-regulated.

Another study tried to understand the underlying biology regulating the effects of dietary restriction and subsequent re-alimentation in the ruminal epithelium papillae of Holstein Friesian bulls. Researchers found out genes like CRYAB protein which exhibits chaperon activities and performs to limit protein aggregation in a wide range of conditions. HSPH1 and HSPB8 genes were down-regulated in skeletal muscle tissue during dietary restriction. Genes involved in cell proliferation transcription factor ZNF516 was down-regulated. Genes related to signalling processes involved in growth and cellular proliferation, such as tyrosine phosphorylation regulated kinases DYRK1A, DYRK1B, PIK3C2G, which is of vital importance in PI3-kinase signalling, expressions were reduced. Additionally, up-regulation of genes like MANF and ID2, associated with cellular proliferation inhibition was identified at the end of the restricted diet period in hepatic tissue.

6.7 Conclusion

Besides heat stress, several stressors are hindering livestock production in the current climate change situation. Animals can counterbalance efficiently without fluctuations in production capacity due to the stored body reserves when exposed to a single stress. However, the cumulative effects of the various stressors may damage if animals are exposed to multiple stressors. Reports evaluating the effects of multiple stresses in cattle are limited. More research efforts are essential to understand the mechanism of how multiple stressors affect the adaptive and productive capability of cattle, such that policymakers can amplify the endeavours of researchers to extra more data on the impact of multiple stressors on cattle. On the other hand, cattle can efficiently counter heat stress, provided nutrition is sufficient to maintain the normal homeostasis mechanism. Nutrition, particularly during heat stress, plays a major role in the adaptive capabilities of cattle. Dietary nutrients wield their roles through numerous nutrient-metabolic and cell signalling pathways; our current information is still not deep enough to untie the complexities of dietary nutrients and animal genome expression.

Learning Outcomes

- In the extensive system of rearing, climate change not only causes the usual suspect heat stress but also leads to nutrition, water and walking stress to grazing animals.
- The multiple stressors induces very high magnitude of adverse impacts on the production and adaptation capacity in cattle than heat stress alone.
- Quantification of multiple stressors impact could be the most appropriate way to support future policies for sustainable livestock production in the changing climate scenario.

6.8 Future Perspective

Multiple stresses concept is gaining significance among the researchers in the changing climatic scenario. Presently, various researchers are concentrating primarily on data generation on heat stress and its impact on animals' productivity and adaptation. However, other stresses like nutrition, walking, water, and many more are to be acknowledged and addressed. Through collaborative research (animal, plant and allied sciences), we can understand the mechanism through which animals and plants adjust to the multiple stresses. Parallelly, studies on ameliorative strategies to counteract the multiple stresses, which are economical and practical at the field level, should be devised and propagated through various extension services. Research on multiple stresses should be initiated in tropical regions as early as possible, given that the agricultural land and water resources shrinkage are catching up speed due to climate change, resulting in increased nomadic behaviour of animals in search of pasture and water. Considering the emergency to address the multiple stresses in livestock, especially in the extensive rearing system, might threaten global food security, policymakers should make rational managerial decisions and fund more studies on multiple stresses than on single stress.

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Comparative Assessment of Thermo-Tolerance of Crossbred and Indigenous Cattle Breeds

7

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Abstract

One of the important strategies to find solution to the climate change associated livestock production is to identify the ideal livestock breed to survive in a specific location. This warrants research efforts to screen the available breeds to identify climate resilient breeds. Further, comparing the crossbred animals with indigenous breeds can help to understand the real differences in livestock adaption in addition to identifying quantifiable biomarkers covering productive and adaptive traits. This chapter is an attempt to project to the readers the differences in adaptation between crossbreds and indigenous cattle. Indigenous cattle possess various unique characteristics which imparts them the potential to survive in tropical climate. However, the crossbred cattle which are well known for their production struggles in harsh climatic condition which ultimately culminates in

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V. Sejian et al. (eds.), *Climate Change and Livestock Production: Recent Advances and Future Perspectives*, https://doi.org/10.1007/978-981-16-9836-1_7

reduced production and welfare. Crossbred cattle shows higher physiological and biochemical reactions, as well as a lower total antioxidant capacity, as compared to native breeds, indicating that they were more susceptible to heat stress. Further, heat shock protein 70 (HSP70), toll-like receptor 2 (TLR2) and TLR4 which is considered as potential markers for temperature adaptation in livestock was highly expressed in less adapted crossbred than indigenous breed. More such efforts are needed to identify more thermo-tolerant breeds to propagate them in specific agro-ecological zones. Such efforts can ensure the livelihood of poor and marginal farmers in the changing climate scenario.

Keywords

Adaptation · Breeding · Climate change · Heat stress · HSP70 · Thermo-tolerance

Abbreviations

%	Percentage
°C	Degree Celsius
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
APP	Acute phase proteins
AST	Aspartate aminotransferase
DAHD	Department of Animal Husbandry, Dairying
DC	Dendritic cells
GDP	Gross domestic product
HF	Holstein Friesian
HPA	Hypothalamic pituitary adrenal axis
HSPs	Heat shock proteins
IL	Interleukin
MAS	Marker assisted selection
ml	Millilitre
mRNA	messenger ribonucleic acid
ng	Nanogram
PBMC	Peripheral blood mononuclear cells
RR	Respiratory rate
RT	Rectal temperature
T_3	Triiodothyronine
TLR	Toll-like receptors
TNF	Tumor necrosis factor

7.1 Introduction

India, a mega diverse country, accounts 8% of all recorded species within 2.4% of the world's land area. The overall climate ranges from humid and dry tropical in the south to temperate alpine in the north with a great diversity of ecosystems. Agriculture, the most climate-dependent economic activity is considered to be the chief source of livelihoods for about 70% of Indian population. In the past four decades, the livestock sector's rapid growth has made a significant contribution to agricultural growth both nationally and globally. Livestock sector's annual contribution in total GDP is nearly 4.9% (DAHD 2016). According to 20th Livestock Census (2019), in India, the total livestock population is 536.76 million showing an increase of 4.8%, in which total number of cattle accounts for 193.46 million marking an increase of 1.34% as compared to the last census. In comparison to the previous census total exotic/crossbred cattle population has been increased by about 29.3% whereas the indigenous (both descript and non-descript) cattle population declined by about 6% from the previous census. More over India continues to be the largest milk-producing nation globally since 1998 (estimated milk production of 187.7 million tonnes per annum), with an increase of about 6.45% annually. By 2029, world milk production will increase around 997 million tonnes at the growth rate of 1.6% p.a. and most importantly, India is likely to contribute more than 30% of world production. The livestock products demand is expected to double in next three decades globally. This increase in demand is due to the improved living standard, growing population, ageing, socio-cultural patterns and also the change in food preferences across the world from staple food to calorie-dense and taste-based food items. In order to overcome this growing demand driven by dietary changes and also the increasing population, 60% of food production has to be increased by 2050.

Crossbreeding spread like wildfire across the country, with new crossbred cattle being preferred over native cattle due to their higher milk yield. Most of the genetic resources used in crossbreeding came from temperate countries, where the climate and weather, differ greatly from tropical countries like India. Indigenous cattle are more thermo-tolerant than crossbreds/exotic cattle as they possess various unique characteristics, which make them well adapted to the tropical climate. Furthermore, the impact of climate change is predicted to be more severe in tropical countries where the dairy cattle are primarily reared in an extensive system, unlike that of chicken and pig which follow a semi-intensive system of rearing. As a result of crossbreeding, not only has productivity grown, but it has also brought with it issues of overuse and concerns about crossbred cattle's long-term viability. In thermal stress, animals dissipate excess heat which is exhibited by various physiological and metabolic adaptive mechanisms which are energy consuming, and this is believed to cause the proportional decline in milk yield in these animals.

Furthermore, it is also reported that heat stress can alter the milk composition by reducing total protein content and total fat content in milk. In India, crossbreds are considered to be most susceptible to heat stress in comparison with indigenous cattle as a result of the long-term intensive breeding in order to improve their milk production. India attained top position as highest milk producing country globally because of crossbreeding program of dairy cattle. Despite achieving this position, India still faces many problems in rearing crossbreds compared to those indigenous breeds of cattle. In the case of crossbreds, primary investment and maintenance expenditure are high which is not feasible for all farmers who struggle for their livelihood and also they are highly susceptible to contagious diseases.

With this background, this chapter is primarily targeted to comprehend the information pertaining to comparative assessment of adaptive capability between crossbreds and indigenous breeds. This will give us a clue of what are those biological markers that could be used for future breeding policies to produce well adapted animals along with production traits. It also tries to elucidate the basic and molecular mechanism of adaptation, therefore to access the thermo-adaptability of indigenous and crossbred cattle.

7.2 Assessment of Thermo-Tolerance Between Crossbred and Indigenous Cattle

7.2.1 Physiological Responses

Generally, a thermal steady state is achieved in animal body through equilibrium maintained between heat produced in the body by metabolic activities and heat gained from the environment. Under heat stress, animal undergoes a series of physiological changes to maintain homeostasis and physiological equilibrium. These changes are highly dependent on external temperature and also genetic make-up of an animal. Most of these physiological adjustments are exhibited in order to dissipate heat from the body to its environment and also to reduce the heat production through metabolic activities within body.

In a comparative study by Sailo et al. (2017), the respiratory rate (RR) in Sahiwal were found to be 15.738 ± 0.795, 18.158 ± 0.795 and 29.818 ± 0.795 and in Karan Fries cattle were 15.779 ± 1.136, 22.979 ± 1.136 and 47.299 ± 1.136 during winter, spring and summer seasons. In same study, the rectal temperature (RT) also showed similar results where in Karan Fries RT (°C) showed 37.492 ± 0.115, 38.398 ± 0.115 and 39.186 ± 0.115 while Sahiwal showed 37.300 ± 0.095, 38.178 ± 0.095 and 38.810 ± 0.095 during winter, spring and summer respectively. When high yielding Holstein Friesian (HF) cows were subjected to high temperature conditions, their rectal temperature increased significantly from 37.3 to 39.3 °C (Koubkova et al. 2002). According to Khan et al. (2018) the intensity of changes in physiological parameters were found to be in the order of HF > Crossbreds> Sahiwal which shows adaptive capability of the breeds. Results from the above studies reveals that indigenous cattle breed respond better and more tolerant when compared to that of crossbreds.

7.2.2 Hormonal Responses

Both long and short term environmental stress on animals affects the endocrine glands through the activation of hypothalamic pituitary adrenal axis (HPA) which is a key component for heat stress. It is the hallmark response since it reflects the physiological status of animals responding to heat stress. In the process of acclimation, the secretory patterns of glands changes due to the activation of HPA and there is consequent change in the plasma level of different hormones along with their receptors in their target tissue. Endocrine system works to reorient the internal milieu that is being disturbed during heat load. Hormones responsiveness to heat stress stimuli primarily includes glucocorticoids, thyroid hormones, growth hormones, mineralocorticoids, prolactin, catecholamines and anti-diuretic hormones.

Khan et al. (2018) observed the results indicated that level of cortisol was more prominent in HF and crossbred than *Bos indicus*. These results were more consistent with the results of Mc-Manus et al. (2009) showing increased level of cortisol in case of heat stressed cows that is more severe in *Bos Taurus* when compared to that of *Bos indicus***.** Tejaswi et al. (2020), observed that the serum cortisol level and T_3 were significantly higher in crossbred (Hariana × Holstien Friesian/Brown swiss/Jersey) when compared to Tharparkar and Sahiwal during both the winter and summer seasons. According to Yousef et al. (1997), the concentration of cortisol increased to 29 ng/ml from 11 ng/ml in Friesian calves and T_3 showed a decline in T_3 levels from 151 to 126 ng/ml under heat load.

7.2.3 Biochemical Responses

Bhan et al. (2012) reported that the ALT and AST values of growing and adult Sahiwal was significantly increased during afternoon compared to that of morning. Georgie et al. (1973) and Shaffer et al. (1981) also reported the significant increase in AST during exposure to different seasonal changes in crossbred cattle. Studies are stating that there is seasonal variation that usually influence biochemical parameters in HF heifers and also reported in the study that there is increase in AST levels in serum during high ambient temperature. Lower levels of serum AST, ALT and ALP activities in the native cattle compared to the crossbred cattle both during summer and winter could be due to their resistance to heat stress which can be attributed to morphological features like higher density, large sized sweat glands and its proximity to the skin's surface, with more layers of cells in the epithelial layer.

7.2.4 Heat Shock Responses

Heat shock proteins (HSPs) are the major cellular proteins which spearhead the heat shock responses for stress adaptation in animals. In normal cellular physiology, there are many HSPs that play a very important role. This is highly conserved protein which is meant to be activated by several environmental stressors. There is an induction of HSPs within minutes after animal exposure, which can go to its peak expression which might be several hours later. In the stressed cell, there is an interaction between denatured protein and HSPs which tends to inhibit the aggregation of cytotoxic proteins and hence maintains the homeostasis of protein in the cell. The improvement in thermo tolerance is seen in case of prolongation or elevation of levels of HSPs. Based on the biological functions and molecular weight, HSPs are divided into HSP10, HSP40, HSP60, HSP70, HSP90, HSP100, HSP 110, and there are other small families of HSPs. Among this HSP 70 is considered as the most sensitive heat stress indicator which acts as a cellular thermometer in livestiock (Dangi et al. 2014; Samad et al. 2019). Different investigators presumes that thermotolerance capacity is associated by assessing intracellular expression of HSP90 in livestock species (Dangi et al. 2014; Deb et al. 2015; Samad et al. 2019; Slimen et al. 2016). In case of farm animals, during heat stress there is an increase in HSP90 and HSP70 which is observed in cattle, buffalo, broilers, sheep and goats (Yu et al. 2008; Slimen et al. 2016; Shilja et al. 2016; Samad et al. 2019; Tejaswi et al. 2020).

Bharati et al. (2017) reported that the HSP90 mRNA is up regulated when the Tharparkar cattle was exposed to 42 °C. Deb et al. (2015) correlates the thermotolerance with the increase of HSP90 mRNA levels in both Sahiwal and Frieswal cattle through *in-vitro* heat stress studies. Kim et al. (2020) also studied the HSP90 mRNA expression in PBMCs and reported that there is an increased level of those proteins in response to elevated temperature in beef cattle. Heat stress responses in different breeds of cattle and buffaloes was studied by Kishore et al. (2013) and documented a rise in HSP90 2 h post heat stress which was highest in Murrah buffaloes, followed by Sahiwal and HF. The study also added the ranking expression between HSPs suggesting that HSP70 was more upregulated and HSP90 was least expressed in thermotolerant animals.

7.2.5 Immune Responses

Immune responses are bodies defensive responses towards infectious and danger signals associated with cellular damage. Chief mediators of these responses include body's physical barrier, chemical barrier, various immune cell and soluble proteins like cytokines. Heat stress associated cellular damage releases HSPs which have the tendency to activate various immune cells mainly Dendritic cells (DC) and Macrophages thereby inducing a cascade of inflammatory responses (Lecchi et al. 2016; Hop et al. 2018). Cytokines are the key secretions of these immune cells that activate the immune cells as well as transmit downstream signals for ultimate response. Based on the major response Cytokines are grouped into pro-inflammatory (IL1 β, TNF α) anti-inflammatory (IL10), which are there to counter act each other in order to maintain internal cellular homeostasis (Cannon 2000).

Bharati et al. (2016) reported that there is a significant rise in levels of IL-2 and IL-6 at various increased temperature of 37 °C, 39 °C and 42 °C in Tharparkar cattle. The expression of pro-inflammatory cytokines and anti-inflammatory molecules was higher in crossbred compared to native breeds. The low negative-Acute phase Proteins (APP) concentrations like lower values of albumin due to elevated levels of cytokines may cause an increase in disease incidences in crossbreds.

Learning Outcomes

- The indigenous livestock breeds possess unique adaptive characteristics which imparts them the potential to survive and produce optimally in harsh climatic condition.
- HSP70 is the most studied molecular chaperon and considered to be the ideal cellular and molecular marker for heat stress in farm animals.
- As the crossbred animals are predominantly targeted for production traits, they cannot withstand harsh climate resulting in loosing even the productive potential when exposed to heat stress for a pronged duration.

7.3 Conclusions

Crossbred cattle shows higher physiological and biochemical reactions, as well as a lower total antioxidant capacity, as compared to native breeds, indicating that they were more susceptible to heat stress. Native cattle had much lower HSP70, TLR2, TLR4, and cytokine expression than crossbred cattle, indicating that they are more resistant to temperature stress at the cellular level. Indigenous cattle exhibited more tolerance to heat stress than crossbred during summer, and hence indigenous breeds are better adaptable to tropical climate.

7.4 Future Perspectives

In the current climate changing scenario, one has to primarily look upon to develop breeds that are more thermo-tolerant along with production traits. Very limited studies are conducted in comparing the thermo-tolerance of crossbreds and indigenous cattle. The advanced biotechnological tools should be employed in identifying more traits like adaptation and production. The results mentioned above in comparing crossbreds and indigenous breeds needed to be extrapolated in identifying agro-ecological zone specific breed. The baseline information is available for only few indigenous breeds and in case of studying all other breeds will give us clues in obtaining breed that will have the ability to survive in multiple locations with optimal milk production. After obtaining the baseline information, different approach of testing their adaptive capability by shifting them to different agro-ecological zone and find which breed can survive better. This way those breeds can be used for marker assisted selection (MAS) breeding with the amalgamation of productive and adaptive traits. This can only be the solution of producing breeds that can survive in any environment along production traits. Efforts are much needed not only in ensuring livelihood security of poor and marginal farmers but also makes the livestock farming more profitable, As world is going to face huge food demand of growing population by 2050, these efforts of bringing up thermo-tolerant breeds will be beneficial in ensuring food security as well.

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Part III

Methods to Quantify Heat Stress Response in Livestock

Non-Invasive Methods to Quantify the Heat Stress Response in Dairy Cattle

8

Gundula Hoffmann, Mullakkalparambil Velayudhan Silpa, Roman Mylostyvyi, and Veerasamy Sejian

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V. Sejian et al. (eds.), *Climate Change and Livestock Production: Recent Advances and Future Perspectives*, https://doi.org/10.1007/978-981-16-9836-1_8

Abstract

The main focus of our research lies on livestock-environment interactions and how heat load in dairy cows can be measured. Several studies have used animal-related parameters to measure heat stress in individual cows. In this report, an overview of different heat stress indicators is given, focusing on non-invasive indicators that analyze different physiological, ethological and endocrine responses of livestock animals. However, potentials of digitalization, e.g. by means of sensor technology, for recording animal-specific adaption reactions of dairy cows are also described. Sensor information of animals such as milk yield, vitality (respiration rate, body and skin temperature) and behavior (standing, lying time) are analyzed taking into account the barn climate. The investigations contribute to a better understanding of heat stress in farm animals and to the improvement of animal welfare.

Keywords

Dairy cow · Heat stress · Indicator · Sensors · THI

Abbreviations

%	Percentage
°C	Degree celsius
AT	Air temperature
ATB	Leibniz Institute for Agricultural Engineering and Bioeconomy
BT	Body temperature
DIM	Days in milk
ETIC	Cattle equivalent temperature index
Fig	Figure
GMT	Greenwich Mean Time
H	Humidity
HR	Heart rate
HS	Heat stress
IRT	Infrared thermography
LR	Linear regression
min	Minutes
NRC	National Research Council
NVB	Naturally ventilated buildings
pH	Potential of hydrogen
R^2	Coefficient of determination
RH	Relative humidity
RR	Respiration rate
SR	Solar radiation
T	Temperature
THI	Temperature humidity index

UK	United Kingdom
USA	United States of America
USB	Universal serial bus
WS	Wind speed

8.1 Introduction

Global climate changes are becoming more and more tangible for the world's agriculture and the increasing heat load plays a significant role in determining animal welfare in livestock husbandry. Dairy cows in naturally ventilated barns are particularly affected by the climate change and will suffer under heat stress. As cows are relatively cold-tolerant but heat-sensitive, the extreme heat periods in the summer months increasingly lead to restrictions in animal welfare, health and production performance. Heat-stressed dairy cows dissipate surplus body heat into the environment and reduce the heat generation rate by adjusting their behavior (body posture, lying bout, movement, etc.), showing physiological responses (e.g. increased body temperature and respiration rate, decreased dry matter intake and milk production, loss of weight, sweating, etc.) and immune responses (changed blood hormone concentration). In order to keep body functions in a steady state, it is necessary for homeotherms to maintain body temperature within a narrow range. A deviation from the set point of the body temperature in a stressful, hot environment leads to an impairment of the physiological processes and thus has a negative impact on the productivity of the animals (Naqvi et al. 2012). Literature studies have made it possible to determine physiological-ethological reactions of dairy cows that correlate with climatic conditions (Hoffmann et al. 2020). The particular difficulty is to identify the corresponding meaningful reactions. The objective of this work was to bring together intensive monitoring of the microclimate and various physiological, ethological and endocrine reactions of lactating dairy cows. Therefore, several animal-individual characteristics as well as the microclimatic conditions inside the barns were taken into account.

8.2 Significance of Non-Invasive Methodologies to Quantify the Heat Stress Response

The field of science and technology has observed a huge progress over the decades. These advancements have aided researchers across the globe in several ways like ensuring quicker and accurate results. However, along with this, there is an increasing rise in animal welfare and ethics especially for the researches involving the use of animals, be it laboratory animals or even farm animals. All the heat stress (HS) studies in livestock face a primary obstacle on obtaining ethical clearance as these experiments subject the animals to severe distress. Having said that, it is inevitable to conduct such live animal studies as they yield the most vital solution to mitigate the rising concern on livestock associated climate change impact. At this point, the use of non-invasive technologies to quantify HS is crucial. As these techniques do not add on to any further stress in animals, adoption of non-invasive techniques over invasive approaches are more preferred from an ethical point of view.

Non-invasive methodologies also have an added advantage of being more accurate in estimate the HS impact in animals. Most of invasive technologies require intense animal handling. These approaches thereby add on to the stress in animals, which is also an issue from the animal welfare perspective. Since non-invasive methods require minimal animal handling, they are proposed to depict relatively accurate stress profile in animals. Furthermore, apart from being accurate, some of these non-invasive methodologies also have an advantage of being inexpensive and of high correlation to assess HS both in producing and non-producing animals.

Time invariably, a number of non-invasive methodologies to assess HS impact in livestock have been adopted. However, with the growing concern on animal welfare and ethics, several improvisations have also been brought about in these methodologies. The non-invasive methods also comprise a number of sensor-based approaches that record series of vital variables in animals. Adoption of such technologies enables early detection of heat load in animals and thereby quicker ameliorative measures can be enforced to reduce its impact on livestock production. Additionally, some of these technologies also aid in predicting the occurrence of stress, be it HS or any other environmental stress, in livestock. Therefore, this gives room for the livestock keepers and other stakeholders in this sector to enforce suitable mitigation measures to combat the predicted adversities. Therefore, the scope of using non-invasive technologies for HS assessment in livestock is massive and thus proper emphasis must be given to propagate its usage among both the livestock farmers and researchers across the globe.

8.3 Measuring Activity and Lying Behavior to Quantify Heat Stress

As a sensitive indicator of HS, dairy cow activities including the resting behavior (total lying time, number of lying bouts, lying bout duration) and number of steps per cow per day were recorded by activity sensors (IceTag3DTM, IceRobotics, Edinburgh, UK). This trial was carried out in the dairy barn of the Agricultural Research and Education Center for Animal Breeding and Husbandry (LVAT, Gross Kreutz, Germany). The effects of HS and individual cow factors (e.g. lactation state, days in milk) on the total daily lying time are shown in Fig. 8.1. It was found that cows with heat load had shorter lying times than cows without heat load. When additional heat load was accumulated three days before the measurement day, reduced activity responses to heat load (i.e. delayed heat load effects) were observed (Heinicke et al. 2019).

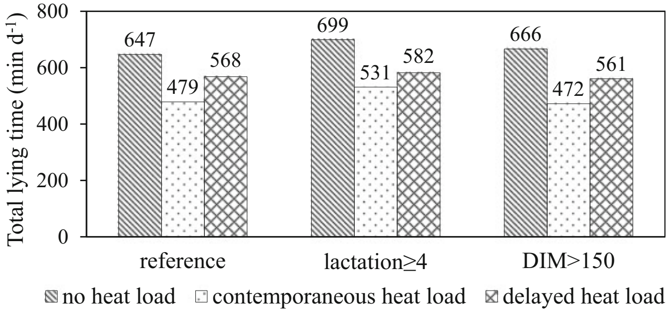

Fig. 8.1 Daily total lying time predicted under different heat load conditions for the reference group, lactation ≥4 group, and DIM (days in milk) >150 group. Reference group has normal milk yield level, lactation state of 1, no pregnancy and estrus (Heinicke et al. 2019)

8.4 Respiration Rate as a Significant Physiological Variable

Another reliable and early indicator of HS in dairy cows is the respiration rate (RR). This was measured by counting the right thoracoabdominal movements for 30 s and multiplying by 2. Depending on the measurement series, these measurements were taken hourly from 0700 h to 1500 h (GMT + 0100 h) or twice daily (morning and afternoon) in the LVAT (Gross Kreutz, Germany). At the same time it was documented whether the animal was in a lying or standing position during the measurement. In addition, the temperature and relative humidity were recorded at fixed time intervals (10 min) and locations directly in the barn (EasyLog USB 2+ sensor from Lascar Electronics Inc., USA). The THI formula according to NRC (1971) was applied:

$$\mathrm{THI} = (1.8 \times \mathrm{T} + 32) - ((0.55 - 0.0055 \times \mathrm{H}) \times (1.8 \times \mathrm{T} - 26))$$

Where, T is the air temperature in °C and H is the relative humidity in %. With the help of the measuring intervals per 10 min, the heat load duration per heat load intensity could also be determined. In addition to the average THI per day, the heat load duration per heat load intensity provide more precise information on how long, how strongly and when the animals were exposed to heat. It could be shown by Pinto et al. (2019) that the RR showed high variabilities for the same THI, especially for higher THI conditions. This illustrates the animal individual influences. The RR increased with the increase of THI for both cow body postures. In addition, it was observed that standing cows had a lower RR than lying cows when THI was less than 80. Since counting flank movements is time-consuming, labor-intensive, and non-continuous, ATB developed a new differential pressure sensor for monitoring the RR (Strutzke et al. 2019). The sensor can carry out automatic, continuous and long-term RR measurements.

8.5 Significance of Hair, Urine and Faecal Cortisol Estimation

Heat stress stimulates a number of endocrine responses in animals. Cortisol is one among the classical HS markers in livestock. Traditionally this glucocorticoid hormone levels are estimated in blood samples. Though the blood samples serve as reliable medium for estimating the endocrinal changes occurring during HS, the methodology is invasive and may add up to the existing stress especially when the animals are not handled properly. Hence, the use of hair, feces and urine samples, as an alternative medium is encouraged. Researchers have reported that these biological samples provide an efficient estimate of cortisol levels in livestock during HS. In most cases, the cortisol metabolites obtained in some studies also correlated with a number of other stress indicators. However, prior to incorporation of these methodologies, one has to be aware of their relevance with HS. Fecal cortisol estimation provides an overview of the acute stress effect in animals wherein a cumulative secretion of glucocorticoids may be observed. This secretion occurs for a few hours coupled with a time delay between plasma glucocorticoid increase and excretion of fecal cortisol that is primarily due to the gut passage time (between 8 and 16 h). Fecal cortisol estimation during HS was reported in a number of studies conducted in cattle, sheep and goats. In a study led by Veissier et al. (2018), an increase in fecal cortisol metabolites were observed with an increase in heat load index in Holstein cattle.

Hair cortisol, another potential non-invasive methodology having wide application in a number of livestock species like cattle, sheep, goat and pig, has been stated to be a biomarker to detect stress. It may, however, be noted that hair cortisol depicts the effect of chronic stress in animals. The glucocorticoids are excreted into the hair shaft and stored within until estimation. The hair samples obtained can be wrapped in clean sample collection papers or bags and kept at room temperature thereby ensuring very convenient sample storage options. Additionally, as the estimation methodology involves extraction of cortisol metabolites stored in the hair shaft, chances of obtaining over-represented values, as a consequence of sample collection, is ruled out. Furthermore, a study by Nejad et al. (2019) also proved HS to act as a strong stressor that can increase hair cortisol levels in Holstein cattle. This methodology however needs to be adopted appropriately, as there are reports stating hair cortisol levels to be influenced by hair color, hair type, body region collected from and also sex. However, the impact of these factors can be nullified when a uniform pattern of hair sampling is followed.

The metabolic products of glucocorticoids, released from the adrenal glands during stress in animals, are excreted in feces and urine. Thus estimating the urine cortisol metabolite concentrations is also a potential non-invasive approach. The urine cortisol metabolites reflect the circulating cortisol levels in animals as it is excreted through the glomerular filtration by kidneys. Similar to the feces and hair cortisol level, urinary cortisol concentrations are also not influenced by animal handling and rather provides an integrated index of cortisol production in the animal. Though the use of urinary cortisol estimation in heat stressed animals is gaining significance only in the recent past, this technique was extensively used in horses as a doping test. However, with the rising concern of animal welfare, urinary cortisol estimations have been conducted in cattle, sheep, goats, pigs and other domestic and wild animals.

Therefore, feces, urine and hair can act as a vital medium to assess the classical HS marker in livestock. Having known about their significance both from the ethical point of view and also its increased accuracy to assess HS in animals, researchers can definitely opt for this non-invasive methodology. However, a parallel study to improvise the use of these techniques by developing quicker and easier protocols that can be used directly at the field levels would be of practical high relevance.

8.6 Opportunities to Measure Body Temperatures for Determining Heat Stress in Farm Animals

Body core temperature is one of the vital variables recorded to assess the impact of HS in animals. A number of methodologies have been adopted to record body temperature depending on the site of recording which may include, inter alia, rectum, vagina, rumen, skin, body surfaces as well as ingestible biosensors and temperature sensing ear tags with a temperature sensor placed within the ear canal. These techniques differ in their degree of invasiveness, accuracy and also time lag on HS response. Body temperature is one vital parameter in farm animals with economic importance due to its association with health, production, and reproduction. Body temperature might be elevated in animals due to illness, HS and other health related issues. Rectal temperature is a routinely used measurement of body temperature. However, it requires animal handling/restrain, which may cause a stress to the animals. To overcome this problem associated with measuring the rectal temperature, a range of subcutaneous microchips and other implantable devices, temperature sensing ear tags, rumen-reticular boluses, intra rectal and vaginal devices with remote data transmission ability have been developed for the continuous measurement of body temperature in farm animals.

Temperature sensitive microchips are injected subcutaneously and a handheld receiver activates the microchip, which transmits a temperature reading to the receiver (Chen and White 2006). Temperature recorded in this method is almost instantaneous, and minimal handling of the animal is required. Using subcutaneous microchips between the shoulder blades, body temperature can be measured comparable to the rectal body temperature.

Rumen boluses, another advanced non-invasive remote sensing technology, are designed to record rumen pH, rumen temperature, and activity in ruminant animals. These devices are sensitive in reading body temperature and already transmit the recordings via reading stations in the barn to a data cloud. Intra-vaginal and intra-rectal thermo-sensor devices are also extremely sensitive to detect changes in body temperature (Burdick et al. 2012). Thus, the use of technologies or tools that detect elevated body temperatures reliably and in time can help to predict and prevent the negative effects of HS.

8.7 Infrared Thermal Image Applications in Assessing Thermo-Tolerance in Farm Animals

Among the recording of body core temperatures, the infrared thermography (IRT) is gaining a lot of importance for its advanced features, reliability, non-invasive approach, wide applicability to assess varied physiological status and diseases in animals and finally for the minimal human animal interaction required. The infrared thermal imager records the body temperature of animals by capturing the radiant heat emitted from them, which involves a combination of body core temperature and blood flow changes. This technology however differs from an infrared thermometer that provides only a point based surface temperature recording. While the infrared thermal imagers, being a field-based temperature measurement system, has a wider area of detection and provides thermographic images having wide applications.

A number of studies have been conducted to assess the reliability of IRT to effectively detect heat stressed animals. In a HS study conducted on Holstein (HO), Girolando (½ Holstein × ½ Gir; considered as ½ HO) and ¾ Holstein × ¼ Gir (¾ HO) dairy cows, Daltro et al. (2017) established IRT to be effective in determining HS in cows. Based on their study, using the IRT results, Holstein cows were reported to be more susceptible to HS than ½ and ¾ Holstein cows. Further, based on this study the authors stated udder region to be the ideal area that can determine the thermal comfort in animals when compared to the other four regions (eye, right-area, left-area and foot). In another study by Montanholi et al. (2008), IRT was stated to have a potential application to detect heat production and methane production along with detection of skin surface temperature fluctuations as a consequence of heat increment due to feeding (physiological event) in Canadian dairy cows.

Figure 8.2 shows IRT images of an own experiment in different body parts of a dairy cow.

Researchers across the globe have employed the use of IRT for HS studies in other livestock species, too. Barros et al. (2016) stated IRT to be a useful and accurate non-invasive methodology to detect variations in orbital area, left flank, right flank and scrotum temperatures in buffaloes, having a positive correlation with temperature-humidity index (THI). In another study in sheep, Kahwage et al. (2017) studied the thermal tolerance of Morada Nova and Santa Inês rams with respect to their ability to maintain body and testicular homeothermy when subjected to thermal stress. The authors emphasized on the role of IRT as an efficient and alternative non-invasive method for thermal mapping the animal's body surface and also stressed upon their usage as an auxiliary tool to detect thermo-tolerant animals. Infrared thermography have also been used extensively in other species like goat and pigs, however, its incorporation in HS studies is minimally documented.

Therefore, IRT can be considered as one of the vital non-invasive methods to assess thermo-tolerance in farm animals. Having said that, this technology has a number of confounding factors which need to be looked into so as to ensure successful outcomes. As this methodology works primarily on the emissions from the animal, the readings obtained thus are influenced by factors like skin and hair variables (thickness, reflectivity, color, etc.), emissivity, health status of the animal,

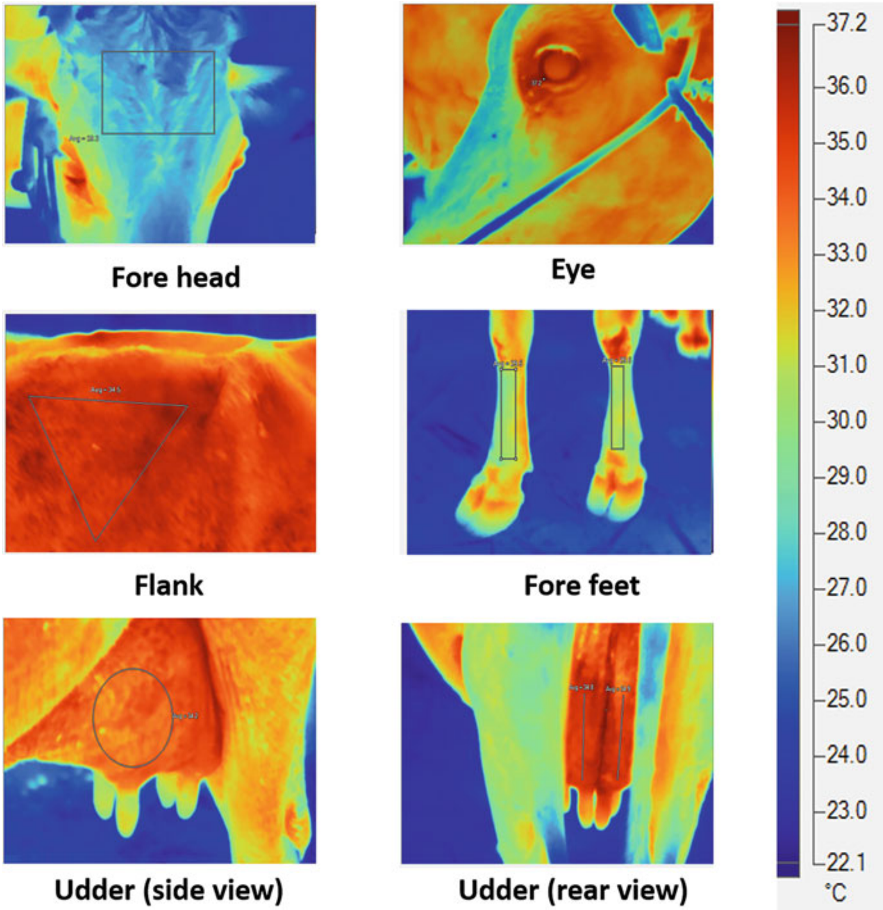

Fig. 8.2 Infrared thermal images in different body parts of a dairy cow

feeding time, climatic variables (relative humidity, ambient temperature, wind speed, sunlight) human factors (user's distance and angle from animal). Therefore, utmost care must also be taken to minimize these factors to lowest levels.

8.8 Models to Quantify Heat Stress in Farm Animals

Considering the existing close relationship between the environment and the body, the direct influence of meteorological factors on the physiological state of productive animals, the assessment of the animals' comfort using integral indicators or indices deserves special attention. The temperature-humidity index (THI), which is based on air temperature (AT) and relative humidity (RH) measurements, has traditionally been used to quantify the degree of HS in animals. THI is easy to calculate and it is rather informative. Numerous studies indicate a close relationship between THI and indicators of body temperature (BT), respiration rate (RR) and heart rate (HR), widely used to assess the clinical state of animals during HS. A sufficiently high correlation between THI and milk yield, as well as the content of milk components, allows this index to be used in predictive models of environmental impact on dairy cattle. Indices that take into account additional variables (solar radiation (SR), wind speed (WS), etc.), which can increase or decrease the heat load, in addition to AT and RH, are also applicable. In particular, Mader et al. (2006) found that the correlations between THI and the degree of dyspnea ranged from $r = 0.47$ to 0.87. Therefore, adjusting the THI equation (for SR and WS) had a higher correlation with the average dyspnea score (from $r = 0.64$ to 0.80), which allows for a more accurate assessment of animal discomfort. Proposed by Wang et al. (2018) Cattle Equivalent Temperature Index (ETIC), including AT, RH, RS and WS, is more accurate in predicting changes in physiological responses. The determination coefficient (R^2) for skin temperature, BT and RR was 0.79, 0.40 and 0.49, respectively, so ETIC can be a useful tool to assess the effect of HS on animal comfort.

HS is an obstacle to the intensive development of animal husbandry, when the livestock is forced to constantly stay in the buildings, not having free access to a pasture. The difficulty is the fact that the microclimate of modern naturally ventilated buildings (NVB) is highly dependent on external conditions that have a direct impact on livestock welfare. Although the use of data from near located weather stations is a recognized practice in modeling the response of animals to HS, the design of the NVB itself (which is associated with indoor air turbulence) can be limiting in building statistical models using such indices (Wang et al. 2018). In addition, the error in the predictions themselves can be associated with a small number of tests, as well as the lack of proper examining of the resulting models in field tests.

A recent study (Mylostyvyi et al. 2020) reported that statistical modeling was effective in predicting THI values in dairy cowsheds based on multiple AT and RH records outside and inside the cowshed over a wide temperature range. The use of linear regression (LR) provided a high forecast accuracy (93–96%) of THI in NVBs of various designs, depending on the external environment. The coefficient of determination between the observed and estimated data was in the range $R^2 = 0.854$–0.921. This approach makes it possible to predict the values of THI in NVBs without the need for constant measurement of parameters (AT and RH) indoors, that makes it possible to decrease animals stress. It also pointed out that one should be careful in predicting the microclimate parameters in NVBs based on weather station data, since there were significant differences between meteorological data and measurements of AT and RH (differences of 1–3 °C and 8–18%, respectively) outside the cowshed during the warm period.

Thus, the use of thermal indices, as well as statistical models for their prediction, can be useful in assessing the possible response of productive animals to HS, given the strong correlation between the value of thermal indices, physiological variables and the level of productivity. However, many uncertainties remain both in the methods of monitoring the microclimate, namely the placement of AT and RH sensors indoors (Hempel et al. 2018).

Table 8.1 Overview of different non-invasive methods and their fields of application in dairy cows

Type of non-invasive method	Type of adaptive response	Field of application
Standing time	Behavioural response	Helps to establish the behavioural reactions of the animals to its microenvironment in a non-invasive way
Water intake		
Feed intake		
Drinking frequency		
Defecating frequency		
Urinating frequency		
Rumination time		
Respiration rate	Physiological response	Helps to establish the physiological response oriented reactions to the microenvironment
Skin temperature		
Infrared thermal images/videos		
Hair cortisol	Endocrine response	Helps to identify the influence of heat stress on the ideal stress marker cortisol
Urine cortisol		
Faecal cortisol		
Temperature-humidity index	Barn climate changes	Indices used for quantifying climatic changes in order to describe the heat load on dairy cattle and its thresholds
Heat load index		
Cattle equivalent temperature index		
Subcutaneous microchips	Physiological response	Quantifying changes in the body temperature of dairy cattle
Implantable devices	Physiological response	Quantifying changes in the body temperature of dairy cattle
Temperature sensing ear tags	Physiological response	Quantifying changes in the body temperature of dairy cattle
Rumen-reticular boluses	Physiological response	Quantifying changes in the body temperature of dairy cattle
Intra rectal and vaginal devices	response	Quantifying changes in the body temperature of dairy cattle

Table 8.1 shows various non-invasive methodologies to quantify HS responses in dairy cattle.

Technological advancements have also resulted to the invention of several non-invasive biosensors like neck collars, leg bands, automated tympanic probes, GPS collars, accelerometers, and so on. All these technologies enable real time detection of a number of behavioral and physiological responses exhibited by animals. However, their usage is mostly limited to the developed or well organized farms. Moreover, these sensors are primarily used to livestock management purposes, their incorporation into HS associated studies are gaining a slow momentum.

Though such advanced biosensor technologies require substantial investments, they can effectively detect heat stressed animals at an earlier stage thereby enabling efficient management intervention to ameliorate its adverse impact that ultimately prevent economic losses to the farmer and ensure animal welfare.

Learning Outcomes

- Animal welfare and ethical issues enforced to find out suitable non-invasive techniques for accurate estimation of heat stress response.
- Non-invasive techniques such as activity sensors (behaviour measurement), urine, hair and faecal cortisol (endocrine responses measurement), are efficient methods to quantify heat stress response.
- Temperature sensitive subcutaneous microchips, ear tags and other implantable devices, rumen-reticular boluses, intra rectal and vaginal devices could be used for the continuous measurement of body temperature in farm animals.
- Infrared thermography is another vital non-invasive tool gaining importance to assess the thermal tolerance in farm animals.

8.9 Conclusions

Cows have different possibilities to react under heat stress. It was shown in different studies that dairy cows under heat stress have higher skin and core body temperatures, higher respiration rates as well as an increase in standing time. All parameters have proven to be useful evaluation criterias for the assessment of physiological-ethological reactions of dairy cows under heat load. The sensors to measure activity (accelerometers) and devices to measure body temperature (e.g., rumen boluses) already used in livestock farming are useful tools for the herd manager and for the scientist. The respiration rate visually counted on the individual animal is very sensitive and offers high potential in real-time monitoring for early detection of heat load. However, visual counting is extremely time-consuming and thus, difficult to implement in everyday life. Therefore, sensor-based parameters are the first choice.

8.10 Future Perspectives

Heat stress in dairy cows is often described with a THI above 68, although this index has some limitations such as the mistaken belief that all animals react in the same way to thermal factors, and it does not include other parameters, such as solar radiation, airflow and total hours of exposition. Based on the previously described findings, the individual animal should be of more interest in the future. Therefore, the reactions of individual cows to HS and the genetic background of these animals are the subject of an ongoing study, where the climatic parameters find intensive consideration as well. In this project, data from various digital applications, barn and animal-specific data, will be merged into a complete system in order to better manage, analyze, and interpret the individual information of the previous isolated applications and make them available in the form of a flexible and application-oriented decision support system. All measured data will be stored on a data platform and analyzed in order to find thresholds were the cows show a stress reaction. Furthermore, algorithms should be developed that process physiological and ethological reactions of the cows in a combined way and incorporate them into management decisions, so that complex deviations from normal animal behavior act as a signal.

Acknowledgements The authors gratefully acknowledge the staff of the Department of Engineering for Livestock Management at the ATB (Potsdam, Germany) and the staff of the dairy farm at the Agricultural Research and Education Center for Animal Breeding and Husbandry in Gross Kreutz (Brandenburg, Germany) for their assistance with experimental setup and animal care.

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Applications of Infrared Thermal Imaging and Rumen Boluses for Quantifying Heat Stress in Cattle

9

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Abstract

Heat stress is a leading cause of production and financial losses, not to mention decreased welfare, for animal industries globally. With climate change increasing the threat of heat stress to livestock enterprises, it is expected that these losses will become more apparent and significant in future years. Elucidating the impact of thermal challenges on cattle, and other species, has been a focal research area for many decades and is likely to continue in future years. Technological advancements have improved the capacity to collect large scale individualised datasets, however this then presents challenges for analysis and interpretation of data. Regardless, the increasing capability and sophistication of technology is

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V. Sejian et al. (eds.), *Climate Change and Livestock Production: Recent Advances and Future Perspectives*, https://doi.org/10.1007/978-981-16-9836-1_9

changing the way that thermal challenges are being investigated. Two such technologies that have been increasingly utilised are infrared thermography (IRT) and rumen temperature (T_{RUM}) via rumen boluses. Each of these technologies have been applied in various formats, with IRT in particular having inconsistent results. This chapter, will focus on the applications of IRT and boluses for the monitoring of heat stress in cattle and provide a perspective on the future opportunities and challenges for applying these technologies in the field.

Keywords

Animal sensors · Beef · Dairy · Heat load · Thermal challenge

Abbreviations

°C	Degree Celsius
AM	Ante meridiem
bpm	Beats per minute
BT	Body temperature
h	Hour
IPCC	Intergovernmental Panel on Climate Change
IRT	Infrared thermography
L	Litre
MID	Mid day
min	Minutes
PM	Post meridiem
RR	Respiration rate
SE	Standard error
SH AA	Shaded Angus cattle
SH BH	Shaded Brahman cattle
SH CH	Shaded Charolais cattle
THI	Temperature humidity index
T_{RUM}	Rumen temperature
UNSH AA	Unshaded Angus cattle
UNSH BH	Unshaded Brahman cattle
UNSH CH	Unshaded Charolais cattle

9.1 Introduction

Recent climate change models are predicting an increase in global temperatures by up to 6 °C, before the end of this century (IPCC 2021). Irrespective of the magnitude of this change, it is evident that our global environment is changing, such that over the last 40 years each decade has been warmer than the previous one (IPCC 2021).

The major adverse consequences of climate change include an increase in the number of extreme weather events, and of particular concern is the prediction of more frequent heat waves in conjunction with longer and hotter summers (IPCC 2021). These events are particularly important as climate change will have profound impacts on animals, despite the application of mitigation techniques. The interest in climate change and its impact on animals is not only centred around the fact that animals will be exposed to more persistent periods of chronic heat stress, but also on the ability of livestock to cope with climatic extremes. Specifically heat waves that are longer and hotter in comparison to those events that currently occur. Therefore, there is a need to investigate alternative non-invasive methods to evaluate how animals are coping with their thermal environment. Automated monitoring individual animal responses has the potential to identify animals that are not coping with heat stress (Islam et al. 2021a), and allow for targeted mitigation techniques to be applied. The global community is becoming increasingly reliant on technology and animal agriculture is not excluded from this. The adoption of 'smart' technologies in agriculture, regardless of sector, is increasing to support production efficiencies to achieve food security. Nevertheless, evaluating body temperature has and will always remain an important mechanism to quantify of heat stress in animals. However, collecting body temperature can be challenging under field and commercial conditions (Lees et al. 2018b). Traditional methods of obtaining body temperature has typically involved relocating animals to handling facilities and/or animal restraint, which are both associated with increases in body temperature (Mader et al. 2005; Lees et al. 2020a). Infrared thermography (IRT) and rumen temperature (T_{RUM}) collected by rumen boluses are two current non-invasive technologies that are available and negate the need to handle and restrain animals to measure body temperature.

9.2 Infrared Thermography

The use of IRT as a non-invasive measure of body temperature has been generating an increasing amount of interest. Infrared thermography technology appears to be useful in highly controlled circumstances, although IRT technology is currently unpredictable in field conditions and inconsistencies exists between studies. Infrared thermography measures the infrared radiation emitted from an animal, allowing for the determination of body surface temperature (McCafferty 2007), which can be used as a method for measuring and mapping the radiated heat loss at the body surface (Labeur et al. 2017). Infrared thermography has had extensive diagnostic applications in veterinary medicine (Schaefer et al. 2004; Metzner et al. 2014), particularly inflammation (McCafferty 2007) and disease detection (Schaefer et al. 2004; Schaefer et al. 2012). Furthermore, there have been numerous studies investigating IRT to understand the impact of scrotal heating on male reproductive function and scrotal thermoregulation (Cruz Júnior et al. 2015; Menegassi et al. 2015, 2016a, b; Wallage et al. 2017). Specifically, in regards to using IRT, eye temperature is a common focal region of interest and shows a greater potential to be a viable alternative non-invasive measure of body temperature although inconsistencies still exist (Church et al. 2014). This is likely associated with high blood flow and close proximity to the brain (Church et al. 2014). In addition, animal restraint is still often required, thus reducing the non-invasive aspect of IRT (George et al. 2014). Regardless, IRT has a clear advantage in that it is completely non-invasive and requires little expertise to implement. However, images must be captured at an angle, between IRT camera and animal, with an ideal angel of 90°, otherwise there is a rapid decline in radiation measured by the camera, which subsequently reduces the temperature reading (Speakman and Ward 1998). Nonetheless, most IRT studies show that body surface temperatures generated by IRT are typically generally below internal body temperatures (George et al. 2014; Wallage et al. 2017; Lees et al. 2018b). If a consistent departure from internal body temperature could be accureately determined, then it is feasible that a correction factor could be applied. Regardless, there are still questions concerning the accuracy of data, remembering that this technology was designed for engineering purposes and covering broad temperature ranges. When applied for use in animals it must be considered that small changes in body temperatures have considerable implications for wellbeing and production.

It is important to consider that the surface temperature of an animal is dependent on many separate and unrelated factors such as exposure to sunlight, moisture or dirt in the coat, type of coat and wind speed (McCafferty 2007; Hoffmann et al. 2013; Church et al. 2014). As discussed by Lees et al. (2018b), some consideration also needs to be extended to the emissivity value applied during IRT image analysis. Steketee (1973) describes an emissivity value of 0.98 for biological tissues. However, it is probable that the emissivity of the coat surface can be influenced by contaminants (McCafferty 2007), such as dirt (emissivity between 0.93 and 0.96) and water (emissivity = 0.96; Campbell and Norman (1998)). Therefore, determining the correct emissivity value where infrared thermography is used in field conditions and in animals with differing coat characteristics becomes difficult. However due to the linear relationship between radiative heat transfer emissivity, McCafferty (2007) suggested that the differences in body surface temperature due to environmental contaminants would be ≤0.5 °C, for the typical mammalian coat. Although, all heat stress physiologists would argue that a body temperature difference of 0.5 °C is biologically significant and represents a significant physiological change in body temperature (Lees et al. 2018b). Furthermore, the determination of body surface temperature from IRT relies on the body being a 2-dimensional shape and does not account for the natural curvature of the animal's body. It remains unclear what impact this has on the determination of body surface temperature. Therefore, it is clear that there is a level of assumed errors in the body surface temperature derived from IRT images.

Another confounding factor is that there is no predefined standard for the assessment of IRT images, therefore, it becomes difficult to compare results across studies (Lees et al. 2018b). The analysis, or extraction, of body surface temperatures from IRT images remains a laborious manual task that has been discussed at length between the authors of Wallage et al. (2017, Fig. 9.1), Labeur et al. (2017, Fig. 9.2)

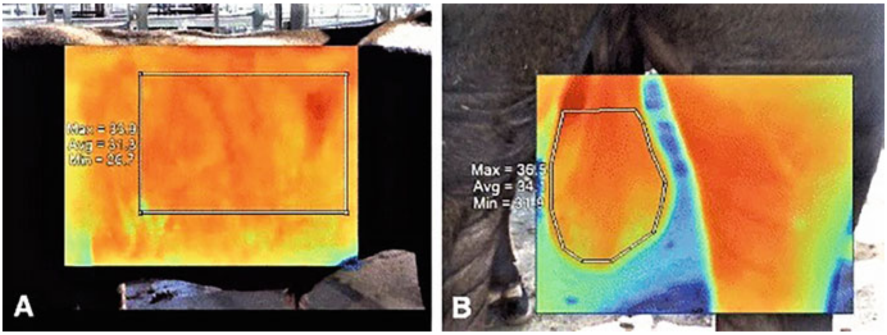

Fig. 9.1 An example of infrared thermal (IRT) image using the FLUKE software, showing an infrared thermal (IRT) image and regions of interest consisting of the flank (**a**) and scrotum (**b**) showing the use of the polygon tool to calculate minimum, maximum and mean temperature of the selected area as described by Wallage et al. (2017)

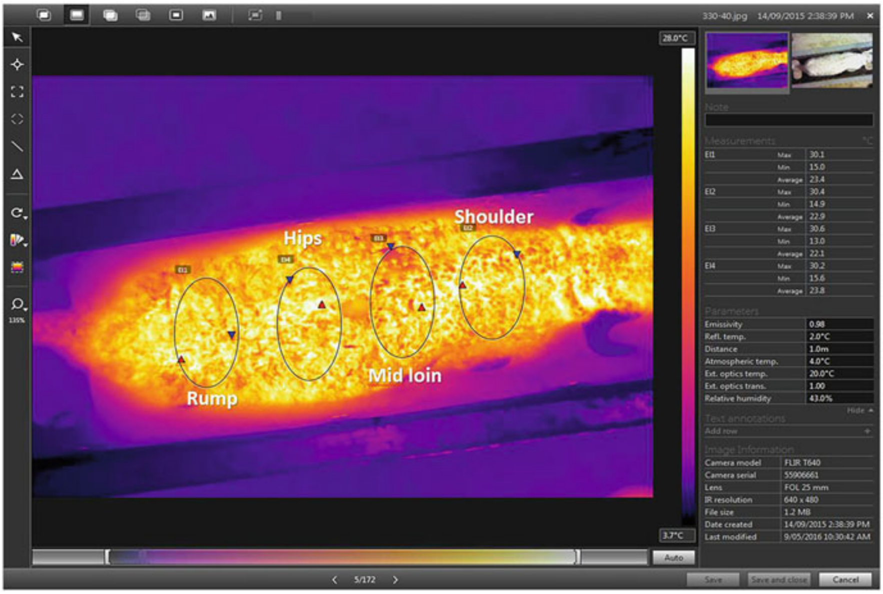

Fig. 9.2 An example of infrared thermal image interpretation and analysis using the FLIR software, displaying the arrangement of four regions of interest across the back of a lamb, being (1) shoulder, (2) mid loin, (3) hips and (4) rump as described by Labeur et al. (2017)

and Lees et al. (2018b) and was explicitly noted by Cuthbertson et al. (2019). Progress towards automating this process would be of benefit for the animal sciences. It is probable, in order to gain a complete understanding of body surface temperature obtained by IRT, that analysis should occur at the individual pixel level.

For IRT to become a commercially viable tool that provides accurate real-time results, an automatic method of data processing is needed (Cuthbertson et al. 2019).

9.3 Rumen Boluses

In many instances technological advancements, specifically rumen boluses, have considerably improved the volume of individualised data that can be collected (Lees et al. 2018a). This is an important consideration as previously studies investigating body temperature have typically been restricted to $\leq$10 days. Rumen boluses present a non-invasive method of obtaining body temperature over long periods of time, months, and potentially years without compromising animal welfare (Lees et al. 2018a). As such, T_{RUM} is rapidly becoming the most prominent method to obtain body temperature data. Two obvious challenges with utilising T_{RUM} are (1) heat generated from digestion (Beatty et al. 2008), and (2) rapid declines in T_{RUM} associated with water intake (Bewley et al. 2008; Ammer et al. 2016; Cantor et al. 2018). Recently, Cantor et al. (2018) quantified that return to baseline T_{RUM} is dependent on water temperature and amount of water consumed. The authors were able to show that 22.7 L of 1.7 °C water took 103 min for T_{RUM} to return to its baseline temperature (Cantor et al. 2018). These findings confirm that water intake results in a dramatic, but temporary, decrease in rumen temperature that is relatively quick to return to baseline temperature. Furthermore, the time required to for T_{RUM} to return to baseline temperature depends on i) the amount of water consumed at each intake and ii) the temperature of the water ingested (Cantor et al. 2018).

As with the majority of data captured by data loggers, T_{RUM} data requires extensive data cleaning and management prior to analysis. Specifically, with T_{RUM} data, decisions regarding the management of water intake need to be considered. Recently Vázquez-Diosdado et al. (2019) developed a thresholding algorithm to detect drinking events. Regardless of the management of drinking events, rumen temperature follows a diurnal rhythm (Lees et al. 2018a, 2019a, 2021) (Fig. 9.3). Across studies, T_{RUM} generally increases between 08:00 h and 20:00 h, and decreases, between 20:00 h and 08:00 h. Similar diurnal trends in alternative measures of body temperature have been reported for T_{REC} (Gaughan et al. 2004), for abdominal temperature (Lefcourt and Adams 1996; Brown-Brandl et al. 2005; Gaughan et al. 2010), and for tympanic temperature (Davis et al. 2003; Mader et al. 2010). Ammer et al. (2016) highlighted that T_{RUM} was influenced by THI, in barn housed lactating cows. Lees et al. (2018a), showed that that breed, ambient conditions, and availability of shade have an influence on T_{RUM}. This suggests that T_{RUM} is influenced by ambient conditions and by mitigation techniques.

In our previous, and current studies, rumen boluses capture data at 10 min intervals, thus capturing 144 data points within each 24-h period (Lees et al. 2018a, b, 2019a, 2020b, 2021). The collection of larger data sets is advantageous as it allows for more reliable statistical interpretations to occur. However, the difficulty becomes identifying appropriate statistical modelling techniques for these large time series data sets. This highlights the importance of consulting with

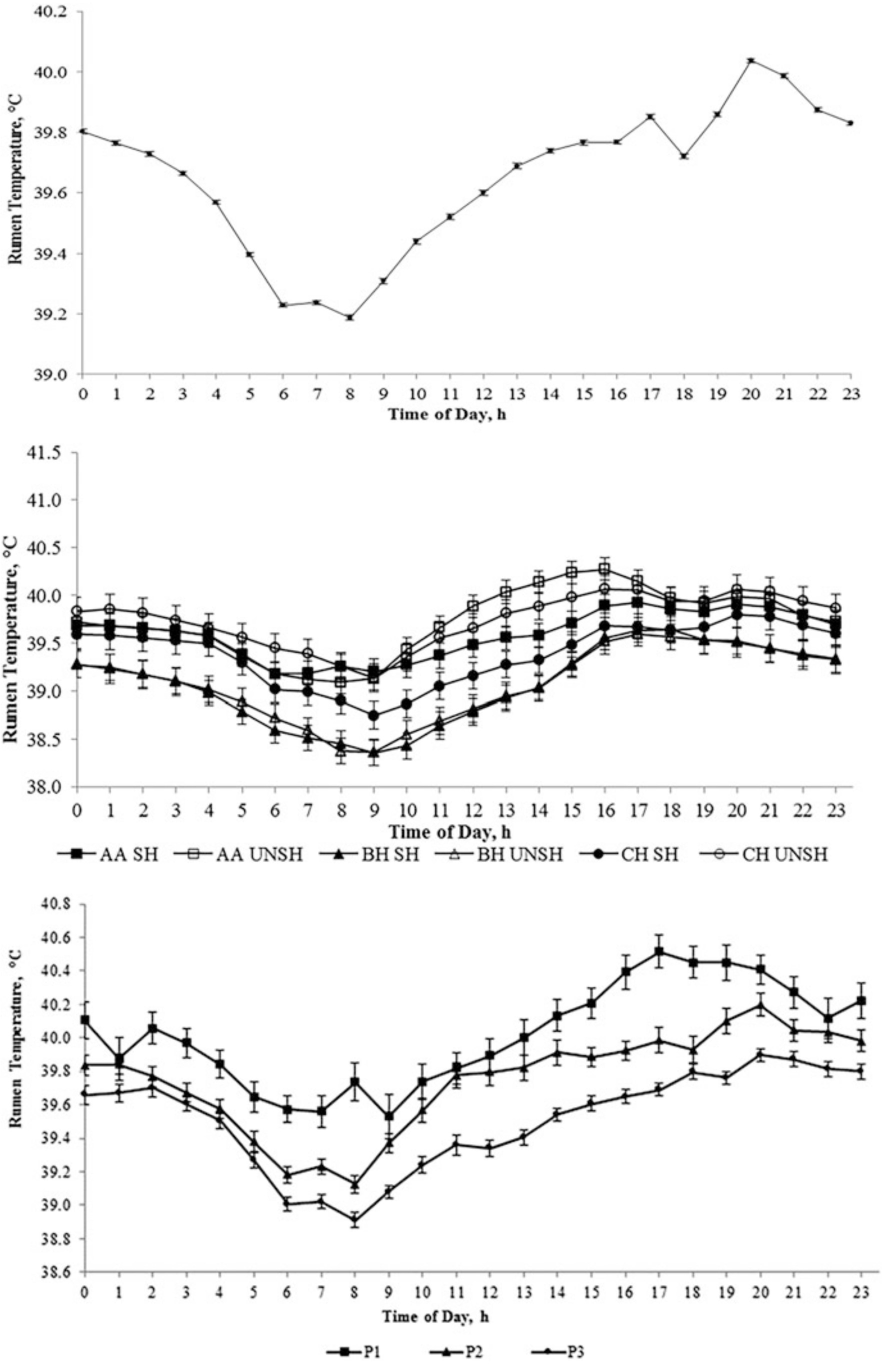

Fig. 9.3 Diurnal rhythm using mean hourly (h) rumen temperature (T_{RUM}) of (**a**) shaded Angus steers over 128 days as adapted from Lees et al. (2019a); (**b**) un-shaded Angus (UNSH AA), un-shaded Charolais (UNSH CH), un-shaded Brahmans (UNSH BH), shaded Angus (SH AA),

biometricians/data scientists that have an intricate knowledge of these types of data sets and is imperative to the successful evaluation of these data. In addition, the generation of these large, individualised, data sets are inherently associated with an increase in computational power required in order to analyse the aforementioned data sets. The generation of these data sets is valuable for research activities, but may be of little value to commercial industries. As such those companies that we have worked within in our previous, and current studies, have established data management software, and/or online databases and mobile applications, that have background algorithms to provide the 'need to know' information for commercial producers, i.e. oestrus detection, high and low body temperature alerts, and insufficient water and feed intake. Thus, provides a versatile and effective animal health management tool that extends beyond a heat stress application.

Learning Outcomes

- Infrared thermography (IRT) and rumen temperature (T_{RUM}) measured by rumen boluses are emerging as viable non-invasive technologies to quantify heat stress response in cattle.
- Both IRT and T_{RUM} are advanced technologies to provide detailed individual animal based datasets for body temperature, however this then presents challenges for analysis and interpretation.
- Automated detection of panting score using accelometer based sensors can also serve as a viable alternative to assess the heat load status in cattle.

9.4 Conclusions

The evaluation of body temperature is a fundamental aspect of quantifying heat stress and numerous methods to determine body temperature exists. Given the changing global environment, the negative impacts of heat stress are likely to increase and expand into more temperate regions. As such non-invasive methods of obtaining body temperature that are fast, efficient and reliable need to be investigated. However, to be considered as an alternative method of determining body temperature, and a viable commercial management tool, new technologies need to be rapid and reliable. Currently, the inconsistencies and the laborious nature of IRT evaluations render this technology not yet suitable for application in commercial settings. However, if IRT image evaluation can be automated and used in conjunction with machine learning techniques as described by Joy et al. (2021), new

Fig. 9.3 (continued) shaded Charolais (SH CH) and shaded Brahman (SH BH) steers over 130 days adapted from Lees et al. (2018a); and (**c**) for three 6 day periods (P1, P2 and P3), adapted from Lees (2016) opportunities will emerge for IRT to become translatable for commercial industries. Rumen temperatures appear to be a reliable method to quantify heat stress, however currently may not be economically viable for commercial industries. Technology certainly has an important role in quantifying heat stress, the continued development and sophistication of these technologies will support its extension and adoption in future years.

9.5 Future Perspectives

In future years, autonomous monitoring for heat stress, and other ailments, are likely to be based on minimally-invasive technologies such as IRT and rumen boluses, and likely accelerometer-based technologies. This will enable real-time solutions to animal responses under various production systems and environmental conditions (Islam et al. 2021a). The future opportunities for 'smart' technologies in animal science are unlimited. Currently, the greatest limitations are associated with our ability to extract and analyse data from research and commercial perspective respectively. Machine learning is the most probable method to support the automation of data analysis moving forward and has been applied to animal behaviour in extensive pastoral systems (Fogarty et al. 2020; Chang et al. 2022). Machine learning techniques are likely to improve the effectiveness of both T_{RUM} and IRT as real time measures of heat stress in ruminants. Recently Joy et al. (2021) showed that machine learning improved the reliability of IRT to monitor body temperature in sheep, although individual IRT images appear to have been manually processed. Thus, automating the processes involved in IRT images and interpretation would be of great benefit.

Body temperature is going to remain the gold standard for determining an animal's thermal status. However, under commercial conditions collecting body temperature may not be a viable option even with technological advancements. Changes in respiratory dynamics, i.e. respiration rate and panting score, also provides a good indication of heat load status in cattle (Mader et al. 2006; Gaughan and Mader 2014), and sheep (Lees et al. 2019b, 2020b). Specifically, under field and/or commercial conditions the assessment of panting score (Table 9.1) is a viable alternative to using body temperature to assess the heat load status of cattle. Gaughan and Mader (2014) showed that there was a strong relationship between body temperature, respiration rate and panting score, confirming that panting score is a good management tool for the assessment of heat stress (Fig. 9.4). Advancements in accelerometer technologies may support the automated detection of respiration rate and/or panting score in cattle. Recent heat stress specific studies are showing that accelerometer-based ear tag sensors can monitor panting scores in cattle under 'mild to moderate' panting (0–2, Table 9.1) (Islam et al. 2020) and 'high to severe' panting (0–4, Table 9.1) (Islam et al. 2021b).

Rumen temperature boluses are likely to be cost prohibitive on a commercial scale and with the laborious task associated with IRT image interpretation, other alternative technologies need to be considered. Consideration is also needed

Table 9.1 Modified assessment of panting score and description of breathing/panting condition

Panting score	Breathing condition
0	No panting
1	Slight panting, mouth closed, no drool, slight chest movement
1.5	Fast panting, mouth closed, no drool, fast easily observed chest movements
2	Fast panting, drool present, no open mouth
2.5	As for 2, but occasional open mouth panting, tongue not extended
3	Open mouth and excessive drooling, neck extended, head
3.5	As for 3, but with tongue out slightly and occasionally fully extended for short periods
4	Open mouth with tongue fully extended for prolonged periods with excessive drooling. Neck extended and head up
4.5	As for 4, but head held down. Cattle "breath" from flank. Drooling may cease.

Adapted from Brown-Brandl et al. (2006); Mader et al. (2006), Gaughan et al. (2008) and Lees et al. (2021)

regarding the implementation of 'smart' technologies in extensive environments, for example the northern pastoral zones in Australia and other regions in the world, that have vast areas without access to electricity or access to internet. Mechanisms to overcome these limitations will provide opportunities to investigate how thermal challenges are impacting animals within grazing pastoral systems. Recently, Mufford et al. (2021) showed that both behavioural and physiological, i.e. respiration rate, heat stress responses could be determined by unmanned aerial vehicles in feedlot and pasture settings. Moving forward, perhaps the combined use of IRT cameras, accelerometer technologies, unmanned aerial vehicles and T_{RUM} will combined become the 'gold standard' for heat stress monitoring in both research and commercial environments as we progress into the age of precision livestock farming.

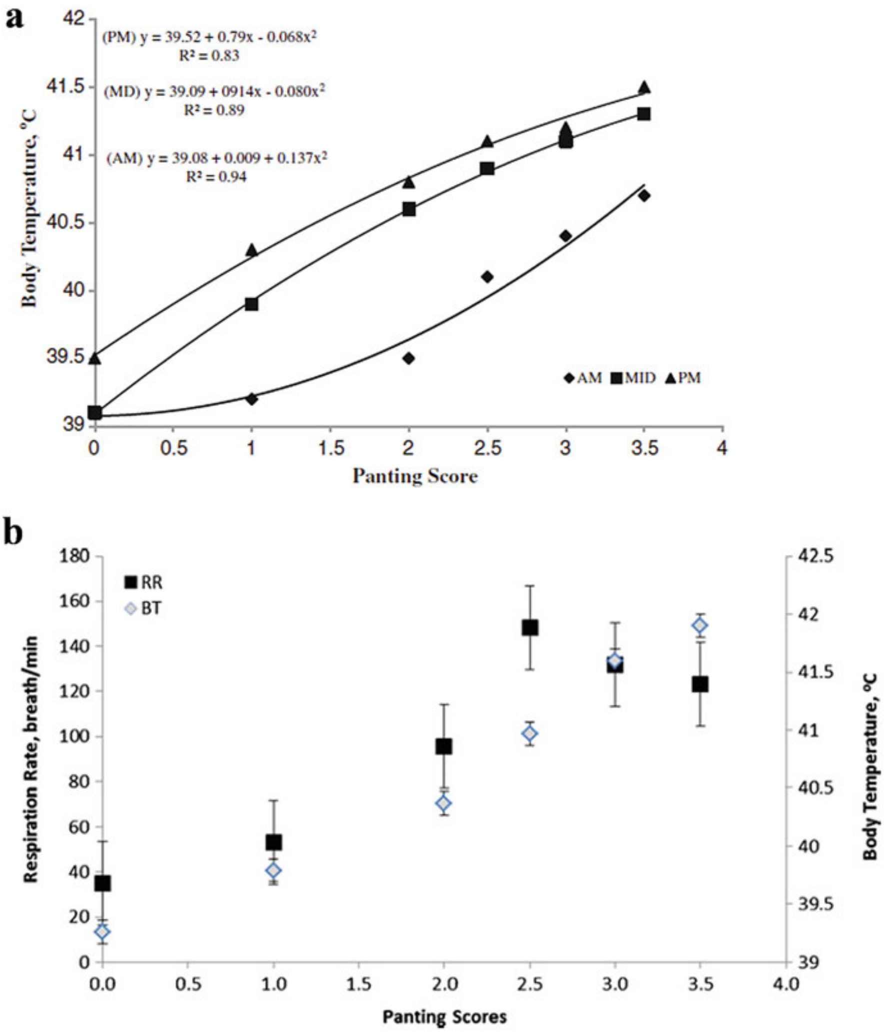

Fig. 9.4 (**a**) Relationship between body temperature and panting score at 06:00 h (AM), 12:00 h (MID), and 16:00 h (PM) and (**b**) Relationship between body temperature (BT ± SE; °C) and respiration rate (RR ± SE; bpm) at panting score values of 0, 1, 2, 2.5, 3 and 3.5. Adapted from Gaughan and Mader (2014)

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Skin Based Novel Approaches for Establishing Climate Resilience in Goats

10

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V. Sejian et al. (eds.), *Climate Change and Livestock Production: Recent Advances and Future Perspectives*, https://doi.org/10.1007/978-981-16-9836-1_10

Abstract

Climate change and food security are the two primary issues that daunt agricultural researchers across the globe. The world is battling to find solution to both these issues as we need to feed the expected human population of over 9.6 billion by 2050. Therefore, it is very vital to sustain agricultural production and livestock sector is particularly considered important for ensuring animal protein. Efforts are also needed to identify the most heat resilient livestock species which could produce optimally during the adversities of changing climate. Goats are considered the ideal climate resilient species. Researchers are currently working on novel approaches to assess thermo-tolerance in livestock. This chapter is one such attempt to elucidate the basic adaptive mechanisms in goats based on skin approaches. The hair and skin are important targets to establish climate resilience as these are the parts in animals which first get exposed to the microclimate. The coat color is a vital variable to establish differences in thermo-tolerance in goats. Further, the hair based variables such as hair characteristics and cortisol could serve as reliable indicators for climate resilience. The skin based variables such as sweating rate, active sweat glands distribution, and infrared thermal images may play vital role in establishing climate resilience in goats. The latest next generation sequencing services such as skin metagenomics, skin transcriptomics and skin epigenetic changes could revolutionize pathways for establishing climate resilience in goats. Thus, this chapter highlights to the readers the above listed skin based novel approaches to establish climate resilience in goats.

Keywords

DEGs · Goat · Hair · Infrared thermal images · Skin · Skin microbes

Abbreviations

ASIP	Agouti signaling protein
CREB3L1	CAMP responsive element binding protein 3-like 1
DCT	Dopachrome tautomerase
DEGs	Differentially expressed genes
DNA	Deoxyribonucleic acid
GDP	Gross domestic product
GHG	Greenhouse gas
GWAS	Genome wide association study
HPA	Hypothalamic-pituitary-adrenal
HSP	Heat shock protein
mRNA	messenger ribonucleic acid
NGS	New generation sequencing
PMEL	Premelanosome protein
RNA	Ribonucleic acid
TRPM 1	Transient receptor potential cation channel subfamily M member 1

TYRP1	Tyrosinase-related protein 1
UV	Ultra-violet

10.1 Introduction

Climate change has emerged as the top most environmental challenge that mankind had to face which is persistently threatening the well-being of future generations. The alarmingly rising trend in global warming, with its erratic outcomes, has been imposing a huge risk on the ecosystem, animal diversity and food security. Nearly 45% of the earth's land surface is occupied by the livestock sector, most of which are located in regions experiencing harsh and variable environments which are unsuitable for other uses. The livestock sector also plays a significant role by contributing to nearly 40% of the global agricultural gross domestic product (GDP).

In addition to climate change, the exponentially rising human population along with depletion in agricultural land use due to human invasion is also exerting a massive pressure on food security. Though the livestock are thought to be least affected due to climate change, when compared to other agricultural sectors, this also holds a unique feature. While the livestock sector faces severe negative implications due to the climatic variability, this sector also contributes to climate change. Livestock production across the world contributes directly and indirectly about 18% of the greenhouse gas (GHG) emissions. Therefore, the livestock sector is of significant importance from the current climate change perspective for its projected role to ensure livelihood and also contribution to GHG emissions.

Numerous studies have been reported over the past decades that would assess the impact of climate change on livestock production, compare this effect across species, breeds and also at individual population level. Furthermore, a set of researchers have also opted for a parallel study to establish ameliorative and mitigation strategies to combat the adversities of climate change. In simpler terms, climate change and livestock production can be considered as an entirely distinct field of science having several branches. There are attempts to screen climate resilient livestock breeds with the intention of identifying agro-ecological zone specific breed. This chapter is therefore an attempt to collate and synthesis information pertaining to skin based novel approaches to quantify heat stress response in goats. Such information could be very valuable for future livestock production as the world is battling to find solution to ensure food security to meet the requirements of growing human population by 2050.

10.2 Advantage of Goat Over Other Species

Among all the livestock species, small ruminants are considered to be the go-to-go species that can relatively adapt well and produce efficiently despite the distressful climatic conditions. Furthermore, goats are proposed to be the ideal climate animal model especially in the tropical environment. For the unversed, a major proportion of the worlds' livestock population is distributed across the developing countries which experience most of the harsh environmental conditions. Additionally, these regions have a prevailing tropical environment wherein livestock farming is mostly characterized by extensive or semi-intensive farming practices involving small scale farmers.

Goats are tipped to thrive better in such climatic conditions for their innate abilities to cope with environmental extremities, sturdy and hardy nature. Goats possess the ability to survive well even on low pasture and additionally they possess unique feeding behavior. The narrow muzzle, mobile upper lip and prehensile tongue in goats gives them an add on benefit to access forage from the soil level and also for that matter, consume fodder resources like, shrubs, thorns spines, etc., that are usually not consumed by other livestock species. Moreover, the bipedal stance behavior that is uniquely exhibited by goats gives them the superiority to have access to tree fodder which are inaccessible to other ruminants due to the height. In addition to these features, goats also possess the ability to travel longer distances in search of feed and water. Moreover, goats are also stated to have better digestive efficiency in comparison to other ruminants.

Moving ahead to the crucial climate resilience traits, goats in general are known for their excellent thermal tolerance abilities. These species possess most of the vital adaptive feature that enable them to survive efficiently in the extremely hot climatic conditions. Moreover, goats are also considered to be extremely draught tolerant species. They possess an inherent potential to conserve its body water reserve (efficient rumen function), suppress evaporative water loss (modulating sweating and respiratory mechanism) and also by reducing the excreted water (through urine concentration and dry feces/pellets). Goats are well known for their better resistance to diseases when compared to other livestock species. This is also a very crucial advantageous trait of goats, especially on being aware of the impact of climate change on health status in animal. Another worthy character possessed by goats is its relatively lower enteric methane production ability. Therefore, goats stand out to be the ideal climate resilient species that not only can sustain the climatic adversities and yield quality products, but can also contribute minimally to GHG emission from livestock.

10.3 Significance of Assessing Thermo-Tolerance in Indigenous Goats

The theory of goats being the ideal climate resilient animal species in comparison to other ruminants has been emphasized and is also gaining concrete evidences for its establishment. Having said there, the concept of breed variations also prevails. Time immemorially it has been proved that indigenous germplasm overpowers the exotic breeds for their immense thermo-tolerance and disease tolerance potential. Majority of the livestock breeding practices have primarily focused to enhance the productive potential in animals via incorporation of exotic blood line that are high producers. Undoubtedly, such approaches did boost the livestock production multifold times, however over the recent past, livestock breeder especially those located in the tropics are raising their concern over such breeding practices. With climate change and its impact on livestock production and food security being inevitable, there is an increasing pressure to conserve and propagate the use of indigenous livestock species.

The productive potential and climate resilience ability of indigenous livestock species need to be studied intensely. There are substantial reports comparing the productivity, thermo-tolerance and also disease resistant abilities between exotic, crossbred and native breeds. However it is necessary to understand the variations among the indigenous germplasm too. Based on the research outputs published from a series of experiments that were under the leadership of Dr. Sejian and co-workers (Shaji et al. 2017; Aleena et al. 2018; Pragna et al. 2018), significant differences in the thermal resilience potential of indigenous goat breeds were observed. The authors compared the adaptive potential of three indigenous South Indian goat breeds, Osmanabadi (Karnataka), Malabari (Kerala) and Salem Black (Tamil Nadu) on exposure to summer induced heat stress in Karnataka state of India. Based on the cumulative results obtained from this project, it was interesting to note that the Salem Black goat outperformed Malabari and Osmanabadi goats (that were native to the experimental location). Thus the Salem Black goats were found to adapt well for heat stress in addition to producing efficiently. This therefore urges the need to conduct similar studies, exploring the indigenous goat breeds, so as identify and promote the use of agro-ecological zone specific goat breeds.

There still lies a lot of hidden intricacies in understanding the thermal resilience potential of indigenous goat breeds which needs to be addressed in the immediate future. Thus, this also opens the scope for identifying and assessing novel adaptive mechanisms that could be adopted by these indigenous goats.

10.4 Inter-Relationship Between Skin and Microenvironment in Goats

Skin is the primary defense barrier that protects the animal from all the external adversities. Unfortunately, skin is an underrated tissue for its proposed significant role in maintaining homeothermy within the individual. Skin is among the very first

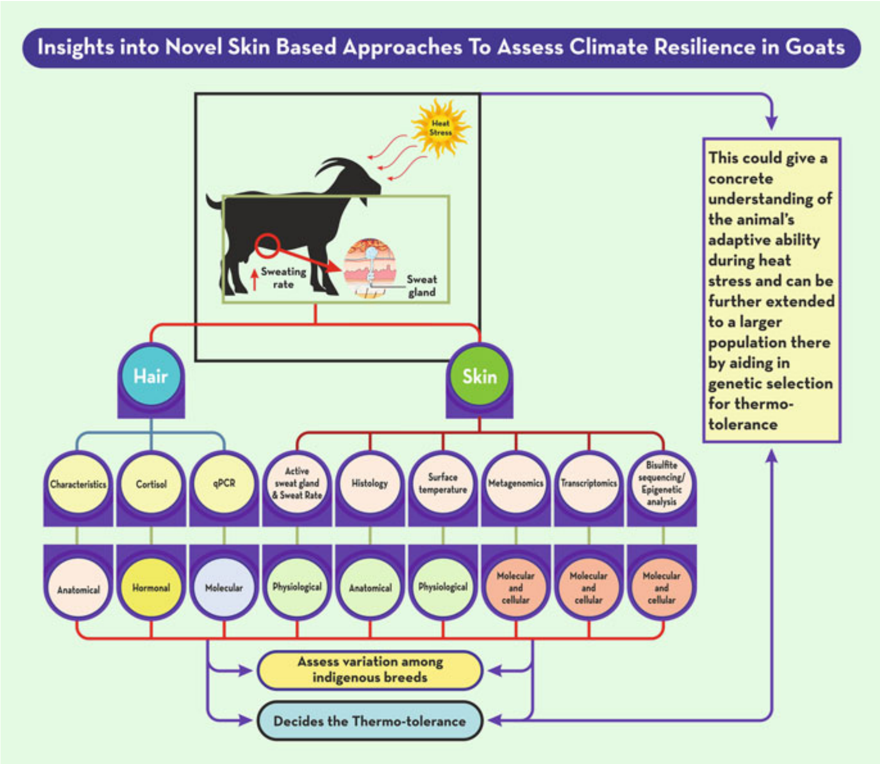

Fig. 10.1 Overview of skin based approaches to assess climate resilience in goats

body component to be exposed to both macro and micro environmental fluctuations. For a matter of fact a portion of micro environmental alterations are effectively tackled at the skin level itself. A number of factors aid to maintain the integrity of this tissue which in itself hosts a complex niche that gets affected due to the slightest alterations in the external environment.

The hair, coat and skin characteristics vary among species, breed and also between individuals. These alterations have a high association with the adaptive response exhibited by an individual. Therefore, unravelling the complexities at the skin level could pave way for path-breaking research findings in the area of climate change and livestock production. There are handful of reports assessing some of the basic skin and its associated characteristics with respect to climate change, more specifically heat stress, in livestock. However, like mentioned earlier, these are quite superficial and needs to be explored further, considering a deeper and holistic approach. Figure 10.1 illustrated an overview of skin based approaches for assessing climate resilience in goats.

10.5 Skin Based Novel Approaches for Establishing Climate Resilience in Goats

10.5.1 Coat Color

Coat color is one of the well established and grossly observable characteristic that has been associated with thermal stress in all livestock species. It is a proven theory that darker color, specifically black, absorbs solar radiation while lighter shades, white, reflect solar radiation. Therefore it is a foregone conclusion that goats with black or darker coat would be more influenced during heat stress event when compared to goats with white or lighter coat. However, researchers have also argued on the protective role of black coats in goats that are found across tropical regions. As one can reckon most of the indigenous goat breeds have colored coats. Additionally some of the breeds that are well known for its hardy and sturdy nature are black in color. The darker coat color in such scenario has been associated with the adaptive response of goats wherein the melanin (responsible for dark pigmentation) produced shields the animal from the harmful ultra-violet (UV) rays of the sun.

In a recent report from Democratic Republic of Congo by Baenyi et al. (2020), the effect of coat color and sex on heat stress response was evaluated in goats. The authors conducted the study in male and female goats with black, grey, brown and white coat color that were exposed to solar radiation during summer. The impact of heat stress in goats was observed through significant alterations in a number of physiological, hematological and biochemical variables. Moreover, female goats having black coat color were observed to be most affected during heat stress.

Furthermore, in a classic study that was dated back to the year 1995 by Acharya and co-workers, heat tolerance in Sirohi goats with relevance to their coat characteristics were assessed. Their study revealed higher alteration in physiological responses to heat stress (rectal temperature, respiration rate and pulse rate) in goats with black coat followed by dark brown, light brown and least in white coat goats. The authors concluded goats with white or light brown coat to possess higher tolerance to heat stress than those with dark brown or black coat.

10.5.2 Hair Based Methodologies

Hair characteristics are another important variable having high relevance in livestock from climate change perspective. The hair characteristics like hair length, diameter, density, and medullation play a crucial role in ensuring thermal insulation/heat dissipation during the climatic extremities. In a study led by Riberio et al. (2018) on Brazilian creole goats, the effect of dry and rainy season prevailing in the Brazilian semi-arid region was assessed on the goats' endocrine and physiological profiles. The authors stressed upon the significance of hair variation to maintain homeostasis in animals especially during seasonal alterations. The authors discussed that animals having short, thick and well-seated hair are better suited for tropical conditions. Furthermore coat thickness is also an important variable that aids in homeostasis during environmental alteration in goats.

Hair also serves as a potential biological sample to assess the cellular and molecular alterations occurring in an animal due to heat stress. Cortisol, considered as an ideal heat stress marker, is usually estimated from blood samples. With the rising ethical and animal welfare concern, researchers are encouraged to adopt non-invasive methodologies during their experimental studies. Hair cortisol estimation is one such approach that is projected to satisfy the animal ethical concern by being non-invasive and also providing accurate results. Based on the experiment conducted by Dulude-de Broin et al. (2019) in rocky mountain goats, hair samples were concluded to be valid biomarkers that can be used to assess the HPA-axis activity provided the confounding variables are taken into consideration.

Nucleic acids, DNA and RNA, which can be extracted from hair follicles, can also be used to assess the heat stress induced molecular changes in goats. The DNA and/or RNA extracted from hair follicles can pave way for molecular studies like, relative gene expression, GWAS, mRNA and other high end next generation sequencing approaches. Though the use of this biological sample for such studies is limited in goats, their adoption is increasing over the years.

10.5.3 Significance of Active Sweat Gland and Sweating Mechanism in Goats

Animals adopt several mechanisms to maintain their body core temperature when exposed to harsh environmental conditions. Increased respiration and sweating are among the many physiological adaptive strategies that aid to dissipate excessive body heat in goats under heat stress. Goats can efficiently utilize their evaporative cooling system-respiratory (increased respiration/panting) and cutaneous (sweating); when exposed to hot climatic conditions. In a study reported decades ago on dehydrated Black Bedouin goats, Dmi’el (1986) proposed that goats enable brain cooling via selective sweating and panting modulation.

One of the simplest methods to estimate sweating rate in goats is by using cobalt chloride impregnated chromatography paper disc (Berman 1957). Though this methodology includes mild human-handling of the animal, yet it still can be considered to be a non-invasive approach. Moreover, being easy-to-use, portable and cheap, this methodology is field friendly and farmer friendly too. Though there are no much studies reported yet, that assess the sweating rate fluctuation in goats exposed to heat stress; there are some reports in sheep (Sejian et al. 2012), cattle (Schleger and Turner 1965; Dikmen et al. 2014) and buffaloes (Debbarma et al. 2020). Therefore, recording both sweating rate and number of activated sweat glands could add on to the novel methodologies to estimate thermal resilience in goats.

10.5.4 Applications of Infrared Thermal Imaging for Assessing Skin Based Thermo-Tolerance

Body temperature is among the primary variable that is recorded in every heat stress/ climate change related study in any species. Though there are several possibilities to record body temperature, recording rectal temperature is the traditionally followed method. Technological advancements have led to the development of infrared thermometers that can record the body surface temperatures. Adding on to this is the development of infrared thermometry, which not only records the surface temperature but also gives the thermal image of an animal. This technology is been widely accepted and credited for its vast application in the livestock research. Not only does it aid in providing an accurate measuring but also is non-invasive.

In an experimental study, the resilience of three indigenous goat breeds, Osmanabadi, Malabari and Salem Black, to heat stress were assessed based on a number of physiological traits and *HSP70* mRNA expression by Aleena et al. (2018). In this study, the authors recorded the body surface temperatures using infrared thermometer. Using this technique, a significantly higher head, should and flank surface temperature was observed in Osmanabadi and Salem Black goats during the afternoon exposure to summer induced heat stress when compared to their control. Furthermore, the heat stressed Malabari goats also exhibited significantly higher head and flank surface temperature when compared to their control however their surface temperature recorded were significantly lower than that of Osmanabadi and Salem Black goats. The authors attributed this significant difference to the coat color wherein Malabari goats have pure white coat while Salem Black and Osmamabadi goats have complete black coat.

In another study by Hooper et al. (2018), the physiological and cellular responses of Saanen goats on exposure to acute heat stress was studied. The Physiological variables recorded included respiration frequency, rectal temperature, dorsal and tail surface temperature (using infrared thermometer); ocular and mammary gland temperature (using infrared thermography). Based on this study, the authors could understand the heat loss dynamics in goats. Infrared thermography therefore can be stated as a potential non-invasive methodology having high relevance for assessing climate resilience in goats.

10.5.5 Application of Skin Based Metagenomics

Metagenomics primarily looks into the isolation of genetic material that is recovered directly from environmental samples. This next generation approach aids in elucidating the microbial communities that inhabits in a specific niche and unravels their population structure, genetic diversity and also their ecological role. Apart from identification of novel organisms, this methodology also gives a sneak peek into the functionalities of the associated microbes (Bashir et al. 2014).

In livestock species, metagenomics studies have been mostly used to assess the rumen, fecal, udder and gastrointestinal microbiome. Narrowing down to studies associated with heat stress, the rumen microbiome is the most explored niche using metagenomics. Apart from influencing the animal, heat stress also leads to significant alteration in the microbial populations that inhabit in an individual. Such alterations based on the niche considered, would have varied implications on the productivity and performance of the host. For instance, alteration in rumen microbiota can impair feed digestion in animals and can also instigate the methanogenic bacteria that harbors in the rumen.

Skin surface hosts a unique ecosystem comprising of varied micro-organism which may also get altered due to heat stress. Alteration in the skin microbiota may affect the skin integrity, skin characteristics and also may impair the primary immune barrier provided by skin tissue. So far there are no studies reported to assess the skin microbial diversity in heat stressed goats using metagenomics approach. This therefore makes it a novel concept to explore and assess thermo-tolerance in goats.

10.5.6 Skin Transcriptomics Based Adaptive Assessment in Goats

As mentioned in the earlier sections, the skin tissue, being the first tissue to be exposed to climatic alterations, undergo a number of molecular changes. Such alterations can be assessed by using the advanced methodology of transcriptomics approach. This again is very minimally explored area in heat stressed goats, however is proposed to provide greater inroad to unravel the hidden intricacies. Over the past few years, there are increasing studies reported that assess the impact of heat stress in goats through transcriptomics approach. However there is a scarcity of reports on skin transcriptomics in goats exposed to heat stress or other environmental stress.

So far, most of the reported studies on skin transcriptomics in goats are associated with either exploring the genes associated with coat color or hair growth. On comparing the skin transcriptomics profile of Laiwu Black and Lubei White goats, 102 genes were reported to be differentially expressed (Peng et al. 2019). Among these, six genes predominantly linked with pigmentation were identified; these included agouti signaling protein (*ASIP*), dopachrome tautomerase (*DCT*), CAMP responsive element binding protein 3-like 1 (*CREB3L1*), transient receptor potential cation channel subfamily M member 1 (*TRPM1*), premelanosome protein (*PMEL*), and tyrosinase-related protein 1 (*TYRP1*). A similar study providing insight into the transcriptional regulation of black and white coated regions in crossbred goats (with black head and white body) was studied by Xiong et al. (2020). Among the 165 differentially expressed genes (DEGs) identified on comparing the black with white coated skin, a number of genes controlling pigmentation of skin and hair follicles were identified. Furthermore, Agouti, *DCT*, and *TYRP1*, were reported as the key DEGs associated with the melanogenesis pathway in the examined crossbred goats.

Though such studies provided some vital information pertaining to the DEGs associated with skin pigmentation, none of these studies assessed the alterations upon heat stress exposure. So far there are no such reports documented in goats however there are very minimal studies conducted in cattle. Therefore, this opens up the urge to incorporate this methodology to assess heat stress impact in goats.

10.5.7 Application of Skin Epigenetic Changes as a Novel Tool for Assessing Climate Resilience in Goats

Acquiring genomic information has substantially increased the accuracy of selection/prediction with an appreciable time reduction, to assess the impact of heat stress on animals and also identify climate resilient individuals. Heat stress induces a number of cellular and molecular changes in an animal some of which is evident from altered gene expression profile. Epigenetics is one such phenomenon that controls gene expression. Epigenetics basically describes the gene-environmental interaction that results to evident alterations at the phenotypic level. Therefore, assessing the epigenetic profile in animals under heat stress, which is a classical host environment interaction, provide a better understanding of the molecular alterations occurring in an individual.

DNA methlyation and histone modification are the common mechanisms that describe epigenetic alterations in animals. Among these, DNA methylation is better studied epigenetic regulatory mechanism in livestock. There are very limited reports assessing the epigenetics profile of heat stressed livestock species. This should thereby instigate researchers working on climate change and livestock production to apply the novel concept of epigenetic profile of animals under environmental stress. Apart from influencing the gene expression, epigenetic alterations that are triggered in an individual due to environmental stress can also be passed down to its future generations. This therefore could explain the epigenetic inheritance acquired by an individual from its ancestors.

In a heat stress study lead by Del Corvo et al. (2021), the methylome patterns of Nellore and Angus cattle were explored. Using Reduced Representation Bisulfite Sequencing, the genome-wide DNA methylation profiles for the heat stress resilient Nellore and heat stress susceptible Angus cattle were obtained. The authors identified 819 genes that were significantly methylated during heat stress and recovery period in cattle. Among these 351 genes were specific to Angus while 366 genes were unique to Nellore. It was observed that heat stress resulted in breed-specific responses wherein genes associated to stress response and cellular defense were under methylated/hypo-methylated in Nellore while some of these genes were hyper-methylated in Angus. This study therefore signifies the impact of heat stress at the epigenetic level in animals and urges the need for extensive future research.

Epigenetic profile is tissue specific and thereby assessing the skin epigenetic profile would give a clear understanding on how this potential tissue, that is the first to be exposed to environmental alterations, responds to the associated stress. So far there are no published reports assessing the epigenetic profile of caprine skin to heat stress. Hence this would also be a novel tool to assess heat stress impact and further screen for thermo-tolerance in goats.

Learning Outcomes

- Skin based novel approaches can be considered as reliable non-invasive tools to identify climate resilient agro-ecological zone specific goat breeds.
- Hair, urine and faecal cortisol and infrared thermal images of skin surface in animals may provide useful information to assess thermo-tolerance in livestock.
- Skin based metagenomics, transcriptomics and epigenetic changes analysis can provide novel insights into goat adaptation to harsh climatic condition.

10.6 Conclusions

This chapter covers in a nutshell the various skin based approaches and variables which could be considered significant in assessing climate resilience in goats. This approach could play a vital role as it is easy to assess heat tolerance in goats both invasively and non-invasively. Hair cortisol and infrared thermal images can be reliable indicators to quantify heat stress response in goats non-invasively. Further, it is very easy to take skin biopsy to subject them for advanced NGS analysis for elucidating the skin microbial diversity, differentially expressed genes and pathways, as well as to establish skin epigenetic changes associated with climate resilience. Therefore, this skin based novel approaches could help to concretely establish thermo-tolerance in goats.

10.7 Future Perspectives

Researchers and policy makers working to sustain livestock production in the changing climate scenario are looking to establish novel ways and means to quantify heat stress response. Although there is evidence that light coat color animals may have the advantage over dark coat color, still this concept has not been fully explored in all farm animals at mechanistic level. This warrants more such research efforts at mechanistic level to explore the unexplored aspects of skin based novel approaches to establish climate resilience in all farm animals. This approach could play a vital role to ensure food security by 2050 by providing clue as to which are all the species/breeds those needs to be taken forward for sustainable livestock production. The skin based approaches may be an important area of research for future to establish the basic and fundamental aspects of livestock adaptation due to the easy accessibility as skin being the first body part getting influenced by the microclimate. More intense research efforts are also needed to compare the adaptive potential of farm animals based on skin based shotgun metagenomics, whole transcriptomics and bisulphate sequencing NGS analysis. Such approaches may yield important biomarkers for climate resilience in farm animals which could be used in future breeding program using marker assisted selection. This could help to revolutionize the future animal breeding for climate resilience in farm animals. Therefore, skin based novel approaches to establish climate resilience in farm animals could be the way forward approach for policy makers to try and establish permanent solution for climate change associated livestock production. Further, these novel approaches can help to identify the best climate resilient breeds specific to agro-ecological zones. By disseminating these breeds to the resource poor small and marginal farmers can help to ensure their livelihood in the changing climate scenario.

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Part IV

Strategies to Ameliorate Heat Stress Impacts

Livestock Shelter Management: Climate Change Perspective

11

Ravindra Kumar, Ankaj Thakur, Rakesh Thakur, and P. K. Dogra

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Abstract

Increased heat stress of the animals is anticipated to jeopardize food security, making climate change a serious global concern for livestock production. Livestock has an immense role to play in the food security in coming years due to

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V. Sejian et al. (eds.), *Climate Change and Livestock Production: Recent Advances and Future Perspectives*, https://doi.org/10.1007/978-981-16-9836-1_11

rising human population. Therefore, development of appropriate strategies to sustain livestock production in the changing climate scenario by gains significance. Altering the housing design is an immediate and cost-effective approach to reduce the magnitude of the climatic change on livestock production. Climate change adaptation through livestock housing can be achieved through appropriate east-west oriented shelter design, improved ventilation, using fans and other cooling devices, and using appropriate roof materials to improve the thermal comfort of the livestock. Naturally ventilated house should always be oriented in an east-west direction in tropical climate as this direction will minimize the possibility of direct sunlight entering the house. Properly designed roof overhang reduces the possibility of direct and indirect sunlight as well as rain water entering the house during hot and rainy weather respectively. Further the shelter design should be oriented in such a way to cater the needs of specific agro-ecological zone. Thus, the chapter provides an overview on appropriate shelter design and management strategies to reduce the impact of climate change associated environmental stresses.

Keywords

Adaptation · Climate change · Cooling devices · Heat stress · Housing · Shelter

Abbreviations

%	Percentage
°C	Degree Celsius
°F	Degree Fahrenheit
BCS	Body condition score
CH4	Methane
FAO	Food and Agriculture Organization
Fig	Figure
ft	Feet
GHG	Greenhouse gas
h	Hour
IFS	Integrated farming system
LCT	Lower critical temperature
N_2O	Nitrous oxide
NAAS	National Academy of Agricultural Sciences
pH	Potential of hydrogen
THI	Temperature humidity index

11.1 Introduction

Climate change which includes increase in earth's near surface temperature, erratic changes in seasonal patterns, increase in frequency of extreme climatic events like heat waves, droughts, floods and precipitation has emerged a serious threat to the environment, economy and welfare of both humans and livestock. As a result, in the Conference of parties 26th meeting at Glasgow 2021, all countries have taken a position at key climate related issues. India has promised a massive one-billion-ton reduction in emission by 2030, a coal phase down, a radical shift to renewable and a net zero emissions target for 2070. The climate change impacts are visible all over the world but South-Asia appears to be most vulnerable region. Almost 70% of livestock in India is owned by small and marginal farmers along with landless livestock keepers. Further owing to limited financial resources and lack of awareness cum adoption of climate resilient technologies their animals are even more vulnerable to adverse impacts of climate change (NAAS 2016).

Increased heat stress of the animals is anticipated to jeopardize food security, making climate change a serious global concern for livestock production. Livestock are key to food security. Livestock produce contribute 34% of the protein and 14% of total calorie consumed globally as well as essential micronutrients such as vitamin B12, A, iron, zinc, calcium and riboflavin. But their contribution to food security and nutrition goes well beyond that, and includes a range of other goods and services, such as animal manure and traction. Hundreds of millions of vulnerable people rely on livestock in a changing climate, because of animals' ability to adapt to marginal conditions and withstand climate shocks.

Livestock has an immense role to play in the food security in coming years due to rising human population; however, its role in contributing to GHG is also being criticized. Livestock accounts for significant amount of methane (CH4) and nitrous oxide (N_2O) emissions worldwide. Ruminants besides suffering from impact of climate change are also contributing to it through enteric CH4 emission and manure management. FAO has proposed the following three ways to substantially reduce emissions from livestock production: productivity improvements that reduce emission intensities; carbon sequestration through improved pasture management better livestock integration in the circular bio economy. Similar alterations like dietary changes, selection for heat resistant germplasm etc. are being put forth for better adaptation and mitigation of impact of climate change. In general, livestock systems under intensive management are less vulnerable to impact of climate change compared to those managed under extensive or semi-intensive management owing to better shelter and adoption of mitigation strategies. This paper focuses on shelter management of livestock from climate change perspectives.

Altering the housing design is an immediate and cost-effective approach to reduce the magnitude of the climatic change. Network project on animal housing and management, India has also put forth the need to generate more information on type of most appropriate housing and management for different species in different climatic zone and different management systems (NAAS 2016). Main goal of housing is to regulate the microclimate of animals and maximize their productivity by shielding them from weather extremes. Selection of site is the preliminary step in livestock housing. Ideal housing can help in providing optimum animal productive and reproductive performance. Ventilation, cost of construction, proper drainage, lighting and ease of cleaning need to be considered while designing house for the livestock.

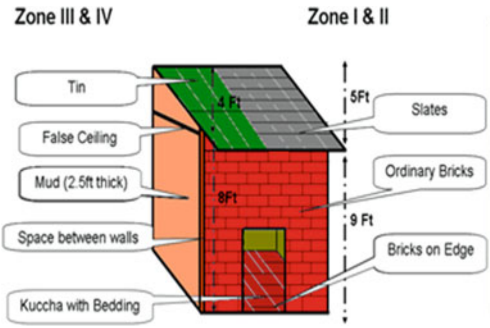
The structure of housing system (combination of the floor-system, manure collection and removal) impacts the level of emission of gaseous compounds especially ammonia. At least one or more of the abetment principles are used in housing systems to reduce gaseous emissions viz. reduction of emitting manure surface, rapid and complete removal of the liquid manure from the pit to external slurry storage, applying an additional treatment, such as aeration, to obtain flushing liquid, cooling the manure surface and changing the chemical/physical properties of the manure, such as decreasing the pH (Melse et al. 2009). Housing systems that use the aforementioned principles can minimize gaseous (ammonia) emissions to the atmosphere from 30% to 80%.

11.2 Shelter Design Considerations

- Recommended eave height is 12 ft. for the structures up to 40 ft. width and 16 ft. for the structure wider than 40 ft.
- Naturally ventilated housed should always be oriented in an east-west direction in tropical climate as this direction will minimize the possibility of direct sunlight entering the house. While in temperate areas orientation may be north to south to get maximum benefit of sunlight.
- Properly designed roof overhang reduces the possibility of direct and indirect sunlight as well as rain water entering the house during hot and rainy weather respectively.
- Cows drink up to 50% more water when the temperature humidity index (THI) is above 80%, hence cool and fresh water should be readily available to the cows during the summers. Also the water point should be located near to the shade areas. In close confinement waterer should be available every 50 feet in case of dairy cows.
- Kumar et al. (2012) proposed different animal shelter designs for different zones of the Himachal Pradesh, taking in to considerations the climatic variations and availability of local materials (Fig. 11.1). Similar studies need to be replicated in different parts of the country so that the impact of the climatic stress can be reduced through shelter management.

11.3 Provision of Shade

Shade is a cost-effective solution for reducing the heat load on the livestock. Total heat load of 30–50% can be reduced with a well-designed shade, but it does not change the air temperature or relative humidity around animals to increase sensible



Agro climatic zone	Agro ecological situation	Height (above MSL)
Zone- I	Low hills, subtropical	Up to 650 m
Zone- II	Mid hills, humid	651-1800m
Zone-III	High hills, wet temperate	Above 1800 m
Zone- IV	High hills, dry temperate	Above 2200 m

Fig. 11.1 Livestock shelter designs for different agroclimatic zones of Himachal Pradesh

heat loss routes. The use of natural shades is not always ideal under grazing conditions, which is why artificial shades have become popular. Artificial shades have been shown to improve animal welfare and milk production when used under grazing situations (Valtorta et al. 1996, 1997). Shade cloth can also be used as an alternative to solid roofing materials for providing shade to the livestock.

11.4 Housing System

Thermal resistance of the roof negatively radiates heat on animal during night hour. Roof creates higher indoor temperature during night time which can result in increased stress to the animals. The outside THI was found to be lower between 17:30 h and 7:43 h, maximum difference was obtained at time point of 6:07 h (Ambazamkandi et al. 2015). Therefore, the loose housing system in the hot humid tropical conditions should be practiced as it allow continuous access to the open paddock. Loose housing system when compared to the tie stall barn system is more appropriate for the livestock. Bamboo domes for the young livestock are used in the low cost rearing system of small ruminants during winter which help in preventing mortality and slow growth rate. This prevents the young one from the cold stress as it maintains higher temperature during extreme cold. If enclosed livestock housing is used, the building design must account for a range of ventilation flow rates. During cold seasons, the minimum ventilation rate should remove the moisture created while retaining as much sensible heat as possible by using thicker walls (Fig. 11.2). The maximum ventilation rate should remove enough of the sensible heat produced to maintain a small temperature difference between inside and outside, usually 2–4 °C.

Fig. 11.2 Inner view of typical animal house (**a**) and multispecies shelter (**b**) in colder areas of Himachal Pradesh

11.5 Roofing

Roof of animal shed should provide sufficient shade, preventing solar radiations from entering the shed. Insulating capacity should be good in the roof materials. Aluminum sheets besides being more durable are more reflective to solar radiations. In small holder production system cheap materials such as palm leaves and dried grass is used for roofing. Skin temperature of the livestock remains higher in the asbestos-roofed shed as compared to thatch roofed shed. In tropical climate thatch and agro-net roof are better when compared to asbestos in preventing the animals from hot and humid environment (Hatem et al. 2015). During the summer, the roof inside surface temperature (°C) of a thatch roof was lower than that of an asbestos roof, and both thatch and agro-net shade material contributed to alleviate thermal stress in crossbred cattle (Kamal et al. 2014).

Gable roof should have slope of 4:12 and a continuous ridge, overshot ridge or raised ridge cap to promote natural ventilation. In houses with uninsulated roofs a steep slope (45°) is highly desirable as it tend to collect the less radiant heat when compared to flat roof and the distance between animal and hot celling is also increased. Painting of the roof (outside or inside with black/white) material can also reduce the stress on the animals by minimizing the radiations. In colder areas the roof height should be low with wide overhang and the roof should be painted black. Roof sprinklers can also be used to reduce the heat gain from the uninsulated roof. When a metal roof is used that isn't insulated, sprinkling during hot weather is particularly effective in reducing heat.

11.6 Insulation and Flooring

In tropical climate the livestock sheds should be insulated to limit the amount of heat entering through wall or roof insulation. Roof insulation can also reduce the solar penetration into the shed, hence should be considered while building some new cattle shed. Spray polyurethane can also be used for insulation of roof. Thermal conductivity of the floor influences the thermoregulatory behaviour of the animals. Straw bedding significantly reduces the lower critical temperature (LCT) as compared to the bare concrete. Gupta et al. (2004) reported that morning rectal temperature was higher in village type closed barn with kutcha floor than the cemented one. Rubber mat floor has been reported to have significantly higher temperature than concrete floor during daytime (Prasad et al. 2013). Though rubber mat flooring offers a lot of obvious benefits, more research is needed to see how it affects thermolysis, animal comfort, and physiological relevance in hot, humid environments. The addition of a thatch ceiling and soft flooring in the form of a sand bed improved the microclimate of the shed, alleviated stress, and increased milk output in crossbred Jersey cows (Sahu et al. 2018).

11.7 Space Requirements

Temperature may rise dangerously if the stocking density is too high for the housing and ventilation system because more metabolic heat will be contributed to the house air than was planned for. Space allowance given to the livestock has an effect on the synchrony of resting and feeding. Floor space availability per animal can be increased by 10–20% during hot summer i.e. stocking density need to reduce by 10–20%.

11.8 Ventilation

The elimination of contaminants originating from animal excreta and the thermal exchange between the animal's surface and the environment are both dependent on ventilation. Assessment of the airflow can be easily done by using smoke cartridge to see where physical improvements can be made before switching to the mechanical ventilation solution. Increasing the airflow above the cows has a dramatic effect on evaporative heat loss from the skin.

Airflow can be increased by two ways, one by installing fans and other by opening sides of the barn. Key areas to keep cool and to determine placement of fans are at feed bunk, holding area and fresh cow pen. Minimum 50% of the floor air covered with the air movement produced by the circulating fans. If the temperature of the provided air is lower than the animal's surface temperature, fans can reduce body temperature by 0.3–0.4 °C. In barns where metal sheet is used for the walls it is practical to remove the sides and install netting. The netting/curtains can be raised to increase the airflow during summer and lowered during the winter.

11.9 Thermal Stress Alleviators

In high-humidity situations, every intervention that introduces water into the environment should be examined because it may have negative consequences as water evaporates, increasing humidity and saturating the air. Evaporative systems are designed to combine forced ventilation with wetness for this reason. Evaporative cooling systems uses the energy in the air to evaporate water, and the evaporation of water into warm air lowers the air temperature while raising the relative humidity. If the area is having higher relative humidity, larger droplets of water with fans are needed but if lower relative humidity is present, a fog or misting system would effectively cool the animals. Evaporative cooling should be used if the temperature remains above 100 °F (38 °C). Sprinkling increases the amount of heat loss from the animal via evaporative cooling while lowering water expenditures (Gaughan et al. 2008).

Combination of intermittent wetting and forced ventilation can dramatically reduce the effects of the heat stress in animals. Spraying should be done in cycles combined with the air movement. Intermittent wetting and forced ventilation using automated device had favourable responses on the physiological parameters and milk production of Murrah buffaloes by alleviating the thermal stress (Sruthi et al. 2019). Fifteen-min intervals have proved suitable for operation, whereby water is sprayed for about 3 min, controlled by a timer, followed by an evaporation time of 12 min. Approximately 1 L of water per square metre of surface area can be sprinkled in this process.

11.10 Thermal Stress Ameliorative Measures Used by the Farmers

Bathing the animals frequently, wetting the body of animals with the gunny bags, hanging the wet gunny bags against the direction of wind, growing tress or increasing green cover around the animals shed or paddocks are the few thermal stress ameliorative measures practiced by the farmers. Buffaloes are also taken to village pond for wallowing during summer to reduce the impact of heat stress in rural areas of north India, which has been proved very cost effective heat stress ameliorative measure.

11.11 Tunnel Ventilation (Fan and Pad System)

Tunnel ventilation technology is widely utilized in swine and poultry houses, but it is new to the dairy industry. It provides air movement and air exchange through fans placed in one end wall of the barn. A bank of high-powered fans is often installed on either the long or short end of a rectangular barn to improve wind speed in the barn at cow level and facilitate cow cooling via conduction and convection (Mondaca and

Cook 2019). Evaporative cooling with cooling pads, misters, or sprinklers is frequently included in tunnel-ventilation barns.

11.12 Integrated Farming System Shelter Models

Integrated farming system (IFS) or whole farm approach is emerging as a nature based solution to boost the productivity and income of the farmers by generating a mix of enterprises viz. fish, pigs, poultry, rice, vegetables etc. in climate stress scenario. IFS involve use of on-farm resources and reduce dependence on external inputs such as fertilizers and pesticides. Several design considerations can be used to integrate to optimize production in the limited space available in the tropics through vertical height utilization (Ambazamkandi et al. 2015). Roof top cultivation in the integrated models can also further help in reducing the thermal stress.

11.13 Cooling Ponds

A cooling pond is a dynamic, man-made structure that is maintained in its dynamic state by adding water to keep a set water level dependent on cow movement and ambient conditions. Within minutes of entering the pond, the internal temperature of the cows dropped by 0.5–1 °C (Bray et al. 1989). Conduction and coefficient of heat transmission to water through skin are the primary ways in which animals in ponds lose heat quickly to cool water. In locations where heat stress is an issue, using a cooling pond on a farm may help both animal comfort and production without harming milk quality (Tomaszewski et al. 2005).

11.14 Manure Management

Manure handling related mitigation practices include reducing the exposure of manure to water (e.g. dry scraping rather than washing into a pond) and changing management from anaerobic to aerobic conditions. Methane emissions from a slurry-based manure management system rise as the temperature of stored slurry rises. The lowering of slurry storage temperature from 20 to 10 °C resulted in 30–50% reduction in methane emissions (Hilhorst et al. 1998). Ammonia and methane emissions from the cow housing can be lowered by more regularly removing dung to a closed storage facility and scrapping the floor on daily basis. In advanced poultry housing belt scrapers can be used efficiently to remove litter/manure continuously, decreasing greenhouse gas emissions (Fournel et al. 2012).

11.15 Interventions for Pastoralism Production System

Grazing systems due to their reliance on climatic conditions and the natural resource base, as well as their limited adaptation options, are expected to suffer the most devastating consequences (Aydinalp and Cresser 2008). Pastoralists stay with their livestock without any shelter for about 4 months in grazing area (in north western Himalayan region) which is devoid of even trees, however providing basic nutritional supplements can also help in maintaining them in healthy conditions. Building the shelters for the accommodation of the livestock along the selected points of the migratory routes can help in building resiliency of nomadic pastoralism during extreme climatic conditions.

11.16 Nutritional Amelioration Strategies in Dairy Cattles

The heat stress is the most detrimental constraint to livestock production in lower gangetic region as the region is affected by both the climatic extremes. Considering the facts, supplementation of Selisseo and Nutri-ferm on both Indigenous and crossbred animals at Bihar Animal Sciences University Cattle Farm, Patna, Bihar as nutritional amelioration strategies for heat stress to sustain milk production in Dairy Cattles showed very encouraging results. The outcome showed that the experimental house temperature and humidity varied between 31–33 °C and 81–85%, respectively, whereas, it was highest recorded in last fortnight of August month of 2021. There were non-significant changes in feed intake, body weight and physiological parameters, however, slight increase in body weight and better feed efficiency recorded in both the treatment groups. Body condition score (BCS) was significantly higher in both treatment group in comparison to control. There was non-significant increase in milk yield and milk composition.

Learning Outcomes

- Scope for better shelter management and amelioration strategies make intensive system more profitable than extensive and semi-intensive systems of rearing livestock in the changing climatic condition.
- Providing artificial shades in the extensive system and intermittent wetting and forced ventilation in intensive system rearing can help to augment livestock production.
- Climate change calls for multidisciplinary approach in designing innovative integrated and self-sufficient multi-climate shelters to ensure optimum livestock production.

11.17 Future Perspective

The vulnerability of livestock to climate change is defined by duration, frequency, severity and sensitivity, location and related assets. Developed countries, in particular, are more inclined to implement costly adaptation measures for climatic change, such as modified housing. New paradigms are required for livestock shelter buildings to enable low greenhouse gas emissions Climate change adaptation through livestock housing can be achieved through improved insulation, passive building designs to improve ventilation, climate control by an appropriate natural ventilation system, passive and active solar design for heating and cooling, cooling pads, earth-air heat exchange, geothermal cooling using underground water and by using smart technologies to improve the thermal comfort of the livestock. Adaptation measures at animal level includes forced ventilation system, cooled lying areas, thermoregulation with water wallows, cool drinking water, fogging, misting and sprinkling system. The sensible heat load in the barn can also be reduced by reducing the stocking density of livestock in summers. Capturing of emissions in the barns and separation of feces and urine has a great potential to reduce the greenhouse emissions. Vertical separation of feces and urine employing plates on solid floor appears promising from animal welfare point of view, as the floor is drier and less slippery as compared to conventional floors. Artificial floor composed of different layers can also help in reducing the ammonia emissions due to separation of feces and urine. Therefore, the climate change's impact on livestock can be reduced using a multidisciplinary approach, innovative, integrated, self-sufficient multi climate shelter designs.

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Nutritional Amelioration of Thermal Stress Impacts in Dairy Cows

12

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Abstract

Heat stress (HS) has been implicated in several negative impacts on farm animal welfare and their production. Some of the negative impacts of HS on farm animals have been alleviated by the advances in management strategies. Despite

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V. Sejian et al. (eds.), *Climate Change and Livestock Production: Recent Advances and Future Perspectives*, https://doi.org/10.1007/978-981-16-9836-1_12

all the advances, HS continues to impact farm animal production during summer heat events, particularly in dairy. Implementation of management strategies, such as providing shade or additional water troughs, and/or nutritional strategies could help the producers when a heat event is imminent. Some of the nutritional strategies that could be utilised include antioxidant supplementation, dietary Cr, betaine and altering the rate of starch fermentation, which have been demonstrated to decrease HS under some circumstances. These nutritional strategies are discussed in this chapter.

Keywords

Antioxidant · Dairy cattle · Heat stress · Nutritional strategies · Starch fermentation

Abbreviations

%	Percentage
Cr	Chromium
CrPic	Chromium picolinate
d	Day
g	Gram
GIT	Gastro intestinal
HS	Heat stress
LWT	Live weight
NaOH	Sodium hydroxide
NEFA	Non-esterified fatty acid
pCO_2	Partial pressure of carbon dioxide
pH	Potential of hydrogen
pO_2	Partial pressure of oxygen
PUN	Plasma urea nitrogen
ROS	Reactive oxygen species
SCFP	Saccharomyces cerevisiae fermentation products
Se	Selenium

12.1 Introduction

Heat stress (HS) negatively affects a variety of dairy production parameters (e.g. milk yield and quality, feed intake) and is already a substantial burden that will only worsen with climate change. While some of the negative effects of HS have been mitigated following development and adopting management strategies such as providing shade, water and cooling systems, but milk production still decreases during HS. A striking decline in feed intake is a clear indication of HS and is thought to be primarily responsible for many of the deleterious effects of HS on cow performance. Another major implication of HS is the significant reduction of milk yield which can decrease by 40–50% in non-cooled dairy during extreme heat events, and by 10–15% in cooled management systems. Therefore, alternative measures such as nutritional intervention are needed to augment the other management strategies. The nature of the nutritional strategies will be targeted at the physiological and biochemical adaptations that occur during HS. This chapter will briefly cover the physiological adaptations to HS and identify targeted dietary strategies to mitigate HS.

12.2 Physiological Impact of Heat Stress

Lactating dairy cattle are especially vulnerable to HS because of the high metabolic demand for milk synthesis and hepatic and gastrointestinal (GIT) metabolism associated with the large feed intakes required for lactation. In dairy cows, sweating (evaporative heat loss) is the major mode of heat loss during HS while 15% of the heat loss is via passive evaporation (Dunshea et al. 2013). While the evaporative efficiency increases with increasing temperature, high humidity may limit evaporative heat loss (Silanikove 2000). HS also increases respiration rate as the dairy cow attempts to dissipate heat through respiration and panting. Therefore, dietary strategies aimed at reducing metabolic rate such as betaine or increasing blood flow to the lungs or skin such as insulin-mimics like chromium offer the potential to mitigate HS in dairy cows.

Despite the reduction in feed intake during HS in dairy cows, there is no change in plasma on-esterified fatty acid (NEFA) concentrations indicating that dairy cows don’t mobilise adipose tissue (Rhoads et al. 2009; Wheelock et al. 2010), which typically should be the case following feed reduction in feed intake to provide energy to support milk production. Also, the decrease in milk production and milk lactose secretion during HS is markedly lower than in pair-fed cows maintained under thermoneutral conditions. Plasma glucose is decreased, insulin is unchanged or increased while plasma urea nitrogen (PUN) is increased. Also, glucose utilisation during a glucose tolerance test increases during HS in dairy cows (Wheelock et al. 2010) while the insulin response to a glucose tolerance test decreases during HS in non-lactating sheep (Dunshea et al. 2017). Taken together, these findings suggest that increased insulin sensitivity may be an essential component of the acclimation mechanism in HS. Increased insulin sensitivity would ensure that cows would reduce adipose tissue lipid mobilisation and the use NEFA as a preferred energy substrate despite a reduction in feed intake during HS. Instead, glucose would become the preferred energy substrate for peripheral tissues increasing the need for gluconeogenesis, as evidenced by increased PUN, and reducing the amount of glucose available for lactose synthesis. Heat stress causes a reduction in blood pCO_2 and increase in blood pO_2 and a resultant decrease in base excess and increase in blood pH in sheep (Gonzalez-Rivas et al. 2017). These changes in blood gas parameters are most likely a result of increased respiration rate during HS. Use of NEFA as a metabolic substrate during HS would result in a low respiratory quotient and would further decrease the blood base excess resulting in respiratory alkalosis. An increase in insulin sensitivity with little change in circulating insulin would inhibit lipolysis and fat mobilization whilst still ensuring hepatic gluconeogenesis since the effective dose to inhibit plasma NEFA concentrations is within the physiological range and less than that which inhibits gluconeogenesis. This suggests that improved insulin sensitivity may reduce the impact of HS on lactating dairy cows. Dietary supplements that may improve insulin sensitivity include thiazolidinediones and Cr (antidiabetic compounds) and as well as the osmolyte betaine.

One of the other implications of HS is the oxidative stress (OS) which if not controlled can lead to oxidative damage. In dairy cows, HS has been implicated to cause OS during transition period (Bernabucci et al. 2002) and other lactating ruminants. Chronic exposure to HS can lead to decreased blood glutathione (reduced form) and increase oxidised glutathione concentration, resulting in OS (Lakritz et al. 2002). Therefore, antioxidant supplementation is one of the potential nutritional strategies to prevent oxidative damage during HS.

The redistribution of blood flow away from the body core to the periphery to increase the radiant heat loss that occurs during HS deprives the viscera of blood flow, causing ischemia/hypoxia leading to increased production of free radicals or reactive oxygen species (ROS) and decreased antioxidant status (Bernabucci et al. 2002). Normally, the free radicals produced are scavenged by cellular antioxidant systems and a balance is maintained. However, when the production of free radicals is much faster than their neutralization by the antioxidant system, this leads to damage of macromolecules, disruption of normal metabolism and physiology and may ultimately lead to loss of cell function. Because of the increased environmental temperature and paucity of green pastures during summer, the antioxidant status of dairy cows is often low and not enough to scavenge the excessive free radicals generated during metabolic challenges (high milk yield) and environmental challenges such as hot summer conditions. Therefore, dietary antioxidant supplementation would be another nutritional strategy to mitigate some of the negative impacts of HS in lactating dairy cows.

12.3 Nutritional Strategies to Ameliorate Heat Stress

12.3.1 Macronutrient Manipulation

There are number of potential nutritional strategies that can be utilised to better manage dairy cows during HS and reduce the losses. Please see review, Beede and Collier (1986) and Dunshea et al. (2013), for more common and detailed feeding interventions in addition to those being discussed in this chapter. Many of the dietary interventions in lactating dairy cows are simply targeted to provide high-energy diets as HS is known to cause negative energy balance. Thus this energy deficit can be ameliorated by concentrate feeding or fat supplementation. For example, dietary supplementation of saturated fatty acids has been reported to reduce rectal temperatures in dairy cattle during the hottest part of the day (Wang et al. 2010). This reduction in rectal temperature can be explained by reduced metabolic heat production associated with these diets and less decline in milk production during HS. Heat load (total heat contributed by both environmental heat and metabolic heat) on cows is likely to be increased over summer not just due to high temperatures but also aggravated by diets which have a high increment of heat of digestion such as fibrous and dry pasture (Silanikove 2000). Therefore, increasing the energy density of the diet (concentrate feeding) and decreasing forage contents of the diet to decrease heat production, are common methods to better manage cows during HS. However, it is equally important to choose protein type and balance the diet of heat-stressed lactating cattle, to minimise the increase metabolic heat production involved in excess nitrogen excretion as urea, following excess protein feeding. During HS, electrolyte and mineral balances are equally important must be carefully managed, particularly of those macronutrients lost via sweat such as potassium. However, cattle are more susceptible to rumen acidosis due to less bicarbonate available for rumen buffering because of excessive salivation and panting during HS (Renaudeau et al. 2012). Therefore, careful formulation and properly balanced diets to avoid high grain content, are required to better manage cattle during HS. Also, as will be mentioned later, the fermentability of the grain or concentrate can impact responses to HS.

12.3.2 Dietary Betaine

HS is known to increase the maintenance requirements of animals during HS due to increased respiration and panting etc. Therefore, feeding methyl donors such as betaine (tri-methyl glycine), a naturally occurring amino acid derivative, are important nutritional interventions to reduce the negative impacts of HS. Betaine has an important role as osmoregulatory action and therefore can reduce energy required for ions exchange (sodium/potassium pumping to maintain cellular osmolarity). For example in pigs, betaine supplementation resulted in improved growth performance which could be attributed to the reduced maintenance energy requirement of pigs (Schrama et al. 2003). Additionally, betaine also has antioxidant properties and can reduce the severity of some enteric infections in poultry by improving the gut membrane integrity (Klasing et al. 2002; Shakeri et al. 2020). While there are limited studies of betaine supplementation in ruminants as compared to monogastrics, but there is evidence that dietary betaine can reduce HS and improve feed intake and growth performance in beef and dairy cattle (Cronje 2005).

It is well established that milk yield increases with supplemental betaine up to 150 g/d in thermoneutral conditions (Wang et al. 2010; Peterson et al. 2012; Hall et al. 2016; Dunshea et al. 2019). While the ability of betaine to mitigate HS has been reported previously in sheep (DiGiacomo et al. 2016), the effects of betaine on milk yield during summer or HS conditions are more equivocal (Zhang et al. 2014; Hall et al. 2016). The multi-faceted dose-dependent response to betaine was anticipated as the underlying factor (Dunshea et al. 2013, 2019). This was evident in a study by

Zhang et al. (2014), where the researchers showed that milk yield increased at a dose of 15 g/d (ca. 0.125 g/LWT$^{0.75}$) during summer, beyond which no effect was observed. A similar quadratic dose-response was observed in sheep during HS where physiological improvements were seen at 2 g betaine/d (ca. 0.125 g/LWT$^{0.75}$) but not 4 g betaine/d (DiGiacomo et al. 2016). Similarly, Dunshea et al. (2019) found that dietary betaine at 15 g/d increased milk yield in grazing dairy cows during summer. In contrast, Hall et al. (2016) saw no improvements at 2 and 4 times this dose in simulated HS conditions. The temperance in response to high doses of betaine during HS may be that the stimulation of hepatic metabolism and consequent increase in heat production the liver may offset the reduction in heat production due to the osmo-protective effects of betaine (Dunshea et al. 2013).

12.3.3 Dietary Chromium

Recall, improving insulin sensitivity may help to reduce HS. Among the essential trace elements, chromium (Cr) is known to mimic insulin action and plays a key role in energy and protein metabolism. We (Dunshea et al. 2017; Hung et al. 2021) have done number of studies on Cr supplementation in ruminants and pigs during HS and have consistently seen positive effects on the physiological and production parameters. Similarly, dairy cows supplemented with dietary CrPic during HS have been reported to increase feed intake and milk yield (Al-Saiady et al. 2004). More recently, supplemental chromium yeast increased feed intake but not milk yield in lactating dairy cows exposed to HS (Shan et al. 2020). Importantly, the physiological responses to HS such as rectal temperature and respiration rate were reduced in those cows receiving Cr while the antioxidant status and immune function were improved.

At least some of the action of dietary Cr may be through improving insulin action. For example, Keshri et al. (2019) found that dietary Cr decreased plasma insulin in dairy calves undergoing HS. Similarly, both dietary Cr and HS increased insulin sensitivity in sheep, with the effects being additive (Dunshea et al. 2017). The mechanism of action of Cr is underpinned by its ability to improve insulin sensitivity during HS.

12.3.4 Antioxidants

Oxidative stress caused by HS can be managed by dietary antioxidation supplementation. However supra-nutritional levels of antioxidants are required to prevent the oxidative damage caused by OS. For example, our studies in sheep have shown that selenium and Vitamin E supplementation can reduce the oxidative stress and ameliorate HS in sheep (Dunshea et al. 2017). While many studies have looked at Se and other antioxidant supplantation in lactating dairy cows, far fewer studies have been conducted under HS conditions. Similarly, a previous study by Calamari et al. (2011), reported increased antioxidant systems of heat-stressed lactating dairy cows fed Se yeast. It appears that organic forms of Se are more beneficial than inorganic forms (Sun et al. 2019). We have found that supplemental Se and Vitamin E reverses the negative effect of HS on antioxidant status in dairy cows although milk yield was unchanged (J. Cottrell, unpublished). We have also found that a proprietary antioxidant supplement containing a mixture of vitamins, betaine and Se (OxiCare, DSM Nutritional Products Pty Ltd) increases milk yield and time spent ruminating in grazing dairy cows (S. Chauhan, unpublished). Further research is needed in this area.

12.3.5 Other Dietary Strategies

Given the significant contribution of metabolic heat to the heat load on farm animals, decreasing the heat of fermentation in ruminants can be one of the simple strategies to reduce susceptibility to HS and improve feed intake (Russell 2007). Another strategy to decrease the heat of fermentation could be to reduce the ruminal fermentation of starch. This can be achieved by increasing the amount of starch that escapes ruminal fermentation (Russell 2007). In Australia, wheat is the most reliable and cheapest grain available for animal feeding which could be indirectly impacting dairy industry over the summer because wheat has a very rapid rate of rumen fermentation, especially when compared to maize (Dunshea et al. 2013). We have found that maize decreased the magnitude of the increase in respiration rate (−22%) and rectal temperature (−26%) as compared to wheat when sheep were fed 50% wheat and 50% maize during thermoneutral and HS conditions (Gonzalez-Rivas et al. 2016, 2017). This response was seen at various feed intakes from 1.3 up to 2.0 times maintenance. We also observed clear differences in flank temperature and the difference between the right and left flank temperatures which clearly indicate the difference in heat of fermentation between wheat and corn fed sheep, and these findings are consistent with observations in dairy cows (Dunshea et al. 2013). Also, treating wheat with 3% NaOH can reduce simulated rumen fermentation and the physiological responses to HS in sheep. Alternatively, rate of rumen fermentation can be reduced by protecting wheat from rumen fermentation. This can be achieved by treating wheat with starch binding agents (Bioprotect™) which may also reduce the heat increment of fermentation (Gonzalez-Rivas et al. 2018; Dunshea et al. 2013). In vitro studies have shown that a starch binding agent (Bioprotect™) can reduce the rate of fermentation of wheat and replacing wheat with wheat treated with the starch binding agent increased milk yield and milk fat % in dairy cows in summer (Gonzalez-Rivas et al. 2018).

Saccharomyces cerevisiae fermentation products (SCFP) have been routinely used to modify rumen fermentation by stimulating cellulolytic and lactate-utilising bacteria, increasing microbial protein synthesis, and stabilising rumen pH. This in turn can promote increased feed intake and milk production and has been particularly beneficial for increasing feed intake in early lactation, likely due to a decrease in inflammation and stress around calving in supplemented animals (Poppy et al. 2012). Optimizing rumen function may mitigate the negative effects associated with HS and benefit lactation performance by improving feed intake and decreasing rectal temperatures. However, the impacts of SCFP on milk yield in cattle exposed to HS are inconsistent, and further research is needed to quantify the effect of SCFP on lactation performance and persistence (Shwartz et al. 2009).

Learning Outcomes

- The higher metabolic demand for milk synthesis and associated demand for large feed intake in dairy cattle makes them the most vulnerable livestock population in the changing climate.
- The essential micronutrients such as chromium, betaine and antioxidant supplementation or other dietary strategies that alter the rate of ruminal starch fermentation proved to be beneficial in relieving heat stress in dairy cattle.
- The additional management strategies (housing, genetic approach, disease management strategies) when clubbed with nutritional intervention may provide rich dividends in terms of sustaining production during heat stress exposure in dairy cattle.

12.4 Future Perspectives

Mitigating HS in lactating dairy cows will require on-farm adoption of multiple strategies including management interventions such as providing shade (to protect cows from direct heat) or additional water troughs (to help cows cool down) and implementing nutritional strategies. Dietary supplementation of antioxidants, betaine and Cr or altering the rate of starch fermentation, depending on conclusive demonstration of benefit in lactating dairy cows, are some of the potential nutritional strategies that could be adopted to ameliorate HS. These nutritional strategies should be targeted towards the physiological and metabolic adaptations that occur during HS. Further work is needed to understand the role of insulin sensitivity and oxidative stress in the aetiology of HS. Further work should also target alternative antioxidant strategies such as plant-derived polyphenols and antioxidants that have beneficial effects in other species. Finally, given the severe negative consequences that HS can elicit on dairy cattle production and welfare, research into the use of combinations of nutritional interventions and other amelioration strategies (such as housing and genetics) are warranted.

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The Role of Napier Grass (*Pennisetum purpureum* Schumach) for Improving Ruminant Production Efficiency and Human Nutrition in the Tropics

13

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Abstract

Napier grass (*Pennisetum purpureum* Schumach) comprises up to 80% of small holder ruminant diets across the (sub)tropics due to its high yield; however, ruminants offered this feed typically have low productivity. The key reason for this low productivity is the grass' low nutritive value under current management. Consequently, high quality milk and meat consumption in the same regions is low with many people suffering from malnutrition, impaired cognitive development, and many children under five are stunted. Therefore, management strategies to improve the nutritive value of Napier grass are urgently needed to increase ruminant productivity, increase associated income and subsequently availability and consumption of animal sourced foods. Currently, well managed Napier grass offered solely to cattle yield 11 L milk/cow/d and gain 0.5 kg/animal/d and has

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V. Sejian et al. (eds.), *Climate Change and Livestock Production: Recent Advances and Future Perspectives*, https://doi.org/10.1007/978-981-16-9836-1_13

the further potential to sustain 14 L/cow/d and 1 kg liveweight gain/d in the tropics and subtropics. This compares against more typical levels of production of less than 5 L/cow/d from this grass. Thus, there is an immense opportunity to at least double levels of ruminant food production through simple changes in Napier grass management from the same land area to improve food security and reduce malnutrition across vast tropical areas.

Keywords

Animal production · Food security · Napier grass · Nutrition · (Sub)tropical regions

Abbreviations

$	Dollar
%	Percentage
ADF	Acid detergent fibre
CP	Crude protein
D	Day
DESA	Department of Economic and Social Affairs
DM/ha	Dry matter per hectare
FAO	Food and Agriculture Organization
FAOSTAT	Food and Agriculture Organization Corporate Statistical Database
FCM	Fat corrected milk
Fig.	Figure
HI	Harvest interval
kg	Kilogram
km^2	Square kilometre
L	Litre
LW	Liver weight
ME	Metabolizable energy
MJ	Megajoule
NDF	Neutral detergent fibre
UNICEF	United Nations Children's Fund
WHO	World Health Organisation
yr	Year

13.1 Introduction

More than half of the worlds humans and cattle are situated in the (sub)tropics. The UNICEF, WHO, and The World Bank (World Health Organization 2018) reported more than 815 million people in the world were hungry and chronically undernourished in 2018 which equates to ~11% of the world's 7.6 billion people. About 23%

of our undernourished people live in sub-Saharan Africa, 12% in South Asia and 6% in Latin America of the sub (tropics) (Concern Worldwide 2014). In addition, 150–151 million children (over 22% of total children) under 5 years old are stunted (low height for age), with half of these children living in Asia and over a third living in Africa. Also, ~51 million children (8% of total children) are wasted (low weight for height) and two-thirds of all these children live in Asia (10% of all children), a quarter in Africa and 1.3% in Latin America. Furthermore, ~38 million babies are born with iodine deficiency and ~250 million preschool children are vitamin A deficient (FAO et al. 2017; FAO 2018a). The highest prevalence of undernourishment in the world occurs in the sub-Saharan region (23%) followed by South Asia (includes India, Pakistan and Bangladesh; 11.5%) and South America (5.6%) (FAO 2017). Geographically, these countries are all located in the tropics and subtropics. Those who live in these regions are typically low to middle income earners, with associated poor food and nutrition security and many live on less than $2/person/day (Table 13.1; FAO 2017). Consumption of animal protein in these regions is as low as 10 kg meat/person/year, ten times less than the 110 kg/person/year consumed in Australia, European and North American countries (Ritchie and Roser 2019; https://ourworldindata.org/meat-production). Thus, almost all stunting, wasting, vitamin A and iodine deficiencies in children under five are due to the lack of animal sourced foods (UNICEF, WHO, and The World Bank 2018; Table 13.1). Importantly, the ruminants in these regions that supply such animal sourced food primarily consume Napier grass. Here, we investigate the current situation of production of milk and meat using Napier grass and its best management practice to improve yield and nutritive quality aiding nutrition security, ending malnutrition, stunting and the wasting of children in these regions.

13.2 Napier Grass Management, Animal Production and Human Nutrition

Napier grass is the main forage source in tropical and subtropical regions, compromising up to 80% of the forage base used by the smallholder dairy farmers (Kabirizi et al. 2017). Thus, Napier grass is also known as ‘small holders grass’ or ‘poor man’s grass’ as it is widely grown and used by those with low incomes. Napier grass is typically harvested when the plant grows to between 2 and 3.5 m high (Zhang et al. 2010; Rengsirikul et al. 2013) to maximise forage yield (10–86 t DM/ha; Cook et al. 2005; Vicente-Chandler et al. 1959) and feed livestock from typically limited (small) areas of land. This robust growth to a late maturity ensures high fibre, and low protein and energy content (Gwayumba et al. 2002; Muinga et al. 1992) with cows offered Napier grass alone producing 8.1 L/cow/d (7.4 L at 4% FCM) but losing ~500 g liveweight/cow/d (Islam et al. 2021; unpublished results). In contrast, average CP and ME content of ryegrass, which is the main grass in the temperate regions are >18% and >10 MJ ME/kg DM respectively which enables the production of 20 L milk from 600 kg dairy cows (Fulkerson 2007). Thus, differences in management are causing large disparities between areas where cattle are located and

Table 13.1 Characteristics of tropical and subtropical countries in the world

	Tropical and subtropical	Rest of the world
Area (km^2)[a]	46,457,647 (36.5%)[b]	80,885,573 (63.5%)[c]
Human (in billion)[d]	4.0 (52%)	3.7 (48%)
Cattle (in million)[e]	900 (61%)	568 (39%)
Milk production share (%)[f]	49%	51%
Income ($)	996–3895[g]	3896 to >12,056[h]
Poverty (million)[i]	767	n/a
Hunger (million)[j]	815–821[k]	>672 (obese)[l]
Undernourished (million)[j]	815[m]	n/a
Stunted (million, children with low height for age)[j]	150–151[n]	n/a
Wasted (million, children with low weight for height)[j]	50[o]	38[p]
Iodine deficiency (million)[j]	38[q]	n/a

[a]Land area, FAO (2018b)
[b]Areas include South Asia, Australia, Latin America and Caribbean
[c]Temperate, Mediterranean, Middle East, Saharan, cold, equators
[d]WHO (2017)
[e]World Cattle Inventory (2018)
[f]Include Asia (29%; India and Pakistan's share 23% of the), Africa (5%), South America (10%), Oceania (5%) for tropical and subtropical. Include EU & USA 35%, China & Japan 8% of the temperate share. The World Dairy Situation (2016)
[g]Low to lower middle income ($996–3895) (upper middle in south American & Caribbean; $3896–12,055)
[h] Upper middle to high income
[i]9767 million people living below poverty line <$1.90/person/day
[j]World Hunger and Poverty Facts and Statistics (2018)
[j, k]815–821 (10.7%; almost all hungry people live in lower to middle income countries)
[j, l]>672 million adults are obese mostly in these countries
[j, m]815 million people (10.7% of world 7.6 billion people) 23% people in sub-Saharan Africa; 11.5% in South Asia; 5.6% in Latin America
[j, n]150–151 million children under 5 globally (over 22%); 1/2 of all in Asia and 1/3rd in Africa
[j, o]50 million children globally (7.5% of total children); 2/3rd of all in Asia (9.7%) and 1/4th in Africa and 1.3% in Latin America
[j, p]38 million under five are overweight mostly in these countries
[j, q]38 million babies born with Iodine deficiency especially in Asia and Africa

their production (Table 13.2). In this regard, FAOSTAT (2018) reported 77% of the word's dairy cattle to be located in Africa and Asia but produce only 33% of the world's milk production. Therefore, improvements in feed quality are critical to the success of the smallholder dairy sector.

Data on animal production performance from a sole Napier grass diets are limited. Available data indicate harvest interval (HI) plays an important role on nutritive value of this grass and subsequently milk yield of cows with Napier grass harvested from 42 to 91 days HI containing 6–8% CP and 7.0–7.6 MJ ME/kg DM and yielding

Table 13.2 Production of cattle meat and milk by region in 2017 (Source FAOSTAT 2018) in relation to world population

	% cattle meat	% whole fresh cow milk	% people in the world[a]
Asia	23	30	60
Africa	10	5	16
Oceania	4	5	1
Europe	16	33	10
Americas	47	27	14[b]

Tropical and subtropical regions are mainly in Asia, Africa and in part of Americas
[a]World population by continent in 2019 (https://en.wikipedia.org/wiki/List_of_continents_by_population)
[b]South America, 6%

4.0–7.2 L milk/cow/d (Table 13.3). However, when this grass was harvested between 28 and 30 days, it contained 7–14% CP and 7.1–7.9 MJ ME/kg DM and yielded 7.8–11.4 L milk/cow/d (Table 13.3). Aroeira et al. (1999) in an experiment conducted over 2 years in Brazil demonstrated Napier grass containing 11–14% CP and 7.9 MJ ME/kg DM, grazed by Holstein Friesian x Zebu cows at 30 days HI can yield on average 11.4 L/cow/d. However, this yield was achieved through the supplementation of 0.9–1.4 kg concentrate during winter and early spring, although no supplementation was required during summer and autumn. The available data, although limited, further indicate for each per cent increase in Napier grass CP (%) from 6.7 to 12.5 and each mega joule increase in ME (MJ/kg DM) from 7.0 to 7.9 corresponds to a milk yield increase of 0.83 and 3.5 L/cow/d, respectively (Fig. 13.1). In line with milk yield, the growth of cattle increases alongside Napier grass quality. Kariuki et al. (1998, 1999) offered Napier grass containing 12% CP and 8.6 MJ ME/kg DM to steer or heifers and reported 390–500 g liveweight gain per cattle/day (Table 13.3). Further, Napier grass quality (Sileshi et al. 1996; Goorahoo et al. 2005) can be maintained at similar levels to that of Kikuyu grass (Fulkerson et al. 2006; Fariña et al. 2011; García et al. 2014) or ryegrass (Fulkerson 2007). Goorahoo et al. (2005) in California for a high input production system showed Napier grass to contain high protein (17–25% CP) and relatively low fibre (49–53% NDF, neutral detergent fibre; 30–35% ADF; acid detergent fibre) as a HI between 14 and 49 days. Similarly, Sileshi et al. (1996) in Ethiopia with three cultivars showed Napier grass to contain high protein (22% CP), low fibre (53% NDF, 29% ADF) and high energy (ME 11.6 MJ/kg DM) when harvested at 28 days (i.e. 13 harvests/yr). In this regard, a sole Napier grass diet could support 24 L milk/cow/d (Islam et al. 2021; unpublished results) provided intake is maintained at >20 kg DM/cow/d. However, such intake levels would be limited by the high NDF levels of Napier grass, but the supplementation of ~2 kg concentrate could enable such levels to be achieved Sileshi et al. (1996) and Goorahoo et al. (2005). Nonetheless, research using Kikuyu and Rye grass suggest that it may be possible to harvest 14–20 L milk/cow/d when Napier grass is managed following principles of Sileshi et al. (1996) and Goorahoo et al. (2005).

Table 13.3 Current cattle production from Napier grass and potential to increase cattle production following examples of Kikuyu grass

	Trial days	Grass harvest interval	CP (%)	ME MJ/kg DM	Cow type	Live wt (LW) (kg)	DMI (kg/cow/d)	Milk yield (L/d/cow)	LW loss or gain (g/cow/d)
Dairy									
Muinga et al. (1992)	50		6.4–6.7					4–6	−4 to −23
Muinga et al. (1992)	98	42	5.6	7.0	Ayr/Brown SxShahiwal	430	6.8	6.9	−490
Gwayumba et al. (2002)	37	70–91	6.1–7.2	7.6	Friesian	400	8.4–9.1	6.1–7.2	−530 to −890
Shem et al. (2003)	56	Flower[a]	7.8	7.1	AyrxFriesian	323		7.8	−87
Muinga et al. (1992)	98	28	7.2	7.6	Ayr/Brown SxShahiwal	430	9.3	8.6	−165
Anindo and Potter (1986)	28		8.6 (7.5–10.2)		Friesian	424–441		10.5	
Aroeira et al. (1999)	730	30	11.2–13.7	7.9	HFxZebu	483	12.9	11.4	+ 450 to +500
Fulkerson et al. (2007)[b]			20.0	10	Holstein		13.0	14.2	
Fariña et al. (2011)[c]	730	20–24	19.0–20.5	10.2	Holstein	613	20.4	25.1	
Beef									
Kariuki et al. (1999)	120	56	11.7		Friesian or Shahiwal	163–181	5.6–6.0		+390 to +420
Kariuki et al. (1998)	104		11.8	8.6	Holstein heifers	144	5.0		+500

[a]Harvested at flowering stage
[b]With kikuyu grass (*Pennisetum clandestinum*)
[c]With kikuyu in spring-summer, ryegrass (*Lolium multiflorum*) pasture in autumn-winter in 65% of land and an annual rotation of maize (*Zea mays*), brassica (*Brassica napus*) and a legume either Persian clover, *Trifolium resupinatum* L or maple pea (*Pisum sativum*) in 35% land to support pasture during scarcity. The system supported 26 t DM/ha/yr to produce 27,835 L milk/ha/yr with forages and 35,000 L/ha/yr when supported with 3 kg grain per cow/d

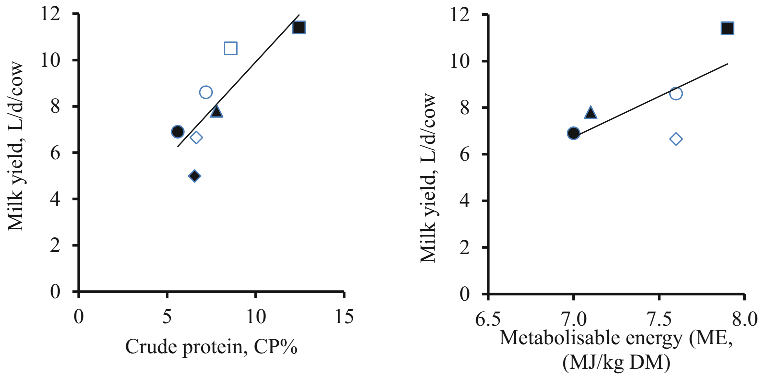

Fig. 13.1 Impact of increasing protein (Milk yield, L/d/cow = 0.83 × CP% of grass + 1.63, $R^2 = 0.69$) and energy (Milk yield, L/d/cow = 3.49 × ME of grass + 17.69, $R^2 = 0.48$) on milk yield of cows using Napier grass (Source: Table 13.3)

Therefore, simple changes in Napier grass defoliation interval are key to improving the quality of Napier grass and in so doing, improve the production of animal protein and the nutrition of people in the (sub) tropics. The dedicated demonstration of such Napier grass management practices in a systems context as part of an adoption strategy for such regions is thus urgently required. The FAO (2019) reported that global livestock product demand was expected to increase by 70% by 2050, and Adesogan et al. (2020) reported that livestock and animal sourced foods play a critical role in improving livelihood, reducing poverty, increasing food security, improving health and nutrition and gender equity and are vital to sustainability.

13.3 The Opportunity to Address Global Malnutrition

The World Bank targeted (Target 2) to end all forms of malnutrition by 2030 including stunting and wasting in children under 5 by 2025. Nutrition is central to these agendas as good nutrition can lay the foundation to achieve these targets. This report also stressed the importance of good nutrition to ending malnutrition, stunting and wasting whilst also enabling healthy lives, a quality education, gender equality, economic growth and for ending poverty. In addition, good nutrition lays the foundation for sustainable development and drives the changes needed for a more sustainable and prosperous future. Adesogan et al. (2020) reported livestock and animal sourced food are vital to sustainability for their critical role in improving nutrition, livelihoods, health and gender equity, reducing poverty and increasing food security. WHO (2014) described animal source foods as the best source of high quality nutrient rich food for children aged 6–23 months and these livestock can play a central role in supplying highly nutritious food such as meat and milk. Thus, we propose that the target to end malnutrition, stunting and wasting can be tackled by increasing the efficiency of livestock production through simple changes in the management of Napier grass as provided here.

13.4 Future Perspectives

The ‘Green revolution’ prioritised plant calories over protein to save millions of lives in Asia and other (sub) tropical continents but in so doing, marginalised ruminant production. ‘Grain’ was viewed as food, but meat or milk were used as buffers for times of shortage. However, with increased income per capita, the consumption of animal protein has increased with ruminants now a key food resource, supported by tropical feeds such as Napier grass. This trend towards ruminant protein production has generally doubled farmer income compared to grain production on the same area of land. Despite this, the management of tropical feeds such as Napier grass could be markedly improved to enable vastly greater levels of animal production. Such research and adoption programmes on improved management of Napier grass are urgently required to support the World Bank program vision to end malnutrition and associated stunting and wasting by 2025–2030.

Learning Outcomes

- Animal sourced foods, milk and meat plays a pivotal role in providing best nutrition to the children and because of which nutrition of ruminant livestock finds its importance.
- Napier grass, occupies 80% of the small holder farmer’s forage in (sub) tropics and due to its low nutritive value, it causes low productivity in ruminants.
- Imperative management strategies such as changes in Napier grass’s defoliation interval is of key importance to improve its quality and thereby increasing the animal’s protein and in turn the nutrition of the (sub) tropics people.

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Strategies to Ameliorate Heat Stress Impacts in Sheep

14

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Abstract

Sheep are continually exposed to fluctuating environmental temperatures and employ multiple behavioural, physiological and cellular mechanisms to maintain a stable core temperature during temperature extremes. Heat stress events during gestation can also impact the health and physiology of the offspring via prenatal

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V. Sejian et al. (eds.), *Climate Change and Livestock Production: Recent Advances and Future Perspectives*, https://doi.org/10.1007/978-981-16-9836-1_14

programming. The clinical responses to heat stress in ruminants include, to a degree, a manifestation of the general stress response. Heat stress can also increase energy requirements due to the processes of sweating, respiration and hormone and cellular protection employed to cope with increased core temperatures in response to heat stress. While there are many studies exploring the physiological and productivity effects of heat stress, less is known about the underlying metabolic and cellular responses and adaptations to heat. Previous research in ruminants, including sheep, has shown that heat stress alters intake as well as nutrient and energy metabolism. Although ruminants often respond to heat exposure by decreasing voluntary feed intake, heat stress increases maintenance energy requirements (due to panting, sweating and the production of hormones), which can in turn lead to production losses and animal health issues. This chapter will examine some of the key nutritional strategies that can be employed to reduce the negative impacts of heat stress on health and productivity in sheep.

Keywords

Climate change · Heat stress · Feed additives · Nutritional supplements · Sheep

Abbreviations

%	Percentage
DMI	Dry matter intake
g	Gram
GPS	Global positioning system
HSP	Heat shock protein
kg	Kilogram
NaOH	Sodium hydroxide
NEFA	Non-esterified fatty acid
NRC	National Research Council
pH	Potential of hydrogen
RFID	Radio frequency identification
ROS	Reactive oxygen species
SCFP	*Saccharomyces cerevisiae* fermentation products
VFA	Volatile fatty acid

14.1 Introduction

The impact of climate change on small ruminants is multifaceted, but includes changes to water availability and quality, land access and soil quality, forage (and feed) growth and quality, changes to weather events, increased ambient temperature extremes and number of extreme heat events; all of which will have direct effects on animal physiology, health and productivity.

Heat stress is caused by a combination of environmental factors including ambient temperature, solar radiation, humidity and wind properties and can impact upon animals in a direct manner by reducing feed intake, altering production traits, negatively affecting reproduction, decreasing disease resistance and consequently affecting the overall efficiency and health of the animal. While sheep are more tolerant to temperature variations than other ruminants, the negative impact of heat stress on sheep productivity are well documented.

The effects of climate change on environments (and hence animals) will differ by region, and it is anticipated that along with an overall rise in global temperatures, there will also be a shift in rainfall patterns and thus water availability for plant and animal growth. This will impact all aspects of ruminant production, from the regions in which they can be grown, the species and breeds that are utilized, the types of feed that can be grown and accessed and the housing requirements for the animals. The combination of changes to the available forage type and quality and the reduction in intake and changes in nutrient utilization and partitioning changes that occur in response to heat stress in sheep highlight the important role that nutrition and supplementation will play in ensuring that sheep production can continue in response to climate change. It is also clear that nutritional interventions that can improve water balance in ruminants will be highly valued in response to climate change.

Ruminant livestock are essential for the provision of high-quality protein and other essential nutrients (meat and milk) and fibre for human consumption and manufacturing use. By converting ligno-cellulose plant matter to nutrients that can be digested by humans, ruminants occupy a valuable niche and it is unlikely humans would cease to produce ruminant products in response to climate change, although production practices may require adaptation and change. Small ruminants provide food security for lower socio-economic populations, particularly in underdeveloped nations. Thus, producers are seeking methods that can be utilized to maintain, and perhaps even improve, the productivity of sheep in warmer climates. Numerous reviews, including those from authors of this chapter (Dunshea et al. 2017; Joy et al. 2020; Zhang et al. 2020), have outlined some nutritional supplements that have been successfully employed to improve production in heat stressed ruminants. This chapter will briefly outline some of these key supplements and interventions. As other chapters in this book have covered the effect of heat stress on sheep behaviour, welfare, meat quality, immune system, monitoring of heat stress, breed differences and genetic selection, shelter management, rumen microbial adaptation and impacts on reproduction these topics will not be covered in depth here.

14.2 Management and Housing

While modifying the environment around animals is a faster alternative to employing breeding or selection strategies, environmental alterations are expensive and thus not always economically viable for producers. A successful method to alleviate heat stress is the provision of shade. Shaded dairy cattle have lower rectal temperatures and respiration rates and increased milk yields than those not given access to shade (Collier et al. 2006). However, the provision of shade does not always lead to reduced core temperatures as the usage of the shade will vary amongst individuals. Sheep that use shade have similar rectal temperatures to that of animals that remain out of shade (Johnson 1991) and shaded and unshaded sheep spend comparable amounts of time grazing, drinking and ruminating (Johnson and Strack 1992). The construction of shade must be carefully designed to account for the environmental conditions likely to be experienced by the animals, as well as the breed of animal being cooled. For example, shade must be purposely orientated, adequately insulated and must be large enough to allow sufficient space per head to not increase the radiative heat load upon the animal. As trees can both protect from sunlight and act as a heat sink by removing heat from the air, shade provided by tress are the most effective form but requires forward planning and is not always possible due to paddock or enclosure designs. In more humid regions the provision of shade is less important as the effects of wind speed and humidity are more damaging than that of solar radiation. Although shade provision may not always lead to improved production and decreased core temperature, shade does reduce the mortality rate of animals in higher environmental temperatures. The cost of construction use and maintenance of these modifications must be accounted for to ensure that its use is economically viable. A simpler and potentially more cost-effective method of improving animal production during thermal stress is the modification of the diet and/or the provision of nutritional supplements during stress events.

14.3 Nutrition

The heat increment of feeding describes the heat produced internally when an animal digests feed and is influenced by the type and quality of feed consumed, as well as the specific rumen environment of the animal. This form of heat gain is of particular importance in ruminants due to the fermentation processes of the gut. Often summer pastures are fibrous and dry and thus have a high heat increment of feeding, adding to the heat load on the animal (Silanikove 2000). Excess protein in the diet of ruminants can further exasperate the heat increment of feeding, as the energy required to excrete excess nitrogen as urea increases the internal heat load (metabolic heat) (NRC 2007). Fat and protein supplementation was shown to increase the respiration rate, rectal temperature and heart rate of sheep in a meta-analysis, while concentrate and non-fermentable protein supplementation reduced rectal temperatures (Slimen et al. 2019). Additionally, concentrate supplementation will increase the ratio of propionate to acetate in the rumen, and propionate produces a lesser heat increment of feeding (accounting for fermentation and nutrient metabolism) than acetate. The mixture of feeds and ration balance will likely need to be adjusted to suit what is available in a climate-altered world. Research is required to examine novel feeds and feed combinations to ensure that animal production efficiency can be maintained in a changing climate. For example, producers may need to adapt to grow crops native to their specific region to ensure fodder can thrive in the climate and soil type etc. Native non-traditional feeds have received limited research to date, but certainly warrant further attention globally.

14.3.1 Water

Water is a vital component for health and often called the 'forgotten nutrient'. Despite the clear importance of animals maintaining adequate hydration, especially during heat stress, producers often do not consider water access or quality when designing animal rations. Although some breeds of sheep, particularly those native to tropical regions, can be well adapted to water deprivation and as ruminant animals can quickly rehydrate after a bout of dehydration as the rumen can store water and release it slowly, water remains an essential ingredient for maintaining adequate production. Moreover, water requirements are increased in growing, pregnant and lactating animals. Increased ambient temperatures will impact an animal's water use by decreasing DMI and thus decreasing water gained from feed (preformed water), and by increasing water use via the evaporative cooling mechanisms employed by the animal to dissipate excess heat. Breed differences exist in water intake in sheep. For example, sheep from mountain and desert conditions have increased water turnover than that of other breeds (Marai et al. 2007). As reviewed by Naqvi et al. (2017), the effects of climate change on water availability and quality will impact sheep physiology from metabolism to endocrine function and reproduction. Intermittent water supplies and fluctuations in ambient temperature are likely to lead to a plethora of impacts on water balance in sheep, including increased consumption of saline water, consumption of contaminated or toxic water (via contamination caused by flooding and other natural events) and decreased overall access to water. It is also likely that changes to weather and precipitation patterns will increase animals' exposure to mycotoxins and contaminated feed as wet/warm climates can promote toxin contamination and growth.

14.3.2 Nutritional Supplements

The provision of nutritional supplements to ameliorate the effects of heat stress is potentially the fastest and easiest method for producers to adopt, particularly for feedlots and dairy animals. Nutritional supplements include additives such as amino acids, vitamins, minerals, osmolytes, buffers and electrolytes. Supplements can improve both animal performance and also animal health, for example by improving the immune system and reducing oxidative stress (Chauhan et al. 2021). Additives such as chromium, glycine and betaine have been examined with varying degrees of success. If successful and cost effective, supplementation provides a management strategy that has the potential to be widely adopted by producers, and hence has great potential to improve production during times of heat exposure. Nevertheless, supplementation must be met with careful consideration as some negative responses to the dietary modification may occur. A recent meta-analysis of the nutritional supplements used to alleviate heat stress in sheep (66 studies examining 38 different heat stress alleviating strategies) found that some supplements actually increased the rectal temperature of sheep (such as concentrates and *Saccharomyces Cerevisiae*) whereas others (such as seaweed and a combination of antioxidants and minerals) successfully reduced rectal temperature to that of or below that seen in thermoneutral sheep (Slimen et al. 2019). Thus, the determination of any naturally occurring and organic compounds that can ameliorate the effects of heat stress is highly desirable. There are a variety of nutritional strategies and supplements that can be utilized to maintain or improve production in sheep. While this chapter cannot adequately describe all published data on currently available interventions, some of the commonly utilized nutritional interventions used for sheep will be briefly described here.

14.3.2.1 Betaine

Betaine, also known as trimethylglycine and glycine betaine, is a non-toxic amino acid found widely in nature. Mammals utilize betaine for two major functions, as a methyl donor participating in protein and lipid metabolism, and when not catabolised, betaine is used as an organic osmoprotectant. Betaine helps to maintain cellular integrity during times of osmotic stress thus sparing energy by reducing the need for cellular ion-pumps. During sustained periods of water loss organic osmolytes, such as betaine, are employed to maintain cellular water balance. Inorganic ions like urea cannot be tolerated for long periods by the cell as they disrupt protein function, while organic osmolytes elicit no such effects, and thus organic osmolytes are employed when long-term osmolytes are required. Moreover, protein synthesis is reduced in cells during times of osmotic stress, and the accumulation of organic ions can permit cells to recover and improve protein synthesis. Additionally, betaine is shown to have anti-apoptotic properties that can promote cell proliferation, which is of particular importance for the maintenance of gut tissue integrity in heat stressed animals (Eklund et al. 2005). In heat stressed sheep betaine (2 g/head/day) reduced rectal and skin temperatures, respiration rates and plasma NEFA concentrations, although a higher dose (4 g/head/day) either had no effect or an additive effect such that responses to heat exposure were increased (DiGiacomo et al. 2016). In lactating cattle, betaine supplementation improved milk yield and quality, feed intake and osmotic balance (Dunshea et al. 2019; Shah et al. 2020). While studies in heat stressed meat-sheep have not been published, betaine supplementation increased feed intake and average daily gain and improved some meat quality measures with particularly favourable responses observed in response to rumen protected betaine (Dong et al. 2020). However, Fernández et al. (1998) demonstrated no improvement in sheep liveweight or muscle area but did show a reduction in fat thickness when supplemented with 2 g/kg betaine.

14.3.2.2 Amino Acids

Heat stress can lead to a negative nitrogen balance due to changes in metabolism and reductions to feed intake. Excess dietary protein can exacerbate the impact of heat stress and further decrease DMI and production (such as milk yield) in ruminants (West 1999). Supplementations of specific amino acids are being increasingly investigated in ruminant models. While the published data to date is in low animal numbers, there is evidence that supplementation of rumen protected choline can improve milk yield in dairy cattle (Holdorf and White 2021). In goats, supplementation of rumen protected choline during the summer period increased feed intake, liveweight gain and feed conversion efficiency (Habeeb et al. 2017). Research into feeding amino acids and combinations of amino acids to heat stressed sheep is required to determine if such responses are also observed in other small ruminants.

14.3.2.3 Fibre and Concentrates

As acetate is used as by peripheral tissues for the de novo synthesis of long chain fatty acids and cholesterol (NRC 2007), acetate metabolism produces more heat than propionate. Thus, reducing the fibre intake of sheep can reduce the heat increment of feeding (West 1999) and decrease the animals' heat load. Although in some cases (depending on the forage type) high roughage diets can decrease body temperature as the overall level of intake, and thus rate of digestion, is decreased. Furthermore, increased water intake during heat stress can dilute feed volume and further impact intake and digestibility. Evidence also shows that VFA production is decreased during heat stress, even when animals are force fed via a cannula, which highlights that changes other than just level of intake are contributing to the change in VFA production and likely relate to rumen microbiome changes. As heat (and other) stressors can reduce intake, more energy dense diets are often provided to meet energy requirements. This can include the addition of short chain fatty acids or fats, which have a low metabolic heat production. However, excess fat supplementation can potentially disrupt the rumen microbiome which can further decrease feed intake. The timing of concentrate feeding may also be important and feeding concentrates at night when ambient temperatures are lower may improve feed intake and energy balance.

14.3.2.4 Grain Feeding

Feed fermentation and the heat increment of feeding are major contributors towards the heat load of sheep. High carbohydrate grain feeds (high in rapidly fermentable starch) have a reduced heat increment from fermentation compared to forages and roughages, but the rapid rate at which they are fermented can increase heat loads. Thus, starch sources must be carefully considered before being fed to heat stressed ruminants. For example, wheat is rapidly fermented and produces a greater physiological response to heat (greater heat load) in sheep compared to corn (Gonzalez-Rivas et al. 2016). Modification of starch to slow the rate of fermentation may therefore reduce heat loads and improve production in heat stressed sheep. A starch binding agent (Bioprotect®) was able to reduce the heat increment of feeding wheat grain to match that produced when feeding corn in sheep (Prathap et al. 2021). In addition, feeding NaOH treated wheat to sheep reduced respiration rates compared to untreated wheat diets with no negative impact on overall starch digestibility (Gonzalez-Rivas et al. 2017, 2021). Research into novel starch sources and starch modification techniques that slow the rate of digestion without negatively impacting digestibility or energy content is therefore warranted.

14.3.2.5 Electrolytes

Reduced intake can lead to decreased mineral consumption, while increased water consumption can alter mineral excretion and changes to metabolism associated with heat stress can alter mineral metabolism. For example, absorption of calcium and potassium are reduced by heat. Thus, mineral supplementation is likely to assist in the maintenance of health and production in heat stressed animals. In sheep, mineral supplementation combined with antioxidants (zinc, cobalt, chromium, selenium and vitamin E) ameliorated the negative impact of heat stress on feed intake and reduced respiration rate and rectal temperatures (Sejian et al. 2014).

14.3.2.6 Essential Oils

While experimental studies examining the feeding of essential oils to heat stressed ruminants are scarce, as essential oils commonly have antimicrobial and antiparasitic properties it is hypothesised that they will improve animal performance and health during times of heat stress. Further, there is potential that essential oils can reduce methane production although the emissions associated with oil extraction techniques might negate any such gains. In lactating sheep in Greece, a combination of cornus ± thyme oil supplementation improved milk yield and milk fat % (Kalaitsidis et al. 2021). Plantain, which contains bioactive components that have free radical scavenging properties, reduced feed intake, rectal temperatures and plasma glucose utilization in response to exogenous insulin infusion in heat stressed sheep (Al-Mamun et al. 2017). Research into novel supplements and native feeds are predicted to elucidate further essential oils that may be useful for heat stress abatement and even rumen modification to reduce methane production. However, current industrial extraction methods used to isolate the oils are harmful to the environment and technological advancements in extraction methods are required if extracted oils are to be fed. Future research might therefore also examine the feeding of whole fresh or dried herbs, rather than extracts.

14.3.2.7 Antioxidants

Heat stress can induce oxidative stress which contributes to the associated production losses. Antioxidants such as selenium and vitamin E are able to act as scavengers for reactive oxygen species (ROS) and can maintain redox balance, protect membrane integrity, modulate the immune response, alleviate leaky gut and improve heat shock protein (HSP) expression in stressed animals (Chauhan et al. 2016). In heat stressed sheep, supranutritional doses of selenium and vitamin E reduced respiration rates, rectal temperatures, plasma ROS and the oxidative stress index which resulted in supplemented sheep maintaining feed intake under heat stress compared to non-supplemented sheep (Chauhan et al. 2014). According to the recent meta-analysis, the feeding of a combination of antioxidants and minerals produced the greatest reduction to rectal temperature in heat stressed sheep (Slimen et al. 2019).

14.3.2.8 Minerals

Chromium plays an important role in the metabolism of fat, carbohydrate and protein and is also involved in glucose metabolism. Chromium has demonstrated beneficial physiological and production responses in sheep exposed to various stressors, including heat. Chromium can improve energy utilization, augment the actions of insulin (Gardner et al. 1998), reduce the hyperinsulinemic effects of heat and may enhance skin micro-circulation and vasodilation to enhance heat loss from the skin. Nano-chromium picolinate reduced rectal temperatures and respiration rates and improved average daily feed intake (Hung et al. 2021) and improved insulin sensitivity (Dunshea et al. 2013) in heat stressed sheep. While further research is required to confirm appropriate forms and dose rates of chromium, and to compare responses in different sheep breeds and physiological states (i.e. lactating and growing animals), currently available evidence suggests chromium supplementation has beneficial impacts on thermal tolerance and production in sheep.

Zinc supplementation can improve intestinal barrier function in animals, and as heat stress can damage gastric and intestinal tissues in response to blood flow redistribution to the extremities, zinc supplementation may improve animal health and performance under heat stress conditions. In steers, a zinc and amino acid complex supplement reduced rectal temperature and improved some blood gas markers (Opgenorth et al. 2021).

A combination of supplements may be beneficial in combating the negative effects of heat stress by targeting the different metabolic and physiological pathways impacted. For example, Sejian et al. (2014) observed an improvement to feed intake and a reduced respiration rate and rectal temperature in heat stressed sheep fed a combination of minerals and antioxidants (zinc, cobalt, chromium, selenium, vitamin E).

14.3.2.9 Saccharomyces Cerevisiae Fermentation Products (SCFP)

Saccharomyces cerevisiae fermentation products (SCFP, yeast) supplements have been utilised in cattle to improve rumen stability and maintain milk production in lactating animals. While there are no published experiments examining SCFP in heat stressed sheep, one experiment in dairy cattle demonstrated improved milk yield and feed efficiency (Zhu et al. 2016). In a meta-analysis of the impacts of SCFP in ruminants (cattle and sheep), SCFP overall increased DMI, increased rumen pH and VFA concentrations, tended to decrease rumen lactic acid concentration, increased milk yield and tended to increase milk fat (with no impact on milk protein concentrations) (Desnoyers et al. 2009). As heat stress can disrupt rumen stability, it is likely that SCFP supplementation can improve health and production in ruminants and thus warrants further research in small and large ruminant species.

14.4 Breeding/Selection

Breeding animals to improve production and efficiency is the aim of most producers, as animals that are better able to convert feed to product are more cost effective. Additionally, animals that are better able to cope with the external environment will have improved production output as they dissipate less energy maintaining thermal homeostasis. Nevertheless, it is generally concluded that animals bred to embody better thermoregulatory control and are thus better able to cope with extreme environmental temperatures will offset this coping mechanism with a decline in overall production. Research into breeding and selection programs, particularly those that might employ the use of native and adapted breeds of sheep, are continuing globally. In the future researchers and producers will need to work together across regions and countries to ensure that breeding programs are utilizing the most appropriate technology and genetic material to maintain production in a climate changed world.

14.5 Precision Feeding

Technology developments in agriculture are required to ensure that production can continue in a changing climate, particularly those that can assist in resource management (feed, land and water). Advances in technology in agriculture has driven the development of an emerging industry and body of research called precision livestock farming (or precision agriculture). Precision livestock farming is the application of technology such as real-time sensors on farm that can be used by the producer to help make decisions and/or directly manage animals or groups of animals on farms. As the cost of feeding animals is estimated to be 60–80% of the cost of production, technological tools that can limit feed wastage and maximise nutrient usage are highly sought after by producers. However, adoption of technologies on farm remains relatively low as producer's acceptance towards these new technologies is limited. Innovations in animal feeding (such as automatic feeders that can be programmed to tailor a diet to each specific animal or group) and virtual fencing are becoming increasingly important in a resource limited modern farming world. While the greatest uptake of such technologies has been in dairy cattle, other ruminant systems (including pasture-based systems) are gradually increasing technology into their daily animal management, although use remains limited by costs. A combination of tools such as RFID ear tags, GPS, walkover scales, automatic feeders, automatic drafters and virtual fencing are key technologies that can be utilized on farm to manage feed (and supplements) and increase feed efficiency in small ruminants (Odintsov Vaintrub et al. 2021).

Learning Outcomes

- Heat stress is one of the primary factors which negatively influences the health and performances of sheep.
- Managemental (shade and shelter, breeding programme) strategies could help to relieve heat stress in sheep.
- Nutritional strategies (grain feeding, precision feeding, amino acids, antioxidants, vitamins, electrolytes and minerals) could be employed to alleviate the negative impacts of heat stress on health and productivity in sheep.

14.6 Future Perspectives

While much has been learned regarding the impacts and thus management of heat stress in sheep, much remains unknown and research is required to fill the gaps and ensure production of sheep meat, milk and fibre can occur in a world adjusting to climate change induced increases in heat events. There remains a knowledge gap regarding the metabolic responses to heat, particularly in non-lactating ruminants. While the actions of insulin play an important role in the metabolic adaptations to heat stress, the exact mode of action of these responses remains to be elucidated. Further studies examining the metabolic responses to heat stress are required to fully discern these processes and advancing our knowledge in this area may lead to improved feeding and breeding practices to improve production during heat stress. While no one intervention in isolation is likely to ameliorate all the negative responses to heat stress, to date there is a lack of published data examining a combination of interventions in sheep. Research and commercialisation of new feed supplements and technologies are required to allow production to continue, and perhaps improve in efficiency, during higher temperature environments. This might include things like precision feeding that would allow for timed delivery of feed and/or supplements to sheep. As sheep are generally housed in extensively managed/grazing systems, the delivery of feed and feed supplements is a major constraint and any advancements in this space have the potential to rapidly improve the ability to manage heat stress in sheep.

While this review briefly described some commonly utilized supplements that can mitigate the impacts of heat stress in sheep, there are others that were not covered in detail and it is likely novel supplements will be discovered in the future. Further, there is a lack of research into potential to combine supplements to have an additive response. This has generally been prevented by the cost required to provide multiple supplements ensuring that producers would be unlikely to adopt such measures; but as the impacts of climate change become more prevalent and damaging there might be an increased cost benefit to providing a combination of feed supplements. The rumen microbiome and its potential manipulation will be described elsewhere in this book and remains another area of rapid discovery and advancement. Given the large contribution that the rumen activity and heat increment of feeding contributes to heat loads in ruminants, methods that can improve fermentation efficiency (and reduce heat production) can likely reduce heat production and improve productive efficiency.

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Strategies to Ameliorate Heat Stress Effects on Sheep Reproduction 15

Susan Robertson and Michael Friend

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Abstract

The reproductive performance of sheep will be challenged by increasing temperatures and more extreme weather events due to climate change. Heat stress potentially reduces the mating behaviour and fertility of ewes and rams, while increasing embryonic mortality, impairing fetal development and reducing lamb birth weight, and increasing perinatal lamb mortality. Heat stress can occur above 32 °C, but the degree, duration, and timing of heat stress determines whether reproductive performance is reduced, and to what extent. This chapter considers

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V. Sejian et al. (eds.), *Climate Change and Livestock Production: Recent Advances and Future Perspectives*, https://doi.org/10.1007/978-981-16-9836-1_15

practical husbandry and nutritional interventions to optimise sheep reproduction in extensive grazing systems. Strategies include genetics, choice of month of mating and lambing, shearing date, provision of shade, nutritional supplements and targeted nutrition.

Keywords

Fertility · Fecundity · Lamb mortality · Nutrition · Ovulation

Abbreviations

%	Percentage
°C	Degree celsius
cm	Centimeter
e.g.	Exempli gratia (for example)

15.1 Introduction

Maintaining or increasing the reproductive rate of sheep is required to meet future demand for animal protein and to retain profitability for sheep breeders. An increase in reproductive efficiency is desirable, rather than a simple increase in the number of ewes, to minimise methane emissions/kg meat produced, offset reductions in the availability of agricultural land, and minimise capital outlay and risk for producers.

The predicted global increases in temperatures can be expected to negatively impact on sheep reproduction, although temperatures in some regions are already sufficient to impair performance. Reduced rates of reproduction decrease the profitability of sheep farming, the rate of genetic gain, and can be an animal welfare concern, which in certain cases will increase mortality. Identification and implementation of suitable management strategies to minimise any adverse effects of heat stress on reproduction across the range of global production systems and environments is required. This chapter will briefly outline the possible impacts of heat stress on ewes and rams, then consider key on-farm strategies to increase reproductive rates in extensive grazing systems, including time of mating and shearing, provision of shade and nutritional management.

15.2 Impacts of Heat Stress on Reproduction

Heat stress occurs when sheep need to use energy to dissipate heat from the body into the environment. The thermoneutral zone for sheep has been cited in a review by Al-Dawood (2017) as 12–32 °C, and heat stress is a function of temperature, humidity, but also solar radiation. Clearly, grazing sheep can be exposed to prolonged periods of heat stress in many locations.

The impacts of heat stress on reproduction in ewes and rams have been reviewed and described in detail elsewhere (Al-Dawood 2017; Krishnan et al. 2017; van Wettere et al. 2021), and are summarised here. Temporary ram infertility or sub-normal sperm production or quality can result from heat stress, and may persist for approximately 50 days, which is the duration of spermatogenesis. Ambient temperatures above 32 °C applied to rams have been reported as reducing fertilisation or pregnancy rates, with the reduction in ram fertility increasing with the severity and duration of heat stress. Both fertilisation rate and embryo mortality can be adversely affected, evident in increased returns to service if further mating opportunities are available, or reduced pregnancy rates. Under field conditions the impact of heat stress on ram fertility is less clear, since diurnal variation in temperature provides relief, and extended mating periods to multiple rams can mask short-term impacts on the more susceptible individuals. It is clear however, that minimising the risk of heat stress in rams for around 8 weeks prior to and throughout the mating period will optimise pregnancy rates.

Heat stress in ewes can impair cyclicity, oestrous behaviour, alter follicular development and reduce oocyte quality, ovulation rate, fertility and increase embryo mortality. Pregnancy rates have been reduced by up to 100% when ewes are exposed to continuous 32 °C conditions. The impact appears to be most severe where heat stress occurs in the period from 5 days before to 5 days after oestrus, as the susceptibility of the embryo declines with maturity. However, sufficient diurnal variation in temperature, as may occur under field conditions, may prevent most of this impact. In naturally cycling flocks in environments where heat stress is not a daily event, only a portion of ewes may be exposed to heat stress in the days around oestrus. Re-mating of non-pregnant ewes in the subsequent oestrus cycle may mask any impact on pregnancy rates. However, where oestrus is synchronised, or where heat stress is frequent, avoiding or mitigating heat in the days around insemination is important to achieve high rates of pregnancy.

Prolonged periods (approximately $\geq$50 days) of heat stress in pregnant ewes can reduce placental and fetal growth, and while feed intake is also reduced by heat stress, the reduction in growth appears not to be due to reduced intake. Reductions in the birthweight of lambs of 16–74% have been reported. The extent of reduction depends on the severity, timing and duration of heat stress. Lower birthweights increase the risk of lamb mortality, and more than a 25% increase in lamb mortality has been reported for ewes heat stressed during the last two thirds of pregnancy (van Wettere et al. 2021). Heat stress can also reduce immunity levels, increasing the risk of disease, and reduce milk production, largely associated with the reduction in intake. Both of these factors contribute to an increased risk of post-natal lamb mortality.

15.3 Strategies to Optimise Reproduction

Extensive grazing systems dominate the large sheep-producing nations such as Australia and New Zealand. The extensive nature of these enterprises and the large scale of operations means any intervention to ameliorate heat stress requires minimal infrastructure and sheep handling or labour, in addition to being cost-effective. A number of practical methods are currently available for implementation by sheep breeders.

15.3.1 Heat-Tolerant Genetics

The consequences of heat stress are optimally avoided by using sheep well adapted to their environment. While an increased risk of more extreme and longer duration of hot temperatures in summer months may occur with climate change, in some locations more extreme chill events may also occur. Sheep in these scenarios need to be resilient to both heat and cold, whereas sheep in warmer environments may only require greater tolerance to heat to maintain welfare and productivity. As such, care is needed to select sheep with the appropriate traits for their production environment.

Breeds derived from tropical regions have higher resistance to heat stress than those from cooler zones (Slimen et al. 2019). However, heat resilient breeds may be less productive or lack particular traits for target markets e.g. hair sheep are not alternatives for fine wool production. There is potential for within-breed selection if appropriate traits are identified for each breed. For example, in the Australian Merino, rams with higher levels of skin wrinkle are more susceptible to heat stress, displayed by a 20% lower conception rate from summer matings (Fowler and Dun 1966). The identification and use in breeding programs of traits or genetic markers for heat resilience must balance any benefit from resilience against other production consequences relevant to each breed.

15.3.2 Time of Mating and Risks

One complication of managing sheep under extensive conditions is optimising weather conditions for the duration of the reproductive cycle, and this difficulty will vary with the climatic location. The potentially adverse effect of heat stress from 50 days pre-mating for semen viability, throughout 150 days of gestation, and through a lambing period of several weeks means sheep will be at a susceptible reproductive stage for approximately 8 months per year.

In some locations, heat stress around the mating period may be easily avoided by not mating during the summer months. However, if the consequence of this is to incur heat stress during later pregnancy or for ewes to lamb during inclement (hot or cold) weather, resulting in poor lamb survival, this may not be advantageous. Mortality of new-born lambs due to hypothermia can exceed 60% in extreme conditions, with lambs born as twins or multiples to Merino ewes more susceptible than singles and crossbred ewes (Donnelly 1984). While hyperthermia during the lambing period does contribute to perinatal lamb mortality, there is insufficient data to inform actual levels of loss under field conditions, since extensive grazing flocks typically are not mated to lamb in summer months and estimates are derived from housed studies. Van Wettere et al. (2021) have predicted a 26% reduction in lamb survival associated with lower birthweights when ewes were continuously exposed to 32 °C for various stages during pregnancy. In practice, the impact of heat stress during pregnancy on lamb survival will depend on the timing, severity and duration of heat, and diurnal variation will reduce the effect. The seasonality of the ewe breeding cycle and potential ovulation rate are other factors which influence choice of month of mating. A decision is therefore required as to what month of mating the flock will optimise the overall reproductive rate. Location-specific assessments of the monthly climatic risks are needed to enable this, along with consideration on the feed supply to meet demands, and in some cases market demand at the point of sale, as discussed later.

Extending the period of mating may allow non-pregnant ewes another mating opportunity, although extended joining and subsequent lambing can complicate flock management. An alternative strategy to reduce climatic risk is to split mating over more than one period. Simulation studies, based on a field experiment in southern Australia, have shown a February split with April mating, compared with mating all ewes in April, resulted in similar lamb production but reduced production risk (Robertson and Friend 2020). In addition to spreading the risk due to month of mating, such a system allows re-mating of ewes failing to conceive or losing embryos from the earlier mating, and as such may increase the number of lambs born, without disrupting the farm management schedule. However, multiple mating periods do require a higher managerial skill, and pregnancy scanning is recommended to identify the ewes to re-join.

Month of mating will also alter the potential stocking rate, weaner growth rates and risk of mortality, the need to supplementary feed ewes or lambs, and ability to meet particular market targets, all of which impact on risk, cost and potential income (Robertson and Friend 2020). Minimising the risk of heat stress is therefore only one of several key factors which must be considered in choice of month of mating.

Use of higher ram percentages than normal (1–2%) may reduce the risk of using individual rams with heat-impaired fertility or performance. However, this may not be cost-efficient or effective if the heat stress is too severe, or the timing of heat stress is inopportune relative to the mating period. The use of several rams with a mob of ewes reduces the risk that sub-fertile rams will reduce conception rates, since ewes may mate with more than one ram.

15.3.3 Shearing and Shade to Protect from Solar Radiation

Time of shearing wool sheep such as the Merino can be used to reduce heat stress resulting from solar radiation. Increasing fleece length up to 4 cm reduces heat stress due to radiant heat, by providing insulation, although the optimum wool length may vary with breed and the density of fleece (see review by Al-Dawood 2017). However, under hot but humid conditions (33 °C dry bulb temperature; 55% relative humidity) Merino sheep with a long fleece length (>8 cm) were less able to regulate body temperatures than recently shorn sheep (Beatty et al. 2008). Therefore, very short wool during hot months is desirable in humid, but not hot-dry environments. Many factors contribute to the choice of shearing date, including disease risk associated with fleece length (e.g. flystrike, fleece rot, grass-seeds, sunburn), contamination with vegetable matter, shearer availability, and the timing of other farm management activities, and as such will also need to be considered.

Provision of shade also reduces heat resulting from solar radiation, and has been reported to reduce heat load by up to 50% (Al-Dawood 2017). Adequate air movement and ventilation is necessary to allow heated air to escape if structures are used. Sheep readily seek shade in warm to hot weather, modifying behaviour to seek water and graze during the cooler periods of the day while idling in shade during the heat. In the extensive grazing situation, trees or shrubs may be the only cost-effective form of shade.

15.3.4 Nutritional Interventions

The reduction in feed intake resulting from heat stress is accompanied by an increase in maintenance energy requirements reported as between 7% and 30%, meaning sheep are likely in negative energy balance (Al-Dawood 2017; Krishnan et al. 2017). Nutritional restriction during heat stress therefore exacerbates potential impacts on productivity (Slimen et al. 2019). As such, improving nutritional status can be an effective means of reducing the impact of heat stress on reproduction.

Numerous nutritional interventions have been reviewed and proposed to mitigate the production impacts of heat stress, including feeding of energy-dense concentrates, non-fermentable protein, fat (Slimen et al. 2019), feeding during cooler parts of the day and feeding in smaller feeds more frequently, use of antioxidants including vitamin C and E, adequate supply of water (Al-Dawood 2017), higher quality forages, minerals including sodium and potassium, and ascorbic acid (Krishnan et al. 2017). In the extensive grazing systems, not all of these strategies are practical, as sheep are usually grazing pastures, rather than being fed complete rations. Additionally, economic viability must be considered. There may be opportunity however, to supplement sheep at specific stages of the reproductive cycle, or to alter the forage base to improve nutrition.

15.4 Targeted Nutrition to Increase Fertility and Fecundity

Feeding of a grain supplement, such as lupins (*Lupinus angustifolius*), to rams for 8 weeks prior to the mating period has long been recommended to promote spermatogenesis. This strategy has not been evaluated for mediation of heat stress and may not be effective if this period coincides with ample high-quality pasture, or if feeding occurs outside the natural breeding season in unresponsive breeds. However, it will assist in overcoming the reduced intake due to heat stress. Well-nourished rams in good but not over-fat condition are required to optimise both semen quantity and ram behaviour.

Similarly, it is well known that undernutrition impairs the ability of ewes to cycle and conceive. Ewes in higher condition score also have a higher ovulation rate. However, there is opportunity to target nutrition around mating to increase ovulation rate even when ewes are in optimal condition. Ovulation rate sets the maximum potential number of offspring. Our group has shown that grazing high quality green feed for 1 week before and after the start of mating during the natural breeding season improves fecundity by 10% or more, through targeting the critical period of days 10–14 of the oestrous cycle for the majority of ewes mated (Robertson et al. 2014). While feeding an energy supplement is an alternative strategy, grazing quality pasture reduces labour and feed costs, and allows sheep to modify behaviour to compensate for heat (e.g. grazing at night). The short duration of increased nutrition improves the efficiency of resource use, but could be extended under hot conditions. This would improve the nutrition of ewes during the period to 5 days after oestrus when embryos are most susceptible to heat stress (van Wettere et al. 2021). The benefit of improved nutrition under heat stress may mean producers reconsider the forage base available at the time of mating, although soil and environmental constraints may limit the type and quality of forage able to be produced.

15.5 Targeted Management and Nutrition to Improve Lamb Survival

Peri-natal mortality has historically been a significant concern for the global sheep industry, even prior to the added risk of reductions due to heat stress. Mortality causes substantial reductions in potential income for producers, and is increasingly being perceived as an animal welfare issue, with future risks in the social licence of husbandry and market access. Preventing increases in mortality due to rising global temperatures is therefore critical.

The lower birth weight of lambs born to heat-stressed ewes poses a risk of reduced perinatal survival (van Wettere et al. 2021), particularly if lambs are born during adverse weather. Changes to climate, resulting in milder temperatures during lambing, may mean that lamb survival may not be reduced to the extent indicated by birthweight alone, since hypothermia is also a significant contributor to mortality in some locations. Warmer weather may also improve pasture growth and ewe nutrition at some locations during the lambing period, potentially improving colostrum production and maternal behaviour.

Hyperthermia can cause mortality in new-born lambs, regardless of whether ewes were heat-stressed during pregnancy. Strategies which reduce mortality during hot weather involve limiting heat stress through provision of shade, but also limiting the potential for excess movement of ewes to access feed or water. The need to move large distances or in difficult terrain in heat exposes new-born lambs to the risk of heat exhaustion and mismothering whilst attempting to follow their mothers. Congregation of large numbers of ewes and lambs at feeding sites or water points at the same time, as occurs when a mob seeks water at dawn and dusk, may also lead to mismothering and mortality. Paddock selection, smaller mob size, and feeding strategies are factors which may reduce the risk of mortality in new-born lambs born during hot weather.

Adequate nutritional supply of energy, protein, minerals and vitamins and maintenance of a suitable condition score of ewes during pregnancy and lactation are required to optimise perinatal lamb survival. Where fetal growth and development has been compromised due to heat stress during pregnancy, or heat stress is occurring during the lambing period, adequate nutrition increases in importance to promote appropriate ewe-lamb behaviours, bonding, and colostrum and milk production.

The reduced intake and higher maintenance requirements associated with heat stress also increase the risk of metabolic disorders in ewes. Recent research has shown that the energy balance of ewes and the immune response in lambs is increased by feeding above-maintenance levels of calcium and magnesium (Ataollahi et al. 2018). Although not measured, these changes could potentially improve lamb survival, particularly under heat stress since heat stress reduces immune function (Al-Dawood 2017). The study also indicates that the current estimates for mineral requirements may be too low to maximise ewe and lamb survival.

Learning Outcomes

- Extreme weather events greatly challenge the reproductive performances of sheep.
- Heat stress has the potential to significantly impair the reproductive behaviour and fertility of ewes and rams, increase embryonic mortality, and impair fetal development, growth and perinatal lamb survival.
- Husbandry strategies (month of mating, lambing and shearing, provision of shade) and nutritional intervention including targeted nutrition could optimize the sheep reproduction.

15.6 Future Perspectives

Excessive heat stress is undeniably a threat to maintaining the reproductive performance of sheep at many locations around the world. The susceptibility of sheep throughout the reproductive process means adaptive practices are needed for each stage. Breeding sheep which are resilient to heat is a viable and attractive goal, although long-term. For selection to be a successful strategy, resilience needs to be gained without significant negative consequences on other traits relevant to the particular production system.

A clearer definition of the temperature regimens which are detrimental under extensive grazing conditions is required to allow managers to know when and what level of intervention is warranted. Provision of shade is required to maintain animal welfare, but additional targeted nutritional modifications have the potential to reduce the impacts of heat stress and improve reproductive performance. While a number of supplements have been identified, the effectiveness, cost and delivery of these has to be considered. Improving nutritional levels at key times through strategic allocation of the existing forage base and feeds, and timing mating and lambing to minimise the risk of extreme weather, may be sufficient in some locations. In locations with prolonged, severe heat stress, further strategies may be required.

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Applications of Genetic Selection in Breeding for Thermo-Tolerance in Livestock

16

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Abstract

Climate change and rising global temperature are expected to exacerbate further the problem of heat stress which is very well known to affect livestock production, reproduction, and welfare negatively. Despite the tremendous advances in

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V. Sejian et al. (eds.), *Climate Change and Livestock Production: Recent Advances and Future Perspectives*, https://doi.org/10.1007/978-981-16-9836-1_16

strategies to alleviate the impact of heat stress on livestock production, heat stress remains a substantial challenge for sustainability of global livestock production causing substantial economic losses to livestock industries in the warmer parts of the world. Physical modification of the environment, nutritional interventions, and genetic selection for heat tolerance are strategies that could be used to attenuate some of the negative impacts of heat stress. While genetic selection for heat tolerance would be the best long-term strategy to produce heat resilient future animals, identification of heat-tolerant animals is a big challenge due to the negative association between production traits and heat tolerance. Due to this unfavorable correlation between production traits and heat tolerance, a sustained reduction in heat tolerance has occurred due to continuous selection for increased production over the last 50 years. More work is thus needed to find phenotypes that are more convenient and economically viable to measure and identify heat-tolerant animals under farm conditions. Similarly, more advancements and cost reduction in data collection and genotyping are needed to develop genomic breeding values for heat tolerance. Genomic selection for heat tolerance in high-producing dairy cattle breeds such as Holsteins and Jerseys may be useful for the developed nations with more advanced data collection, efficient management systems, and genomic technologies. However, less developed countries with adapted local breeds may have better crossbreeding options between local stock and high producing exotic breeds. Nevertheless, selection for heat tolerance will improve the heat resilience of future animals and play an important role in supporting sustainable livestock production under changing climates.

Keywords

Breeding · Genetic selection · Global warming · Heat stress · Livestock

Abbreviations

°C	Degree celsius
ADG	Average daily gain
AUD	Australian Dollar
BCS	Body condition score
BV	Breeding value
CO_2	Carbon dioxide
GEBV	Genomic breeding value
GWAS	Genome Wide Association Study
H+	Hydrogen ion
H_2CO_3	Carbonic acid
HCO_3-	Bicarbonate
HS	Heat stress
HSP	Heat shock protein

NEFA	Non-esterified fatty acids
pH	Potential of hydrogen
SNP	Single nucleotide polymorphism
THI	Temperature humidity index

16.1 Introduction

Heat stress (HS) caused by increased environmental temperature is one of the greatest challenges livestock production faces in the warmer parts of the world. Increased environmental temperature compromises an animal's ability to dissipate heat from the body, and core body temperature exceeds the physiological limits for the species causing HS (West 2003). HS has negative effects on animal physiology, welfare and production, which will be exacerbated by global warming (Osei-Amponsah et al. 2020). Significant progress has been achieved in understanding the impacts of HS on various livestock species, economic losses associated with HS, and the potential strategies for amelioration of HS (Dunshea et al. 2013) and have been thoroughly discussed by other authors in this book. However, HS remains a significant challenge for sustainable livestock production and continues to cause a substantial financial burden to global livestock production, especially in warmer climates. There are three types of strategies: physical modification of the environment, nutritional interventions, and genetic selection for heat tolerance, which could be adopted to ameliorate HS in livestock species. Significant progress has been achieved in the physical modification of the animal's environment (providing shade and shelter, cooling down during hot times) and nutritional interventions (feed supplements) which we and other authors have discussed in separate chapters in this book. This chapter summarizes the genetic manipulations to improve heat tolerance, particularly recent advances in genomic technologies and their application for selecting heat-tolerant animals.

16.2 Identification of Thermo-Tolerant Phenotypes

One of the biggest challenges of selecting animals for thermo-tolerance is the lack of convenient, efficient, and economically viable methods to identify heat tolerant versus heat-sensitive animals under farm conditions (Carabaño et al. 2019). Different methods of measuring heat tolerance are used, which are mainly based on animal's physiological and behavioral responses or ability to maintain homeothermy and production performance when exposed to hot conditions. One of the common behavioral mechanisms exhibited by most livestock species on exposure to high ambient temperature is reduced feed intake. For example, in sheep, various adaptive responses, including reduced feed intake, rumination time and frequency, and increased water intake, are invoked to cope with the increased environmental temperature (Joy et al. 2020a). Such responses are invoked to reduce metabolic heat production, one of the important contributors to heat load on animals, the other being ambient temperature. Research involving various livestock species has consistently reported a significant decline in feed intake (West 2003; Dunshea et al. 2013; Chauhan et al. 2014; Joy et al. 2020b; Zhang et al. 2021). In addition, animals exposed to HS tend to increase their total water intake as a compensatory mechanism to restore the elevated fluid loss through respiratory and cutaneous routes to cool the body. Apart from maintaining the body water status, the increased ingestion of water also helps the heat-stressed animals cope with the adverse conditions by immediate rumen-reticular cooling, which effectively reduces their core body temperature. However, genetic differences among different breeds show a variable decline in feed intake. For example, our recent work on HS in sheep in Australia, involving Dorper and second cross lambs [Poll Dorset × (Border Leicester × Merino)], showed reduced feed intake in second-cross Merino lambs, but not in Dorper lambs when both breeds were subjected to HS in the climatic chambers (Joy et al. 2020b). Thus, reduced feed intake in second cross lambs under HS could be attributed to them being more heat sensitive and on the other hand, Dopers can be identified as heat tolerant, given less reduction in feed intake and less increase in water intake, only 27% in Dorper lambs compared to 63% in second cross Merino lambs in this study.

Physiological and metabolic responses of the animals following exposure to higher environmental temperatures are also used to measure heat tolerance. Physiological measures of heat tolerance include respiration rate, rectal temperature, and pulse rate (Chauhan et al. 2014). The magnitude of increase in these standard physiological measures can be used to evaluate HS in livestock. Increased rectal temperature on exposure to heat indicates that the thermoregulatory responses are insufficient to maintain homeothermy. Generally, in hot environments, increased respiratory rate is the first thermoregulatory mechanism recruited by the animals to lose heat and to maintain their body temperature. Panting is another response recruited by cattle and sheep in response to severe heat, which marks a substantial increase in the respiratory rate (Joy et al. 2020b). Our recent summer study (Osei-Amponsah et al. 2019) involving 120 lactating dairy cows in Northeast Victoria, Australia, showed that the cows experienced significant increase in average body temperature (11%), respiration rate (67%) and panting scores (64%) on exposure to summer conditions with temperature humidity index (THI) ranging 72–84. Similarly, sheep studies have demonstrated an increase in respiration rate, rectal temperature, and panting scores over the summer season. However, variation in physiological changes on exposure to heat can in different genotypes and, therefore, compare heat tolerance. For example, Dorper sheep exhibited lower rectal temperature, respiratory rate and skin temperature than Merino cross lambs which indicated better heat tolerance of Dorper lambs (Joy et al. 2020b).

Production traits are also used to measure thermo-tolerance, as HS influences the animal's performance negatively. For example, in dairy cattle, reduction in milk production is one of the most significant implications of HS, and up to 30% decline in milk production has been recorded (West 2003). Cows that show a greater reduction in milk production on exposure to heat can be termed as heat-sensitive, while the cows with smaller decline in milk production are termed as heat tolerant (Nguyen et al. 2016). This is a cumulative effect of reduced grazing in extensive system and declined feed intake in the intensive system. Our recent study (Osei-Amponsah et al. 2020) in dairy cattle in Australia, showed a 14% decline in milk yield despite the provision of the shade and cooling system in the milk parlor, which clearly showed that multiple HS mitigation strategies are required to ameliorate the adverse effects of HS. On an average, on a hot day with an ambient temperature above 32 °C, 95% of the cows sought shade and were reluctant to go grazing, and 60% of the cows spent most of the day near the water bodies to cool down.

Similarly, a reduction in production performance occurs in sheep as an effect of harsh conditions and reduced feed intake. This then leads to a drop in body weight and average daily gain (ADG) (Zhang et al. 2021). Loss of body weight during HS might also be attributed to the increase in energy expended for heat dissipation through respiratory evaporation and, subsequently, the reduction in the amount of water available for storage. Additionally, the warm environment also causes a decline in body condition score (BCS) due to insufficient fat storage and compromised energy availability. The reduction in feed intake for an extended period may have implications for carcase and meat quality characteristics. Activation of the adrenergic system, initiated by HS, leads to peripheral vasodilation and muscle glycogenolysis, resulting in higher ultimate pH and darker meat from heat-stressed ruminants (Zhang et al. 2021). The meat from heat-stressed goats exhibited increased cooking loss, reduced water holding capacity, and increased toughness (Archana et al. 2018). In contrast, a recent study conducted in Dorpers and second cross Merinos, did not show any effect of higher temperature (2 weeks exposure) on meat color, water holding capacity and texture (Zhang et al. 2021). It is worth mentioning that the variation in these results could be attributed to the duration of HS to which those animals were exposed to. It appears that the impact of HS on these parameters is quite limited when the heat exposure time is shorter than 3 weeks.

The biochemical profile of ruminants is also modified under hot environments, with changes in hematocrit values, hemoglobin and lowered blood glucose, protein, cholesterol, and non-esterified fatty acids (NEFA) concentrations (Joy et al. 2020a). Increased respiration rate during severe HS may result in respiratory alkalosis in sheep (Chauhan et al. 2014), greater heat dissipation, and increased alveolar ventilation. An associated elevation in CO_2 excretion shifts the bicarbonate equilibrium to H_2CO_3 from H+ and HCO_3-. At the molecular level, adaptive responses to heat exposure are driven by increased expression of heat shock proteins (HSPs) which function as intra-cellular chaperones and prevent protein and cell damage in heat-stressed animals, along with many other genes (Collier et al. 2008). Research in various livestock species (See in review Chauhan et al. 2021) have also demonstrated compromised immune responses during summer.

While a number of physiological, biochemical, and molecular markers and production responses to HS have been identified across species, better understanding of the genetic basis of thermo-tolerance is needed for accurately phenotyping heat tolerance. Potential tools capturing the changes in biomarkers of HS on farms are required for the successful application of genetic selection for thermo-tolerance in various livestock species.

16.3 Breeding for Thermo-Tolerance in Livestock

Genetic improvement for thermo-tolerance in dairy cattle is more profitable as it produces a permanent and cumulative change in the animal population. Genetic selection for thermo-tolerance may provide a sustainable means of augmenting other HS mitigation strategies such as feeding and/or housing modification to reduce losses. There are considerable genetic resources and local breeds of cattle in tropical and subtropical countries that are well adapted to hot environmental conditions. However, the lower production of these animals is a big challenge due to the negative association between production traits and heat tolerance. Due to this unfavorable correlation between the production traits and heat tolerance, a significant reduction in heat tolerance has occurred in the dairy animals where continuous selection for productivity has been practiced (Pryce and Haile-Mariam 2020). Identifying the stress-adaptive genes in heat-tolerant breeds could provide the selection targets for selection in new breeding populations of high-producing animals. Furthermore, the selection signature for thermo-tolerance can be identified through functional genomics. The heat resilience of high-producing breeds could be improved by introgression of HS tolerant genes or through crossbreeding with resilient genotypes. For example, heat-tolerant Holstein genetics have already been developed through the introgression of SLICK haplotype, initially identified in Senepol cattle. SLICK haplotype Holstein cows have a better ability to regulate the body temperature and experience a less-pronounced reduction in milk yield when exposed to hot conditions (Dikmen et al. 2014). While introgression can be quite useful to incorporate favorable alleles from different breeds, it is usually a very long process involving several generations of backcrossing to restore production traits.

Another option for improving the thermo-tolerance while maintaining or improving production could be crossbreeding involving local breeds and high-producing selected exotic breeds. This could be more appropriate for the tropical regions characterized by relatively high HS environments than the temperate regions. Local breeds, particularly in Asia and Africa, are known for their better thermo-tolerance and ability to thrive under harsh conditions. In general, tropical and subtropical breeds of cattle have greater adaptive capacity to stressful environments than exotic cattle. For example, Zebu cattle (*Bos indicus*) can better regulate body temperature under hot conditions than *Bos taurus* breeds of cattle. *Bos indicus* breeds have been naturally selected for survivability rather than production traits. For example, the majority of the breeds from warm climates prioritize fattening rather than milk production under good management and feeding conditions, clearly indicating that lower production is one of their constitutional characteristics. Additionally, the introduction of high-producing germplasm and genomic selection (described below) may not be best suited to the regions facing scarcity of quality feed and fodder, poor housing management, and greater incidence of diseases.

16.4 Genomic Selection for Thermo-Tolerance

Recent advances in genomics and transcriptomics may help to develop genomic breeding values for heat tolerance across breeds. Genomic selection uses genome-wide DNA markers to capture the effects of many mutations that influence variation in a complex trait like heat tolerance (Nguyen et al. 2016). Information from gene expression or genome-wide association studies (GWAS) can be used to improve the accuracy of selection further. GWAS has often been used to identify regions on the genome that have a specific effect on a trait of economic importance in ruminant livestock. Genomic selection presents the advantage of accelerated genetic gain, as livestock can be selected from a young age using their estimated genomic breeding values (GEBVs), rather than waiting to test their progeny. Australia was the first country to release breeding values (BVs) for heat tolerance in 2017, with an accuracy of 0.42–0.61 using high-density SNP genotypes (Nguyen et al. 2016; Garner et al. 2016). GEBVs for thermo-tolerance of Australian Holsteins and Jerseys are relevant because profitability and animal welfare can be improved by identifying the animals that can adapt to current and future climate challenges. When exposed to simulated heatwaves, genomically predicted heat-susceptible and -tolerant animals showed significant differences in their decline in milk production and rectal and intravaginal temperatures (Garner et al. 2016). Heat tolerance ABV allows farmers to identify the animals with greater ability to tolerate hot, humid conditions with less impact on milk production. It is expressed as a percentage with a base of 100. An animal with BVs of 105 is 5% more tolerant to hot, humid conditions than average, and its drop in production will be 5% less than the average. On the other hand, an BVs of 95 means the animal is 5% less tolerant to hot, humid conditions than the average and its drop in production under heat stress is 5% more than the average (DataGene).

Given the negative association between milk production and heat tolerance, the association of GEBVs for thermo-tolerance with BVs for other production traits will help implement genomic predictions for thermotolerance. Preliminary data (Fig. 16.1) from our recent study conducted in a herd of Holstein Friesian cows exposed to HS over Australian summer showed a positive association between heat tolerance GEBVs and Australian Feed Saved breeding values indicating improving feed efficiency can improve thermo-tolerance.

However, because thermo-tolerance is a genomic trait identified only recently, the GEBVs for HT are less reliable than those of already established phenotype-based production traits such as feed efficiency, fertility, and mastitis resistance. The accuracy of GEBVs for thermo-tolerance is further limited using variation in test-day milk production to measure heat stress, as the variation between days is not wholly dependent on ambient conditions alone. Data on core body temperature with increasing temperature and humidity and altered fatty acid profiles in milk using mid-infrared spectroscopy may improve the accuracy of GEBVs. However, the cost

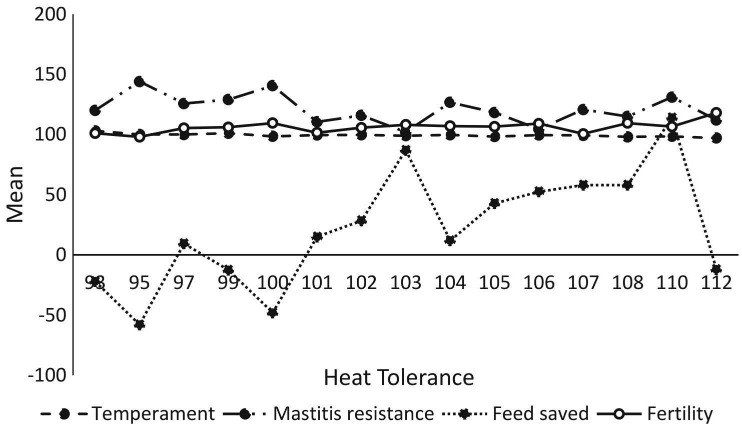

Fig. 16.1 Variation of temperament, mastitis resistance, feed saved, and fertility GEBVs with heat tolerance GEBVs of Holstein dairy cows

of genomic selection is an important issue; if this is too high, farmers may not wish to participate in a breeding scheme involving genomic selection. In 2011, the cost of genotyping was approximately 100 AUD per heifer which was proven to be not economically viable as a selection tool. It is encouraging that the cost of genotyping of cattle has shown a steady reduction, making genome-wide selection more affordable in the future. If the price of genotyping is further reduced in developing countries (perhaps by a subsidy), this would have a major impact on farm profitability by selecting heat-tolerant animals.

Learning Outcomes

- Genetic selection is an very effective tool to identify climate resilient animals.
- Crossbreeding between local breeds and high producing exotic breeds may be better strategy for producing thermo-tolerant animals in developing countries.
- Selection for heat tolerance will improve the climate resilience of future animals and play an important role in supporting sustainable livestock production.

16.5 Conclusion

Despite the significant advances in strategies to alleviate the impacts of heat stress on livestock production, heat stress remains a significant challenge for sustainability of global livestock production, especially in the warmer parts of the world. Physical modification of the environment, nutritional interventions and genetic selection for heat tolerance are some of the strategies that could be used to attenuate some of the negative impact of HS. While genetic selection for heat tolerance can provide a long-term strategy to produce heat resilient animals in future, identification of heat tolerant animals itself is a big challenge due to lack of accurate and commercially viable tools for identifying heat tolerant phenotypes, which is further exacerbated by the negative association between the production traits and heat tolerance. Therefore, more research is required and should be supported by stakeholders to improve the phenotyping for thermo-tolerance and improve the understanding of the genetic basis of thermo-tolerance.

16.6 Future Perspectives

Global warming and HS will continue to challenge our livestock industries, compromising animal welfare and farm productivity. HS is and will remain a complex issue eliciting multiple responses and triggering various physiological pathways in the animal body, negatively impacting their production. Genetic improvement of animal populations and physical modification of their environment and feeding strategies to reduce the negative impacts of HS will play an important role in ameliorating HS in animals. Existence of considerable variation in heat tolerance between and within the breeds of cattle provides an excellent opportunity for the selection of thermo-tolerant livestock and will be an important step towards breeding resilient animals. While the genetic selection for thermo-tolerance will help produce heat tolerant animals for the future, more research should be aimed at accurately and conveniently identifying the heat-tolerant phenotypes. In this regard, global strategic collaborations would be critical to generate and share quality data on thermo-tolerant phenotypes that could be used for genomic predictions. While genomic selection will play a key role in future breeding programs, its application and adoption by different countries will depend upon the type of resources available. Genomic selection for thermo-tolerance within high producing animal populations will be an ideal strategy for producing heat tolerant animals in resource-rich countries. On the other hand, resource poor nations will need to focus on better identifying and characterizing breeds that are best suited for their production environments where nutritional deficiency and disease burden might be bigger challenges than HS. In those countries, introgression of the slick gene for instance and crossbreeding between local and high producing exotic breeds, will play an important role in improving thermo-tolerance and production of more resilient genotypes for climate change adaptation.

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Part V

Enteric Methane Emission and Amelioration

Enteric Methane Mitigation in Livestock: An Indian Perspective 17

Raghavendra Bhatta, Pradeep Kumar Malik, and Veerasamy Sejian

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Abstract

An interdependent relationship exists between livestock and climate change, where one adverse event can have a ripple effect on the other. Methane (CH_4) emissions from livestock can occur due to enteric fermentation and manure management; nevertheless, nitrous oxide (N_2O) emissions are entirely due to animal manure management systems. In general, Indian livestock emits 8–10 Tg enteric CH_4 per year, with cattle and buffaloes generating more than 90% of the total enteric CH_4 emission from livestock in India, while small ruminants emit 7.7%. Reducing cattle numbers, feeding quality concentrates, fodders, supplementation of Ionophores, balancing ration, Defaunation of protozoa, reducing acetogenesis, use of plant secondary metabolites, bio-hydrogenation, and active use immunisation and disabling of surface proteins are the essential ameliorative techniques for reducing enteric methane emissions. The primary greenhouse gas

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V. Sejian et al. (eds.), *Climate Change and Livestock Production: Recent Advances and Future Perspectives*, https://doi.org/10.1007/978-981-16-9836-1_17

emitted by manure is CH_4, which is emitted during the decomposition of organic matter anaerobically, while N_2O during storage and soil application. Standard management procedures could lower both CH_4 and N_2O emissions from manure. Considering that India has the world's most enormous livestock population, reducing livestock-related GHG emissions has to be the key focus. This might improve farm profitability by diverting all energy into productive use, significantly minimising livestock-induced climate change.

Keywords

Climate change · Enteric methane · GHG · Manure · Nitrous oxide

Abbreviations

%	Percentage
$B_{o(T)}$	Maximum methane producing capacity for manure produced by livestock category *T*
CH_4	Methane
CO_2	Carbon dioxide
$EF_{(T)}$	Emission factor for livestock category *T*
EPA	Environmental Protection Agency
FAO	Food and Agriculture Organization
Fig.	Figure
GHGs	Greenhouse gases
H_2	Hydrogen
ha	Hectare
kg	Kilogram
MCF	Methane conversion factors
MS (T,S,k)	Fraction of livestock category *T*'s manure handled using manure management system *S* in climate region *k*, dimensionless
N	Number of head of livestock species
N_2	Dinitrogen
N_2O	Nitrous oxide
N_2O_D	Direct N_2O emissions from manure management
Nex	Annual average N excretion
NH_3	Ammonia
NO_X	Nitric oxide
S	Manure management system
T	Species/category of livestock
Tg	Teragram
VS	Volatile solids
WRI	World Resources Institute
yr	Year

17.1 Introduction

During 2005, the global greenhouse gas (GHG) emission was around 49 Gigatonnes (WRI 2011). China ranks first in methane emission, followed by the United States of America and the European Union (WRI 2011). India accounts for only 4.25% of global GHG emissions (Fig. 17.1). Livestock is considered a crucial component of agriculture and provides subsistence for over a billion people. This sector alone provides 13% of calories and 28% of protein requirements word-wide. Global demand for milk, beef and eggs is predicted to increase by 30%, 60%, and 80%, respectively, by 2050, compared to 1990, due to rapidly changing food patterns. This additional demand can be met by either expanding the number of animals or strengthening current livestock stock productivity. FAO estimates that 1526 million bovines and 1777 million bovines are maintained, while these populations are anticipated to expand around 2.6 and 2.7 billion by the end of 2050, respectively.

Livestock and climate change are inextricably linked via a complex process in which one's adversity impacts the other. Climate change is a constant threat to the livestock sector, but the severity of adversity varies according to the prevailing agro-climatic conditions. Climate change, directly and indirectly, impacts livestock production through changes in the atmosphere (stress), nutritional and volumetric changes in fodder crops, health, and other factors. In other words, we can consider livestock as both culprit and victim as far as climate change is debated. Climate change's deleterious effect on livestock productivity, either directly or indirectly, is outside the scope of this chapter; so, only the involvement of livestock in global greenhouse gas (GHG) emissions and mitigation methods will be discussed.

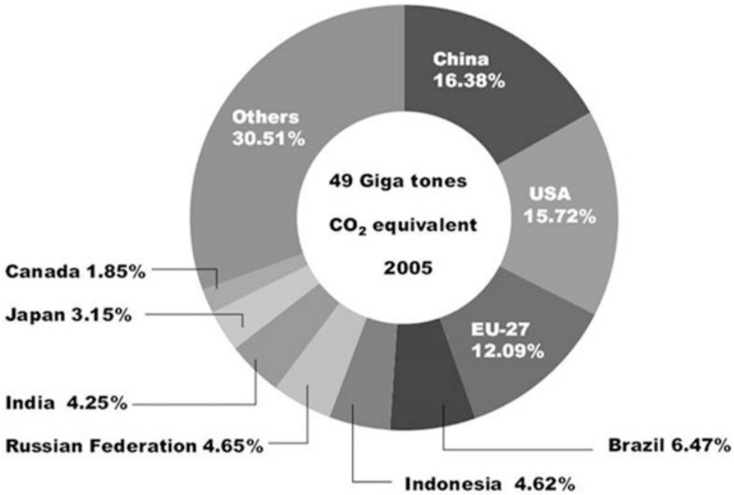


Fig. 17.1 Nation wise greenhouse gas emissions (WRI 2011)

17.2 GHG Emissions from Livestock

Livestock produces a significant amount of carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O). Emissions of CO_2 from livestock are considered part of the perpetual cycling biological system, in which plant absorbed CO_2 is metabolised by livestock through digestion and subsequently released into the atmosphere via respiration to be reabsorbed by plants (FAO 2006). The emitted and absorbed CO_2 is considered equivalent, and therefore, the release of CO_2 from livestock on a net basis is almost zero. Due to the net-zero contribution, CO_2 as a byproduct of respiration is excluded from the calculation of livestock GHG emissions (Holtkamp et al. 2006). Agriculture, including livestock, is the third-largest sector after power and land-use change to emit greenhouse gases in the atmosphere (Fig. 17.2).

On the whole, a total of 14% of global GHG emissions are attributed to agriculture (Fig. 17.2). Soil alone is accountable for 38% of the GHG emissions (as N_2O) from the agriculture sector; however, enteric fermentation is also not far behind and contributes 32% of the total GHG emission from the agriculture sector (Fig. 17.3). Rice cultivation, biomass burning and manure management constitute the rest 30% of the sector.

Enteric fermentation and manure management contribute to CH_4 emissions from livestock production, but N_2O emissions are solely the result of livestock manure management systems. Large populations and high emissions of CH_4 from ruminants make them a crucial CH_4 source in many countries (Bhatta et al. 2009). CH_4 emissions from manure management are comparatively very less than enteric emissions. Confined animal management operations deal with liquid-based manure management systems, where large amounts of CH_4 are released into the atmosphere. The amount of N_2O emitted by manure management systems varies significantly according to different management systems and may also result in indirect emissions due to various kinds of nitrogen loss from the system. Globally, livestock only accounts for 9% CO_2 emission, but this sector contributes 35 and 65% of CH_4 and N_2O from anthropogenic sources, respectively.

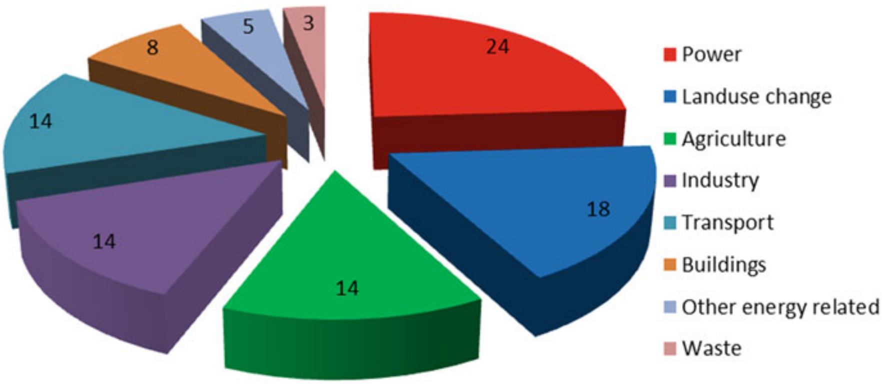
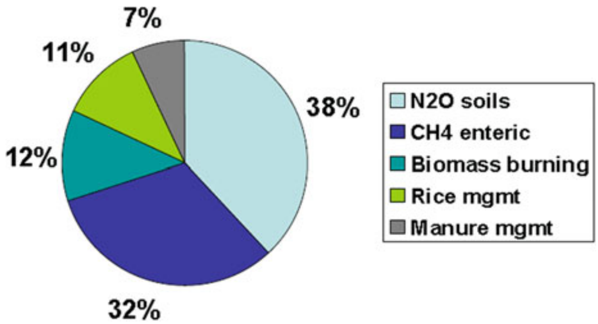


Fig. 17.2 GHG emissions according to different sectors

Fig. 17.3 Global agricultural GHG emission



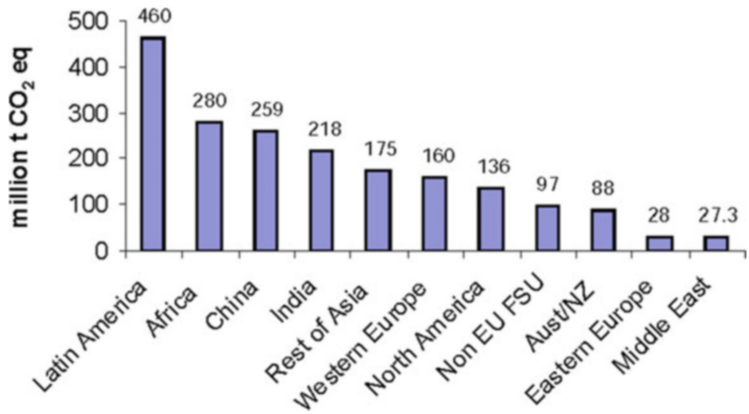


Fig. 17.4 Region wise enteric methane emission (modified from O'Mara 2011)

According to Fig. 17.4, livestock in Latin America release the most enteric CH_4, trailed by Africa and China. With an annual emission of 8–10 Tg, India contributes significantly to world enteric CH_4 emissions. Projections indicate a substantial increase in enteric CH_4 emission from 2010 to 2030 (Fig. 17.5). However, the increase in CH_4 from manure management is not much. Likewise, an increase in N_2O emission is also predicted (Fig. 17.6) by the end of 2030. China's N_2O emissions from excrement will also increase significantly. The increase in enteric CH_4 emissions in the next 15 years is likely due to the increasing livestock numbers and their continuous low productive performance.

17.3 Enteric Methane: A Necessity for Ruminants

Rumen serves as a home for a diverse range of microbes that perform various functions, ranging from complex carbohydrate degradation to the removal of fermentation metabolites syntrophically under a strict anaerobic environment. Enteric fermentation produces a considerable volume of H_2 that is effectively disposed of

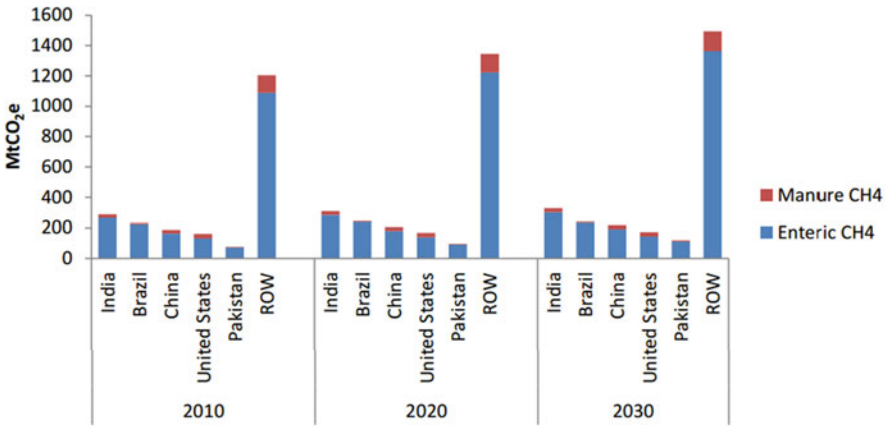


Fig. 17.5 Projections for CH_4 emission (2010–2030)

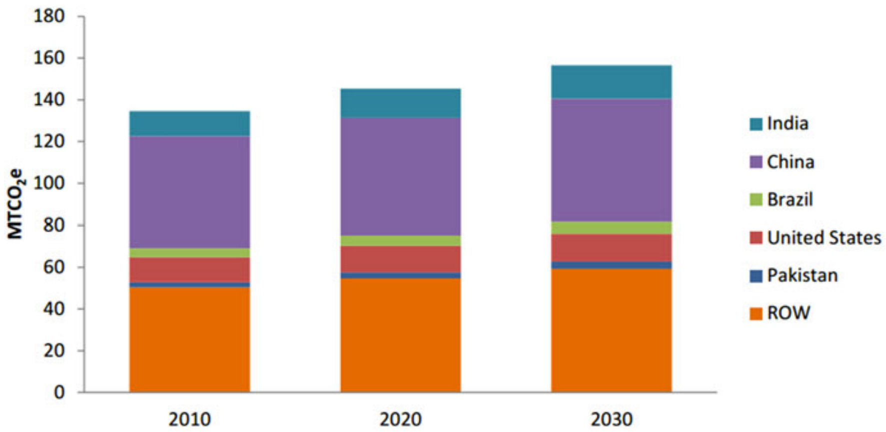


Fig. 17.6 Projections for N_2O emission (2010–2030)

from the anaerobic vat to carry out various duties and maintain the favourable rumen conditions for the dwelling bacteria and the host animal. In a healthy rumen, metabolic H_2 reduces CO_2 to CH_4, which is then vented into the atmosphere by the mouth and nostrils. The bacteria referred to as 'archaea' are the rumen's methane-producing machinery. Although these archaea or methanogens were previously classified as bacteria (prokaryotes), recent classification indicated that they belong to archaea's domain, which is markedly distinct from the bacterial domain. Among the numerous end products of rumen fermentation, H_2 is an essential metabolite since its partial pressure in the rumen controls the extent to which methanogenesis occurs and the extent to which feedstuffs can be oxidised (Hegarty and Gerdes 1999). H_2 is commonly referred to as the *currency of fermentation* in the rumen (Hegarty and Gerdes 1998). A ruminant can produce around 6–12% of its total energy intake in the form of methane through methanogenesis, which is necessary yet inefficient.

17.4 Enteric Methane Emission: Indian Scenario

India is home to 512 million animals (19th Livestock census, Govt. of India). Cattle and buffaloes account for 60% of the livestock population and produce the most methane (CH_4) than any other livestock species. Estimates from various organisations indicate that Indian livestock produces 8–10 Tg of enteric CH_4 annually. More than 90% of the enteric CH_4 emissions from livestock in India come from cattle and buffaloes (Fig. 17.7); Small ruminants, such as sheep and goats, make up only 7.7% of total methane production. Species such as yaks, mithuns, and others found only in a few places provide the balance of the emissions. Crossbred cattle produce far more enteric methane than indigenous cattle (46 kg/ha/yr vs. 25 kg/ha/yr).

17.5 Enteric Methane Amelioration: Challenges and Opportunities

As far as enteric CH_4 emission and its amelioration is concerned, these are the challenges that should be addressed in a planned manner for achieving long term CH_4 reduction from livestock

- Uncertainty over the validity of the country-specific data
- Improper methodologies for methane prediction
- Shortage of feed and fodder
- Quality of feed and fodder

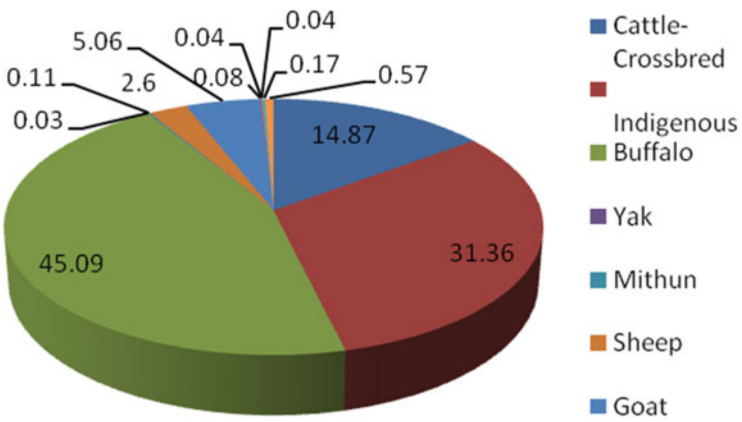


Fig. 17.7 Species wise % enteric methane emission (modified from Kamra 2014)

- Low functional productivity
- Twinkling methane reduction attributes
- Complicated rumen microbial system
- Unexplored community of rumen archaea

In order to reduce CH_4 emissions from the rumen successfully, any technique under evaluation must fulfil the criteria of shrinking H_2 production (Bhatta et al. 2008, 2013a). A brief overview of the potential opportunities to minimise enteric methane emission and ameliorative measures (Table 17.1) is presented below.

- Cutting down on the number of unproductive or under-productive animals.
- Dipping of H_2 production in the rumen to obstruct the supply
- Diverting H_2 utilisation from methanogenesis process
- Targeting H_2 utilising microbes for indirect inhibition of methanogenesis
- Targeting rumen archaea for direct inhibition

17.6 GHG Emissions from Manure Management

Soil quality, tilth, and productivity can all be improved by using livestock manure as a supplement to the soil. CH_4 and N_2O are the major GHGs produced by manure during the anaerobic decomposition of organic matter during storage and soil application, respectively. Indirect sources of nitrous oxide include the gases NH_3 and NO_X generated by manure, which also contribute to the odour. Estimated and projected CH_4 and N_2O emissions from manure management are presented in Table 17.1. It is clear from the table that the CH_4 emission from manure management in the country is about 9.6% of the world's emission and projected to be remaining 9.5–10.2% in the next 40 years. N_2O emission from livestock waste was estimated at 3.9% of the world's total (Table 17.2).

Manure management is essential in minimising GHG emissions caused by microbial activities during manure decomposition. The decomposition of dung under anaerobic conditions during storage produces CH_4. These conditions occur most readily when manure is disposed of in liquid-based systems. The bulk of dung produced and the portion of dung that decomposes anaerobically are the main factors that influence CH_4 emissions. When manure is stored or treated as a liquid in lagoons, ponds, tanks or pits, it decomposes anaerobically and can produce a significant quantity of CH_4. The temperature and the retention time of the storage unit significantly affect the amount of CH_4 produced. Handling dung as a solid (e.g., in stacks or piles) or when it is deposited on pastures and rangelands, it tends to decompose under aerobic conditions and hence, produce very little CH_4. The anaerobic decomposition of the faeces produces CH_4 due to its volatile solids (VS). There are both biodegradable and non-biodegradable fractions of VS in animal manure. The VS excretion rates can be derived from publicly available sources for country-specific estimations. Feed intake levels can be used to determine country-specific VS excretion rates if daily average VS excretion rates are not readily

Table 17.1 Ameliorative measures for enteric CH_4 mitigation

Measures	Opportunities/limitation	Remarks
Reducing the livestock numbers	CH_4 emissions per kilogramme of livestock product are high because of the large number of low- or non-producing ruminants. It is impossible to slaughter these animals because of a ban on cow slaughter in India	Animals that are not productive should be graded up through rigorous, selective breeding to increase their productivity and produce less methane
Feeding of quality fodders, concentrate	To reduce methane emissions, feed interventions are the most effective method. The uninterrupted availability is a question mark. Since the last three decades, the area under pasture and permanent fodder production has decreased or remained flat. About 7–8% of the arable land in the country is used to feed livestock	With an ever-increasing human population and food-feed-fuel competitiveness, it appears impossible to increase the availability of excellent fodders
Ionophore	Selective inhibition of microbes and failure to achieve long-term reduction are major issues. After a short period of time, animals return to their normal emission levels. It is prohibited in several European nations to use them	Rotation and combination may be used to sustain the long-term decrease
Ration balancing	Ration balancing with feed resources available at the farmer's doorstep will improve the productivity with concurrent methane reduction at a low input level	Ration balance is critical for farmers, and they must be made aware of the financial benefits that accrue from it
Removal of protozoa	Lower levels of CH_4 are produced when the rumen is freed of ciliate protozoa. Fibre digestibility may be reduced. However, maintaining rumen free of protozoa is nearly impossible	However, even after complete removal, enteric methane reduction can be achieved partially through defaunation without compromising fibre digestion
Reductive acetogenesis	The rumen's methanogenesis is favoured by thermodynamics. When it comes to H_2 substrate affinity, acetogens have a far lower affinity than methanogens. It will not function until the rumen's methanogens are targeted	By simultaneously targeting rumen archaea, a reduction in acetogenesis can be encouraged. As a result, there will be less methane and more acetate for the host animal
Use of plant secondary metabolites	The use of PSM as methane mitigation agents is a viable option in the event of a lack of quality fodder. Dose optimisation and validation of methane migration potential *in vivo* on a large scale should be compulsory before recommendation	Enteric methane amelioration may be possible if the degree of inclusion is safe and does not adversely influence feed fermentability—the combined effect of PSM on *in vivo* methane emission warrants further investigation
Nitrate/Sulfate	To a more considerable extent, nitrate and sulphate can reduce methane emissions. Thermodynamically, these	Slow-release sources for these chemicals may lessen the risk of toxicity from intermediate

(continued)

Table 17.1 (continued)

Measures	Opportunities/limitation	Remarks
	reductive mechanisms outperform methanogenesis. The outcome of this production process will have no benefit to the animal in terms of energy. Intermediary products are toxic to the host animal	metabolites. A safe inclusion level must be determined and tested on many animals, taking into account all of the species accountable for methane production
Active immunisation	This strategy has the potential to reduce methane emissions significantly if the methanogenic archaea of the rumen are thoroughly studied to identify the best candidate vaccination target	For consideration of this technique for the amelioration of enteric methane, it is necessary to gather information on the diversity of methanogenic archaea
Disabling of surface proteins	Identifying and disabling the methanogens which adhere to other microbes surfaces for H_2 transfer through surface proteins might reduce enteric methane emission	In order to investigate the possibilities, significant fundamental and advanced study is required in this relatively unknown region
Bio-hydrogenation	Restricting the H_2 supply to methanogens through alternate use in bio-hydrogenation decrease enteric methane amelioration. The use of fat/lipids at a high level depresses fibre digestion. Of the total, about 5–7% of H_2 is only utilised in this process	This approach is not practical due to the high cost of fat/lipids and fibre depression at a high level of use

Source: Bhatta et al. (2000, 2001, 2005, 2012a, b, 2013b, c, 2015) and Malik et al. (2014, 2015)

Table 17.2 Estimate and projected emissions of methane and nitrous oxide from manure management

	Manure methane (kg × 10^5)		
	Estimated	Projected	
Methane	2010	2025	2050
World	11,414	12,849	15,046
India	1096	1221	1543
% of total	9.6	9.5	10.2
	Manure nitrous oxide (kg × 10^5)		
	Estimated	Projected	
Nitrous oxide	2010	2025	2050
World	383	445	516
India	15.3	17.5	21.4
% of total	3.9	3.9	4.1

Source: Patra (2014)

available. The ‘Enhanced’ characterisation approach can estimate cow and buffalo feed intake. The VS content of dung is the percentage of the animal’s food that isn’t digested and is excreted as faecal matter. The following equation can be used to figure out the rate of excretion of VS.

Volatile solids excretion rate

$$VS = \left[GE \cdot \left(1 - \frac{DE\%}{100}\right) + (UE \cdot GE] \cdot \left[\left(\frac{1 - ASH}{18.45}\right)\right]$$

Using the VS excretion rate, the CH_4 emission factor from dung can be calculated using the equation presented below.

$$EF_{(T)} = \left(VS_{(T)} \bullet 365\right) \bullet \left[B_{o(T)} \bullet 0.67 kg/m^3 \bullet \sum_{S,k} \frac{MCF_{S,k}}{100} \bullet MS_{(T,S,k)}\right]$$

Where:

$EF_{(T)}$ = Annual CH_4 emission factor for livestock category *T*, kg CH_4 animal^{-1} yr^{-1}

VS (T) = Daily volatile solid for livestock category *T*, kg dry matter animal^{-1} day^{-1}

$B_{o(T)}$ = Maximum methane producing capacity for manure produced by livestock category *T*, m^3 CH_4 kg^{-1} of VS excreted

0.67 = Conversion factor

MCF (S,k) = Methane conversion factors for each manure management system *S* by climate region *k*, %

MS (T,S,k) = Fraction of livestock category *T*'s manure handled using manure management system *S* in climate region *k*, dimensionless

Nitrification and denitrification processes work together to produce N_2O emissions directly from manure management processes. During the storage and treatment of manure, the amount of N_2O released depends on the nitrogen and carbon content of the dung, as well as the storage period. Nitrification (the oxidation of ammonia nitrogen to nitrate nitrogen) is a necessary prerequisite for the emission of N_2O from stored animal manures. Nitrification is likely to occur when stored dung has a sufficient supply of oxygen. In order to release N_2O from stored animal dung, ammonia must be oxidised to nitrate nitrogen. When dung is appropriately stored, nitrification is more likely to occur as long as enough oxygen is available. Anaerobic denitrification produces N_2O and dinitrogen from nitrite and nitrate. Direct N_2O emission from manure management may be estimated using the following equation.

Direct N_2O emission from manure management

$$N_2O_{D(mm)} = \left[\sum_S \left[\sum_T \left(N_{(T)} \bullet Nex_{(T)} \bullet MS_{(T,S)}\right)\right] \bullet EF_{3(S)}\right] \bullet \frac{44}{28}$$

Where:

$N_2O_{D\ (mm)}$ = direct N_2O emissions from Manure Management, kg N_2O yr^{-1}

N (T) = number of head of livestock species/category *T* in the country

Nex (T) = annual average N excretion kg animal^{-1} yr^{-1}

MS (T,S) = fraction of total annual nitrogen excretion for each livestock species/ category *T* that is managed in manure management system *S* in the country, dimension less

EF3 (S) = emission factor for direct N_2O emissions from manure management system *S* in the country, kg N_2O-N/kg N in manure management system *S*

S = manure management system

T = species/category of livestock

44/28 = conversion of (N_2O-N) (mm) emissions to N_2O (mm) emissions

17.7 Measures for Reducing GHG Emissions from Manure Management

Table 17.3 lists preventive and ameliorative techniques for reducing manure-related emissions of CH_4 and N_2O.

17.8 Conclusions

Livestock produces methane and nitrous oxide due to enteric fermentation and manure management, which are considered the primary sources of anthropogenic GHG emissions. These GHGs have a far higher global warming potential than carbon dioxide. Enteric methane emissions from livestock contribute to global warming and rob the host animal of a significant portion of its energy supply. India cannot afford this energy loss since it will require extra feed supplies to compensate for the shortfall. The implementation of mitigation measures to reduce enteric methane should be based on the viability of the intervention(s) in the specific region. Our attention should be on measures that have the potential to endure over time and result in a 20–25% reduction in enteric methane emission. Different storage conditions are necessary for manure management's methane and nitrous oxide emissions. The methane emission from manure in the country is not very alarming, so our focus should be on reducing nitrous oxide emissions from manure management by developing interventions that ensure that methane emission has not gone up while trying to mitigate nitrous oxide emission from manure management. Many strategies have been developed and evaluated in the country. However, their confinement to the laboratory is a crucial issue and needs the attention of the extension workers and line departments for undertaking front line demonstration and popularising the farmers' mitigation strategies. Large enteric methane emissions in absolute quantity from Indian livestock warrants the urgent attention of the researcher and policymakers.

Table 17.3 Precautionary/ameliorative measures for reducing GHG emissions from manure management

GHG	Measures
CH_4	• It might be a viable option rather than storing manure in a lagoon (this may increase N_2O emission), handling manure as a solid or putting it on pasture. • Using manure decomposition as a source of CH_4 to generate renewable energy • Avoid incorporating straw with manure, which acts as a substrate for anaerobic bacteria. • Prolong storage of manure stimulates anaerobic decomposition leading to increased CH_4; it is better to add manure to the soil as soon as possible. • Avoiding the process of manure application when is soil is too damp, which might lead to increased methane production. • Enhance animal feed conversion efficiency by giving high-quality feeds to reduce GHG emissions • Covering the tanks or lagoons with impermeable covers like plastic to capture GHGs.
N_2O	• Apply manure immediately before crop growth to allow the crop to utilise the maximum amount of available nitrogen. • To avoid excessive nitrogen loss during spring and N_2O emissions, do not apply manure in the late fall or winter. • Applying manure during hot and windy or before a storm should be avoided, as these conditions may increase N_2O emissions. • Few soil and water management practices like increasing soil aeration, avoiding soil compaction, improving drainage and using nitrification inhibitors for nitrogen gas production instead of nitrogen oxide. • Distribute manure evenly around the pasture. • Maintaining healthy pastures using effective grazing management strategies contributes to fodder quality improvement. • Including the proper amount of amino acid levels in the feeding regime minimises nitrogen excretion, primarily in the urine. Use phase feeding to match diet to growth and development • Reduce the amount of N_2O emitted by moving fresh manure into a covered storage facility. • Storage at lower temperatures inhibits microbial activity

Learning Outcomes

- Cattle and buffaloes account for more than 90% of the enteric CH_4 emissions from livestock in India, while small ruminants account for just 7.7% of the total enteric CH_4 emissions from livestock in India.
- Methane emissions can be reduced at a low input level if rations are balanced with the feed supplies available at the farmer's doorstep.
- If the methanogenic archaea of the rumen is studied to the full extent, the active immunisation strategy has the potential to reduce methane emissions significantly.

17.9 Future Perspectives

Agricultural GHG emissions come primarily from livestock, producing methane through enteric fermentation and manure management. GHGs have much greater global warming potential than carbon dioxide. Enteric methane emissions from livestock contribute to global warming and divert a significant amount of energy from the host animal. India cannot afford the energy loss in the form of methane since it will require extra feed supplies to compensate for the shortfall. Mitigation options for enteric methane reduction should be chosen based on the feasibility of the intervention(s) in a particular region. Our attention should be on strategies that have the potential to endure over time and result in a 20–25% reduction in enteric methane emission. Different storage conditions are required for methane and methane emissions from manure management to the fact that methane emission from manure is not particularly alarming in the country; our focus should be on reducing methane emission from manure management by developing interventions that ensure that methane emission does not increase while attempting to mitigate methane emission from manure management.

Enteric methane amelioration from Indian livestock is required from monetary benefits to stakeholders and environmental points of view. To achieve the substantial reduction under the present scenario of feed and fodders deficit, a set of likely interventions alone and in combinations need to be explored under different feeding systems and species. The target of the researchers should be long term methane reduction rather than short term. Basic information on methanogenic archaea should be explored to effectively control methane generating machinery in the rumen. Even though the absolute methane emission corresponding to the total livestock population in the country is less, due to high enteric methane emission per unit of product, Indian livestock always remains a matter of discussion. Large enteric methane emissions in absolute quantity from Indian livestock warrants the urgent attention of the researcher and policymakers. Given the acute feed shortage and seasonal variability of the feed resources, the researcher should focus on exploring the prospects of biological interventions for reducing enteric methane emission from the livestock, and efforts should be taken up for the genetic up-gradation of the livestock for low methane emission. Many strategies have been developed and evaluated in the country. However, their confinement to the laboratory is a crucial issue and needs the attention of the extension workers and line departments for undertaking front line demonstration and popularising the farmers' mitigation strategies. Large enteric methane emissions in absolute quantity from Indian livestock warrant the urgent attention of the researcher and policymakers.

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Heat Stress on the Rumen Fermentation and Its Consequence

18

Yutaka Uyeno

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Abstract

Ruminants are considered as one among the most sensitive animals to heat stress. Very little information is available regarding the ruminal microbiota, playing significant role in ruminant digestion, in connection with the physiological changes associated with heat stress. A study was conducted by our team for assessing the effects of temperature and relative humidity on the ruminal microbiome composition using RNA-based method and the results were used to effectively describe the predominant bacterial colony colonizing the rumen. Four female Holstein animals (Heifers) were used in the study, with 2 weeks each animal maintained in a climatic chamber under three different temperatures (20–33 °C). Ruminal fluid samples are collected from each animal on the termination day. In this experiment it was observed that at elevated environmental temperatures and humidity, body weight gain and dry matter intake were decreased and feed digestibility was increased with increasing temperature. Increase in the comparative populations of the genus *Streptococcus* and the *Blautia coccoides-Eubacterium rectale* group and decrease in the genus *Fibrobacter*'s population was observed as a response to rising temperature. It

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V. Sejian et al. (eds.), *Climate Change and Livestock Production: Recent Advances and Future Perspectives*, https://doi.org/10.1007/978-981-16-9836-1_18

was also established that the ruminal microbiota was affected by various factors such as age, diet and stress. These findings suggests that the ruminal eco-system and its microbial community remain in a relatively unperturbed and matured state throughout a longer duration, with typical daily fermentation patterns remaining largely unchanged across the different stages.

Keywords

Heat stress · Holstein heifers · Metagenomics · Rumen · Rumen microbiota

Abbreviations

%	Percentage
°C	Degree celsius
16S rRNA	16 Sedimentation coefficient rRNA
CO_2	Carbon dioxide
CP	Crude protein
DMI	Dry matter intake
Fig.	Figure
H	Hydrogen ion
H_2	Hydrogen
LPS	Lipopolysaccharides
pH	Potential of hydrogen
PS	Persimmon skin
PSM	Plant secondary metabolite
PSS	Persimmon skin silage
RH	Relative humidity
RNA	Ribonucleic acid
rRNA	Ribosomal RNA
SARA	Sub-acute rumen acidosis
SCFA	Short chain fatty acid
VFA	Volatile fatty acid

18.1 Rumen Microbial Community Overview

The complex microbial ecosystem of rumen efficiently converts the carbohydrates from plant source to organic acids. Any disturbances caused to this ecosystem will negatively influence the host by causing diseases or impairing its productivity. Factors such as farm management, feeding practices followed in the farm, and importantly, composition of diet will severely affect the rumen's ecosystem of animal, producing negative effects on animal's health and production. There is a renewed interest in this complex system due to the development of various molecular strategies, with particular mention to the techniques that rely on 16S rRNA genes.

These technologies do not only provide a phylogenetic framework of the microbiome, but also in addition, they describe the impacts caused by environment and host factors on the dynamics and structures of rumen microbiome. Eventually the extreme molecular diversity exhibited by the bacterial community shall be understood. Phylum Firmicutes and phylum Bacteroidetes are the ones under which majority of the ruminal bacteria are affiliated (Uyeno et al. 2007). All the fibrolytic bacteria that contributes to the characteristics of the organ, belong to the lower taxa, such as *Ruminococcus flavefaciens* and *Ruminococcus albus* and the genus *Fibrobacter,* and they also possess niches in the ruminal community. Methanogens are exclusively represented by the archael community of the ruminal microbiota, which is claimed for hydrogen removal. Methane generation, being the crucial component involved in maintenance of the rumen ecosystem's stability, is at its peak of attaining attention worldwide, due to its major part played in contribution to greenhouse effect and global warming. Mitigation strategies implemented to reduce the enteric methane production have increased the energy deposition in the ruminant's body which made ruminal archaea to acquire an indispensable segment of rumen's ecosystem.

Over the years, innumerable numbers of attempts were made by the researchers worldwide to manipulate the composition and functions of ruminal ecosystem for improving the production of animals. Microbial colonization in the rumen that is inherently harmful for the rumen microbiota is usually unsuccessful, because of the robust nature of ruminal ecosystem. However, some common microbial colonization also becomes harmful under certain conditions. For example, pH reduction caused by the organic acids accumulation in the rumen that are absorbed little via the ruminal epithelium. This condition is very commonly observed among the cattle undergoing heat stress and the ones fed with excess amount of concentrates.

18.2 Heat Stress and Rumen Acidosis

The combination of excessively high temperature above the upper critical limit and the higher relative humidity always causes heat stress in animals. Ruminants are considered as one among the most sensitive animals to heat stress that is primarily caused by various environmental factors like relative humidity, temperature, air movement and solar radiation in combination (Collier et al. 2006). Metabolic heat produced in the rumen during rigorous fermentation is the main reason for heat load in case of cattle. The increased temperature attained from this process is nullified by the means of heat loss. (Bohmanova et al. 2007). To get over heat stress, cattle respond physiologically by reducing the exercise and feed intake. Since, mass energy is spent to tackle heat stress effects rather than energy consumption, this process in turn leads to deleterious effects on reproduction, milk yield and animal health. In these occasions, ruminal ecosystem probably exhibits response to heat stress. Changes produced at the group level of ruminal bacteria was monitored by using the ruminal fluid. Total RNA was extracted from the collected ruminal fluid to investigate the changes in bacterial community.

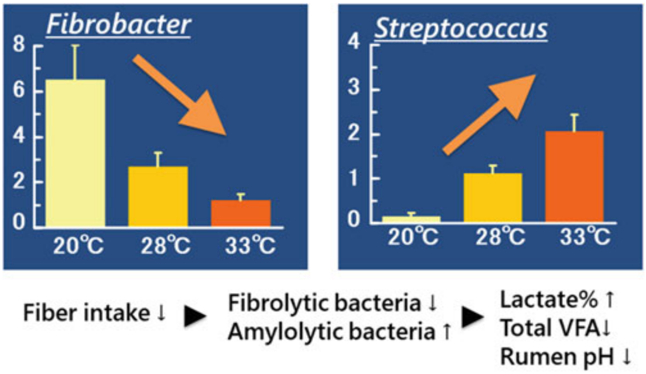
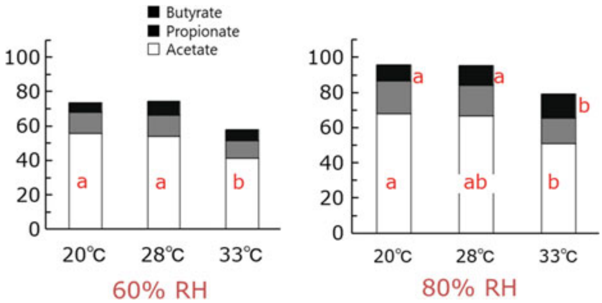


Fig. 18.1 Effect of heat stress [ambient temperature and relative humidity (RH)] on the population of specific bacterial groups in the rumen of Holstein heifers

Fig. 18.2 Effect of heat stress [ambient temperature and relative humidity (RH)] on the rumen VFA level and the composition of Holstein heifers



Our study conducted using rumen-fistulated, four healthy female Holstein animals of age between 9 and 15 months that are exposed to different temperatures, showed reduction in body weight gain and dry matter intake (DMI) particularly with consumption of forages and with increase in temperature (20–33 °C) (Uyeno et al. 2010; Tajima et al. 2007). Profiling of rumen's bacterial colony structure was investigated. Increase in the genus *Streptococcus,* a major saccharolytic bacteria (2.5–10 times) and decrease in the genus *Fibrobacter*, a major fibrolytic bacteria (50–80%) was observed as a response to increment in environmental temperature (Fig. 18.1).

Under heat stress, one can observe the consistent changes in the population level of particular group of bacteria. It is the preferential increase in concentrates consumption justifies the increase in activity of saccharolytic bacterial population and reduction in activity of fibrolytic bacterial population. These changes in turn lead to the notifiable reduction in concentration of SCFA (Fig. 18.2). This reduction in concentration of SCFA could be an attributing factor for impairment observed in growth performance of animal suffering under extreme conditions. Heat stress effect on the fermentation can be indirectly explained by the reduction in pH caused by increment in the activity of bacteria responsible for starch degradation. It is also said

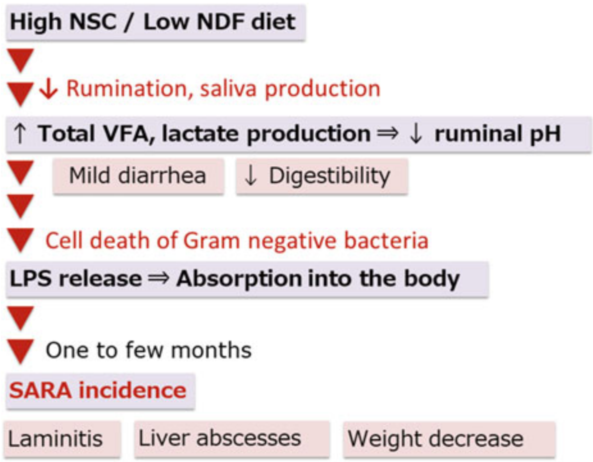


Fig. 18.3 A schematic flow about how SARA occurs due to changing feed

that, may be these bacteria undergoing changes may be small in population, still their comparative population in the rumen holds an important position in deciding the rumen's adaptive response to heat stress. It is very vital to note that the diversity of bacterial community as a whole at both species and genus level, remained intact even under heat stress. If one group gets reduced under certain situation, meanwhile the nullification for this reduction of certain group is taken care under different situation and hence the diversity of ruminal bacterial community is maintained.

From the current understanding of ruminal physiology, it can be noted that the combination of different strategies are required to optimize the animal's production under this hot and humid conditions. Various studies demonstrated the effects of different diets on the composition and diversity of ruminal bacterial community. Knowledge in this perspective is now good enough to have nutritional intervention as a simple approach to tackle the heat stress related challenges. Reducing forage in the ruminant's diet can be a very simple and better way to increase the nutrient density and decrease the adverse effects on productivity. In addition, this forage reduction in the diet, also contributes for increase in the milk production of dairy cattle undergoing heat stress in comparison with forage fed cattle, which can be explained by decrement in the production of metabolic heat. But it should also be understood that at times this feeding strategy may lead to increase in the population of lactate producers (*S. bovis*) and henceforth drop in the pH of rumen will be observed, because of the fact that the absorption of lactate is very much less than that of VFAs by the ruminal epithelium.

There are few other conditions which is associated with ruminal pH reduction but not linked to increase in lactic acid accumulation. Sub-acute rumen acidosis (SARA) is one such condition, which often occurs with accompanying laminitis and liver abscesses during the early and mid phase of lactation and is characterized by the reduction in ruminal pH. Etiology of this condition is related to the factors like shift in ruminal microbe population and immunological responses but not with lactic acid accumulation in the rumen (Fig. 18.3).

It is postulated that the moderate reduction in ruminal pH can increase the lipopolysaccharides production of ruminal bacteria and also the permeation capacity of LPS which then acts as endotoxins for triggering the systemic infection. Under extreme conditions, to maintain the animal's health and productivity, nutritional intervention can be a key option to cater the negative effects on the rumen's function. Application of radio transmission pH sensing system was demonstrated to monitor and assess the changes in pH of cow's rumen in the transition period (Hasunuma et al. 2016). Cows suffering from SARA in transition, early and mid phase of lactation periods were successfully identified through consecutive monitoring of changes in ruminal pH. For easy and better diagnosis of SARA in the on-farm basis, this technique can be considered as a promising strategy to track changes in ruminal pH. Acquisition of vast amount of data along with the study of relationship of ruminal pH changes with various other factors can give better understanding regarding the factors causing susceptibility and tolerance of animals to SARA in near future.

18.3 Enteric Methane Emission and Mitigation

Various forms of carbohydrates are converted into VFAs by the complex ecosystem of rumen to meet out the energy requirement of host through categorical disposal of H by reducing carbon dioxide to CH_4. It is in this step of reducing H, lies the primary concept of manipulating methane generation, which is aimed at diverting this H away from CH_4 production. For instance, alternative pathways of metabolism to divert this hydrogen like pathways generating propionate from succinate. Another vital way is to reduce the H available for methanogenesis which can be done by utilizing the alternate electron sinks. But equivocal results attained from the trails conducted with this concept demands more information regarding the function, composition and interactions taking place within this complex ecosystem of rumen.

In ruminants, methane production principally depends on the dry matter intake (DMI), particularly fiber. The more carbohydrate digestion in the rumen leads to more disposal of reducing power in the form of methane. An inverse relationship has been described long back between the rumen's methane and propionate production, reflecting the competitiveness exhibited by both the processes in consuming the reducing power. Three groups of ruminal bacteria were classified on the basis of its H_2 utilizing/producing ability: (1) bacteria producing lactate, butyrate, propionate and/or ethanol, (2) bacteria producing hydrogen and acetate and, (3) archae producing methane (methanogenic and hydrogen users) (Fig. 18.4). The first classified bacterial community theoretically undergoes fermentation completely without requiring any disposal of hydrogen, which means that they don't rely on methane production and the second classification shows a marked dependency on third classified bacterial community for effective fermentation. Some bacteria are potent in utilizing molecular hydrogen for reductive acetogenesis, and even threshold for this reaction is much higher than the methanogenesis resulting in very poor opportunity to alter methanogenesis with acetogenesis.

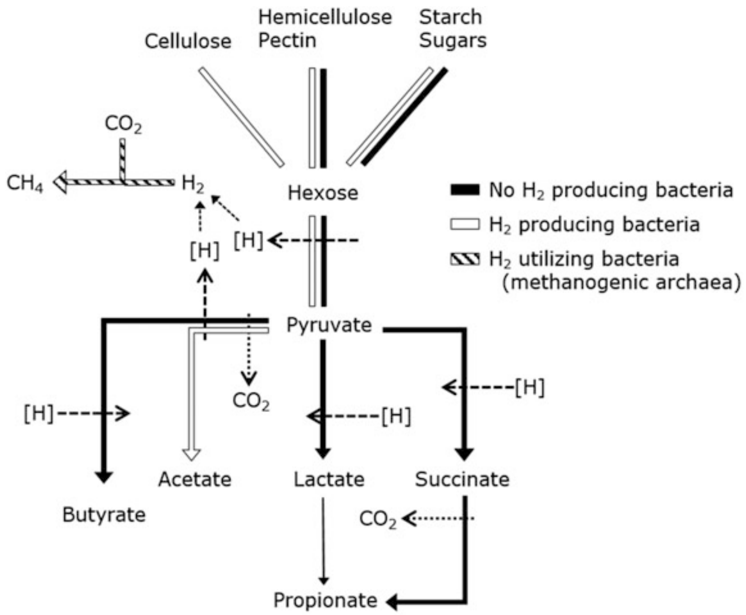


Fig. 18.4 Schematic representation of fermentation of carbohydrates and categorization of bacteria according to their demand for H_2 utilization/production

Direct ruminal interventions aim to manipulate any of the steps, where possibility appears for the mitigation of methane emission in ruminants. Several efforts have focused on decreasing enteric methane emission through the addition of lipids, plant compounds, monensin, and other organic compounds, or by otherwise controlling diet composition. There is a blooming interest in the field of utilizing plant extracts and plants itself for mitigating enteric methane emissions. Interest is gaining in tropical parts of the world to use the plant secondary metabolites (PSM) for mitigation of livestock's methane emission and to improve production performance of livestock. Antimicrobial property of the PSM, makes it a better substitute for artificial feed additives. Major classes of PSM are constituted by saponins and tannins, which are under major area of research among multiple laboratories around the globe (Bhatta et al. 2009). Tannins can bind with microbial proteins and feed and hence it can be used to limit the substrate required by methanogens for methane production and therefore as a better supplement to inhibit enteric methane production without producing any undesirable effects on ruminal function (Patra and Saxena 2011). Much more investigations are required to confirm the feasibility and potential nature of tannins as additives in the feed of ruminants. Effects of tannins are not limited to carbohydrate metabolism alone. Sufficient documentation is available for the effect produced by tannins in the metabolism of nitrogen in rumen. Tannins (phenolic hydroxyl group) react with proteins predominantly through hydrogen bonding. Tannin-protein complex prevents the breakdown of proteins by proteases. This bonding occurs at the ruminal pH which in turn affects the metabolism by ruminal microbiota. This characteristic of tannins can be utilized in the production of rumen escape CP. This complex in turn can be reversed back in abomasum for animal's utilization and this strategy can play a significant role in improving the availability of protein for the host. For more details, please refer to other articles in this book.

A widely distributed fruit in the Asian continent, Persimmon scientifically named as *Diospyros kaki,* occupies majorly the food industries of South Korea and Japan and is produced more than 200 kilotonnes per year. Hoshigaki, dried form of persimmon is consumed as such sometimes. The process of preparing Hoshigaki involves peeling its skin, which is associated with the production of 20% of persimmon skin (PS). This skin of persimmon is very rich in polysaccharides like pectin and various soluble forms of carbohydrates, which makes this as a best suitable source of energy for animals. To understand its effects on CH_4 mitigation, in vitro experiment (incubation study) was conducted using the unfermented and ensiled PS (Mousa et al. 2019). Outcome of this experiment proved that the in vitro production of methane was reduced by PS silage (PSS) and it was also found that this reduction in methane generation is attributed to the carbohydrate composition and changes in the composition of PSS. It can be concluded that the changes occurring during this ensiling procedure is the sole responsible factor for the effect absorbed on fermentation and methane production. Hence, by utilizing different dry absorbents for adjusting the moisture in PSS, reduction in the effluent loss shall be achieved to a trivial amount (Abdelazeem et al. 2020). Furthermore, sheep after consuming a partially (upto 20%) substituted ration with PSS, showed no undesirable effects on palatability and feed intake. Henceforth, detailed investigations along with *intro* experiments (incubation study) are needed for determining the effects of PS on animal's feed efficiency and its impacts on enteric methane production and emission.

18.4 Future Perspectives

Many years of research were aimed at manipulation of composition and function of rumen microbiota for improving its production. Even the minor changes in the rumen's microbiome composition have a potential impact on food intake, satiety and energy expenditure of the animal. Therefore, it can be understood that the animal's health and its productivity is determined by its ability to prolong the balance within rumen's microbial ecosystem. Balance maintained in the rumen ecosystem chiefly determines the animal's health and its productivity. Hence, the fundamental aspect of future research should aim at warranting the interactions between the microbial community and its interaction with the host. The ruminal ecosystem and its microbial community remain in a relatively unperturbed and matured state throughout a longer duration. In order to link the ruminal microbiome composition to the changing rumen metabolites, understanding the effects of different factors, including diet, individual animal variability, and post-feed time, on the rumen microbiome is warranted.

Learning Outcomes

- Factors such as age, diet, and heat stress influence the microbial composition of rumen.
- Heat stress significantly increases the relative populations of the genus *Streptococcus*, and the *Blautia coccoides-Eubacterium rectale* group while decreases the genus *Fibrobacter* in cattle.
- 16S rRNA gene sequencing technology apart from providing the phylogenetic framework of the microbiota, can also provide an insight into the impact of environmental and host factors on the dynamics and structure of ruminal microbial community.
- Phytochemicals like tannin and saponins serves as best feed additives and their antimicrobial property can be used to inhibit enteric methane production in ruminants.

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Comparative Assessment of Rumen Microbial Diversity in Cattle and Buffaloes

19

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Abstract

Cattle and buffalo are essential components of the livestock industry, which contribute significantly to the economy. Globally, the rumen microbiota in general, and methanogen community in particular in buffaloes, have received less attention than cattle. The rumen microbiota has a strong correlation with the digestion and metabolism of host diet. Furthermore, buffalo rumen exhibits higher bacterial, fungal and protozoal populations compared to cows, indicating different metagenome profiles. Firmicutes and Bacteroidetes are the most prevalent ruminal bacterial phyla in both buffaloes and cows, indicating the existence of a core microbiome in ruminants. Diversity of microbes is much higher in buffaloes compared to cattle. Between buffalo and Jersey cows, the methanogen community was comparable, with *Methanobrevibacter* genus accounting for

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V. Sejian et al. (eds.), *Climate Change and Livestock Production: Recent Advances and Future Perspectives*, https://doi.org/10.1007/978-981-16-9836-1_19

more than 90% of the total archaea. The protozoal community are more variable than bacteria and archaea; *Metadinium* being higher in buffaloes, while the *Entodinium* genus predominated in Jersey cows. Fungal diversity has been found to be influenced more by the host rather than effect of diet. Genera *Caeocomyces*, *Cyllamyces*, and *Orpinomycesi* have been principally found in cattle while *Aspergillus*, *Candida*, or *Kluyveromyces* found to be highly abundant in buffaloes. It is critical to characterize the ruminal microbiota differences between buffalo and Jersey cows on a worldwide geographic scale for developing innovative methods to their productivity while minimising environmental impact.

Keywords

Buffalo · Cattle · Metagenome · Methanogen · Microbiome · Rumen microbiota

Abbreviations

%	Percentage
>	Greater than
<	Lesser than
CH_4	Methane
CO_2	Carbon dioxide
FAO	Food and Agriculture Organization
GOI	Government of India
MCP	Microbial crude protein
mL	Millilitre
pH	Potential of hydrogen
RNA	Ribonucleic acid
Tg	Teragram
VFA	Volatile fatty acid

19.1 Introduction

Cattle and buffalo are a vital part of the livestock sector, which contribute significantly to the economy. Cattle are raised across the world, whereas buffalo, which have evolved to tropical and subtropical temperatures, are confined in low-latitude regions (Asai et al. 2021). India is endowed with a vast variety of 43 indigenous cow breeds and 13 buffalo breeds that have endured for hundreds of years due to their adaptability for certain functions in the local environment (GOI 2021a, b). Indian livestock inventory now total 536.76 million head of livestock, including 193.46 million cattle and 109.85 million buffaloes (GOI 2019). The cattle and buffaloes contribute 48% and 49% to the total annual milk production (198 million metric tonnes) in India (GOI 2021a, b).

With the livestock sector's fast growth, various challenges have arisen, including low feed conversion efficiency, nitrogen use efficiency, product quality, and significant methane emissions (Kumar et al. 2013). Ruminants face more of the above challenges than monogastric animals do due to their unique digestive system, which must be better understood in order to handle these concerns. Attempts to understand and manipulate the rumen microbiome for the benefit of global agricultural challenges have been ongoing for decades with limited success, owing primarily to a lack of a detailed understanding of this microbiome and our inability to culture the majority of these microbes outside the rumen.

There have been several reports claiming that buffaloes are better at digesting fibrous foods than cattle, particularly diets rich in cellulose (Norton et al. 1979). Buffaloes are more efficient at using high-roughage feeds (Lapitan et al. 2008); they digest fibrous fractions, crude protein, and crude fibre more efficiently than cattle (Robles 1971). Dry matter, organic matter, crude protein, neutral detergent fibre, and acid detergent fibre digestibility were shown to be higher in buffalo than in cattle (Chanthakhoun et al. 2012), showing clear variations in the rumen microbiome composition or function between buffalo and cattle. Globally, the rumen microbiota in general and methanogen community in buffaloes is particular less characterized as compared to cattle (Malik et al. 2020). Thus, it is critical to characterise the ruminal microbiota variations between buffalo and Jersey cows in order to formulate appropriate feed for them.

19.2 Rumen Microbial System

Rumen is one of the most diverse and complicated microbial habitats, containing both prokaryotes and eukaryotes. Ruminants' ability to utilise a wide variety of feeds is a result of their highly diverse rumen microbial ecosystem, which includes bacteria (10^{10}–10^{11} cells/mL, representing more than 50 genera), ciliate protozoa (10^4–10^6 cells/mL, representing 25 genera), anaerobic fungi (10^3–10^5 zoospores/mL, representing five genera), and bacteriophages (10^8–10^9 cells/mL). These figures might be substantially higher, considering the majority of them are unculturable (Kamra 2005). Due to the enormous variety and functions assigned to rumen bacteria, the rumen microbiota is recognised as a unique organ consisting of billions of microbes with gene content hundreds of times that of host cells (Human Microbiome Project Consortium 2012).

The rumen microbiota generates enzymes that efficiently break down complex lignocellulosic plant components into simpler molecules that the host can easily absorb. Vertebrates evolved without the ability to manufacture enzymes capable of degrading cellulose and other complex polysaccharides. The primary benefit of rumen bacteria is their capacity to absorb non-protein-nitrogen and plant polysaccharides in a diet that cannot be digested and utilised by the host animal. Several microbes in the rumen can secrete cellulase, which is a key enzyme to use plant cell wall materials by ruminants. Rumen fermentation is carried out by bacteria (two main phyla are *Firmicutes* and *Bacteroidetes*), fungi, archaea

(*Euryarchaeaota*), and protozoa, which produce volatile fatty acids, vitamins, microbial proteins (Hobson and Stewart 2012), and amino acids. Certain rumen microorganisms also synthesise their own proteins (referred to as microbial crude protein, MCP) for growth, employing the energy and nitrogen obtained from the diet. The MCP are digested and absorbed by the host in the small intestine, therefore contributing to the host's nutrition also. Additionally, several bacteria are capable of producing vitamins, such as vitamins B and K. Rumen microorganisms have several enzymes involved in the de novo production of vitamin B12, which are absent from the human gastrointestinal microbiome (Seshadri et al. 2018).

Carbon dioxide (CO_2) and methane (CH_4) are the end products of anaerobic fermentation in the rumen, which are excreted into the environment (Morgavi et al. 2012). Enteric fermentation, which emits between 87 and 97 Tg of methane per year, continues to be one of the primary sources of methane in agriculture (Chang et al. 2019). Globally, cattle and buffaloes contribute 77% and 13%, respectively, to yearly enteric methane emissions (FAO 2021). The cattle and buffaloes contribute 48% and 49% to the total annual milk production (198 million metric tonnes) in India (GOI 2021a, b). India is the world's largest milk producer, owns 13% of the worldwide cow population and 53% of the global buffalo population (GOI 2019), which together account for 4.92 and 2.91 Tg of yearly global enteric methane emission from the respective species (Bhatta et al. 2019). These two largest bovine species together accounts for more than 85% of India's total enteric methane output (Malik et al. 2021). Methane is generated entirely by methanogenic archaea during ruminal fermentation.

The rumen microbiota has a strong correlation with the digestion and metabolism of host diet. Numerous prior researches have shown that one or more groups of ruminal microbiota have an effect on feed efficiency, nitrogen digestibility, and methane generation in ruminants (Schären et al. 2018). Rumen methanogenic archaea produce methane mostly from the products of fermentation processes. It becomes critical to understanding the roles played by the constituent microbes for devising any strategies to manipulate the rumen microbiome to improve ruminant production whilst reducing environmental impact.

19.2.1 Bacteria

Despite considerable advances in microbiology, the function of rumen bacteria and their interactions with other members of the rumen microbiome remain poorly understood, and as a result, there are only a few cases of direct modification of this community's composition yielding favourable results. Gram negative bacteria prevail when animals are given a high forage diet, while Gram positive bacteria, such as Lactobacilli, predominate when animals are fed a high grain diet (Hungate 1966). *Prevotella*, *Butyrivibrio*, and *Ruminococcus* are the most abundant bacteria in the rumen, and their community structure is influenced by the host's diet (Henderson et al. 2015; Iqbal et al. 2018). *Fibrobacter succinogenes* and *Ruminococcus albus* are primarily responsible for cellulose breakdown, while *Streptococcus bovis*, bovine amylolytic bacteria, is responsible for starch digestion (Matthews et al. 2019). According to studies, the shape of the microbial community may be determined by diet rather than animal type.

The feature of milk production is closely associated with the rumen microbial composition, particularly with bacteria (Xue et al. 2020). *Firmicutes*:*Bacteroidetes* ratio is substantially connected with milk-fat yield (Jami et al. 2014), but milk yield, protein percentage, fat yield, and bacterial communities all have modest associations (Bainbridge et al. 2016). *Prevotella* sp. and *Succinimonas amilolytica* were shown to have favourable correlations with VFA concentrations in the rumen of high milk producing cows.

19.2.2 Archaea

The archaea found in the rumen are classified as *Euryarchaeota*. These methanogens produce methane in the rumen, which is subsequently eructed and released into the environment, acting as a significant greenhouse gas. Methane is mostly created through the hydrogenotrophic pathway, which involves the reduction of CO_2, and less often via the methylotrophic pathway, which involves the use of methyl groups, or even less frequently from acetate (acetoclastic pathway) (Morgavi et al. 2010). Though ruminal methanogens are not as diverse as bacteria, their necessity for a different substrate makes this community complex (Malik et al. 2021). The primary phylogenetic groupings of methanogens in the rumen include *Methanobrevibacter* (>62% of methanogens), *Methanosphaera* (>15% of methanogens), and the newly described *Methanoplasmatales* (>16% of methanogens). The remainder are classified as minor genera, including *Methanimicrococcus*, *Methanosarcina*, and *Methanobacterium* (St-Pierre and Wright 2013). The *Methanobrevibacter gottschalkii* and *Methanobrevibacter ruminantium* clades, as well as *Methanosphaera* sp. and two *Methanomassiliicoccaceae*-related groupings with variable abundance, dominate the archaeal community in all animal species worldwide (Henderson et al. 2015).

19.2.3 Protozoa

Rumen protozoa account for a significant proportion of the rumen's microbial biomass (about 20–50% in certain circumstances); their involvement in ruminal fermentation, metabolism, and nutrition of the animal remains a source of much debate (Williams and Coleman 1992). They are, together with fungi, the eukaryotic members of the rumen microbiota. Both flagellated and ciliated protozoa have been described in the rumen, with ciliate protozoa (e.g. genera *Diplodinium*, *Dasytricha*, *Isostricyha*, *Eremoplastron*, *Entodinium*, or *Trichostomatia*) being far more abundant and belonging to two distinct orders: the *Entodiniomorphida* and the *Vestibuliferida* or *Holotricha* (Williams and Coleman 1992).

The rumen protozoa engulf organic materials, typically bacteria, and transport it to digestive vacuoles where it undergoes hydrolysis and fermentation. Protozoa have also been shown to boost the rumen pH faster than bacteria do, hence stabilising rumen fermentation (Williams and Coleman 1992). As with other rumen bacteria, certain ciliate protozoa are capable of digesting plant structural polysaccharides such as cellulose and hemicellulose, whereas all ciliate protozoa are capable of engulfing particulate matter. They can engulf and utilise starch grains, insoluble protein and even microbial cells. Ciliate protozoa are capable of fermenting carbohydrates and generating end products such as formate, acetate, propionate, butyrate, CO_2, and hydrogen. Defaunation, a process of protozoa elimination, lowers methane production by around 11% (Morgavi et al. 2010). Rumen protozoa show variability in their contributions to plant degradation and methane production. For example, *Epidinium* spp. play a significant role in plant degradation (Huws et al. 2009), and holotrichs in general encourage methanogens and methanogenesis (Belanche et al. 2015).

19.2.4 Fungi

Anaerobic fungi comprise 10–20% of the rumen microbiome and are critical in the breakdown of low-quality forages (Krause et al. 2013). Fungi are one of the most powerful fiber-degrading organisms known to science, owing to their large and effective array of enzymes for the destruction of plant structural polymers (Solomon et al. 2016). Additionally, their rhizoids are capable of physically penetrating plant structural barriers (Orpin 1977), benefiting other rumen bacteria by expanding the plant cell surface area available for colonisation. Six genera of fungi are found in the rumen: the monocentric *Neocallimastix*, *Caecomyces*, and *Piromyces*, and the polycentric *Anaeromyces*, *Orpinomyces*, and *Cyllamyces*, each with 21 species, and the newly discovered genera *Oontomyces* and *Buwchfawromyces*. As with the protozoal population, rumen fungi's close interaction with methanogenic archaea promotes fungal activity and contributes to methane generation.

19.2.5 Virome

Virome is comprised of the bacteriophage and viral population. Bacteriophage are obligatory pathogens of bacteria that exist in the rumen at a rate of around 10^7–10^9 particles per gram of digesta (Klieve et al. 1996). Although the high prevalence of bacteriophage indicates that they contribute significantly to the rumen system's equilibrium, they remain by far the least well-characterized component of the rumen microbiome. The complete genome sequences of lytic phages, of the order Caudovirales was obtained (Gilbert et al. 2017), that can infect *Bacteroides*, *Ruminococcus* and *Streptococcus*. In the rumen, the majority of viruses are linked with *Firmicutes*, *Bacteroidetes*, and *Proteobacteria* (Berg Miller et al. 2012). Recent research has shown the occurrence of RNA-based viruses that infect fungus (mycoviruses). However, there is a lack of sufficient molecular and physiological information for this class of rumen microbe. The precise function of phages in the rumen, their effect on fungal populations in the rumen, and fibre breakdown all need more investigation to acquire a better understanding of their role and function in the rumen.

19.3 Comparative Microbial Diversity

In India, the buffalo population is growing at a higher pace than cattle (Paul et al. 2018). Thus, it is critical to comprehend and compare their fermentation patterns and microbial community composition in order to fully understand the variations and effects of animal species on their gut microbial community. The rumen and reticulum of grazing buffaloes contained a high quantity of bacteria and a low concentration of archaea (bacteria, methanogenic archaea, and ciliate protozoa). Buffalo had a larger bacterial population and a higher Shannon diversity index than Jersey cows, with the highest abundance of *Bacteroidetes* and *Firmicutes* (Iqbal et al. 2018). *Firmicute*s population in the rumen is proportional to carbohydrate fermentation (Fernando et al. 2010); hence, *Firmicutes* are always more abundant in the guts of efficient feed consuming animals. Buffalo are more efficient in breaking down feed nutrients than Jersey cows, owing to their larger Firmicutes population. Similar to cows, buffalo rumen had a greater number of ruminal cellulolytic bacteria and fungus (Chanthakhoun et al. 2012). This conclusion is bolstered further by the fact that buffaloes have higher ruminal VFA concentrations (Iqbal et al. 2018).

Firmicutes and *Bacteroidetes* were the major ruminal bacterial phyla in both buffalo and Jersey cow, confirming the existence of a core microbiome in the rumen of ruminants (Henderson et al. 2015). *Bacteroidetes* were more abundant in the rumen of cattle, which may account for their tolerance to high roughage feeding. *Prevotella* was substantially less abundant in buffalo than in Jersey cows; however *Ruminococcus* was significantly higher in buffalo. *Prevotella* is engaged in protein breakdown and polysaccharide digestion, but *Ruminococcus* is highly effective at cellulose and hemicellulose degradation (Flint et al. 2012). Bacterial diversity and Shannon index were much higher in buffalo than in Jersey cows (Iqbal et al. 2018), demonstrating that buffaloes have a greater rumen microbial diversity than cattle. *Ruminicoccus albus* and *Ruminicoccus flavefaciens* were found in greater numbers in the rumen of swamp buffaloes than in cattle (Chanthakhoun et al. 2012). *R. albus* levels in rumen fluid were considerably greater in buffalo than in cattle, whereas *Ruminicoccus flavefaciens* and *Fibrobacter succinogenes* levels tended to be higher in cattle than in buffalo. The dissimilar microbiome profiles in cattle and buffalo became more closely related after 16 weeks of similar dietary regime (Asai et al. 2021). The population of *Fibrobacter succinogens* was increased significantly in the tannin and tannin + saponin supplemented groups as compared to control. In contrast, the population of *Ruminococcus albus* and *Ruminococcus flavifaciens* was decreased significantly ($p < 0.05$) in the tannin + saponin (*Ruminococcus albus*) and tannin (*Ruminococcus flavifaciens*) groups respectively, as compared to control; reporting differential response of fibrolytic microbes to tannins and saponins supplementation.

The rumen archaeal population is profoundly influenced by the host physiology. The geographical region and host have an effect on the structure of the archaeal communities. Between buffalo and Jersey cows, the methanogen community was comparable, with *Methanobrevibacter* genus accounting for more than 90% of the total archaea (Iqbal et al. 2018). Six archaeal orders have been recognised in cattle, whereas seven orders have been identified in buffaloes (Malik et al. 2021). Methanogens related with the *Methanocellales* have been found solely in buffaloes. Methanogens belonging to the *Methanobacteriales* were predominant in both hosts, accounting for 72–73% of total ruminal archaea (Fig. 19.1a). *Methanobrevibacter* was the most prevalent genus (55–62%) in both cattle and buffaloes, but *Methanobrevibacter gottschalkii* constituted the largest proportion of the archaeal community at the species level (Trivedi et al. 2020). However, other Indian studies discovered that *Methanomicrobium* dominated the methanogen community in buffaloes (Singh et al. 2011).

Methanotrophic *Methanomassiliicoccales* comprised 10% of the entire archaeal community and had the greatest variation between cattle and buffaloes (hosts) and geographical locations. *Methanomassiliicoccales*, including *Methanosphaera* sp., Group 4 sp., Group 8 sp., Group 12 sp., and Group 9 sp., were substantially more abundant in cattle than in buffaloes. Thus, except for the presence of *Methanobrevibacter gottschalkii*, the host species appears to have minimal effect on the organisation of the rumen methanogens community. A meta-analysis (Paul et al. 2018) demonstrated that the community structure of methanogens differs at the genus and species level between cattle and buffaloes. Buffaloes had a larger abundance of *Methanomassiliicoccales*, *Group 10*, and *Methanobacterium alkaliphilum*, whereas Jersey cows had a lower abundance of *Methanobrevibacter*, demonstrating that host species do have an effect on the rumen archaeal community (Iqbal et al. 2018).

The archaeal diversity between cattle and buffaloes fed on the same diet (Fig. 19.2) demonstrated that *Methanobrevibacter* dominated the archaea community (66–68%) and that its abundance was comparable across cattle and buffaloes (Sirohi et al. 2013; Parmar et al. 2017; Malik et al. 2021). Additionally, *Sulfolobus thuringiensis* was detected in Indian cattle and buffaloes, making this the world's second report and the first from India (Malik et al. 2021).

The Shannon (alpha diversity) and Bray-Curtis (beta diversity) indices at the archaeal genus level in cattle and buffaloes are displayed in Fig. 19.3 and demonstrate that the total diversity of ruminal methanogens was considerably different between cattle and buffaloes. Additionally, the examination of the core methanogens revealed a minor variation in the core microbiome of cattle and buffaloes. *Methanomicrobium* was found only as a component of the cow archaeal core microbiome. Methanogens such as *Methanomicrobium* mobile and *Methanobrevibacter wolinii* were found to be present only in the cow archaeal core microbiome (Fig. 19.4c). In contrast, *Methanobrevibacter smithii* was shown

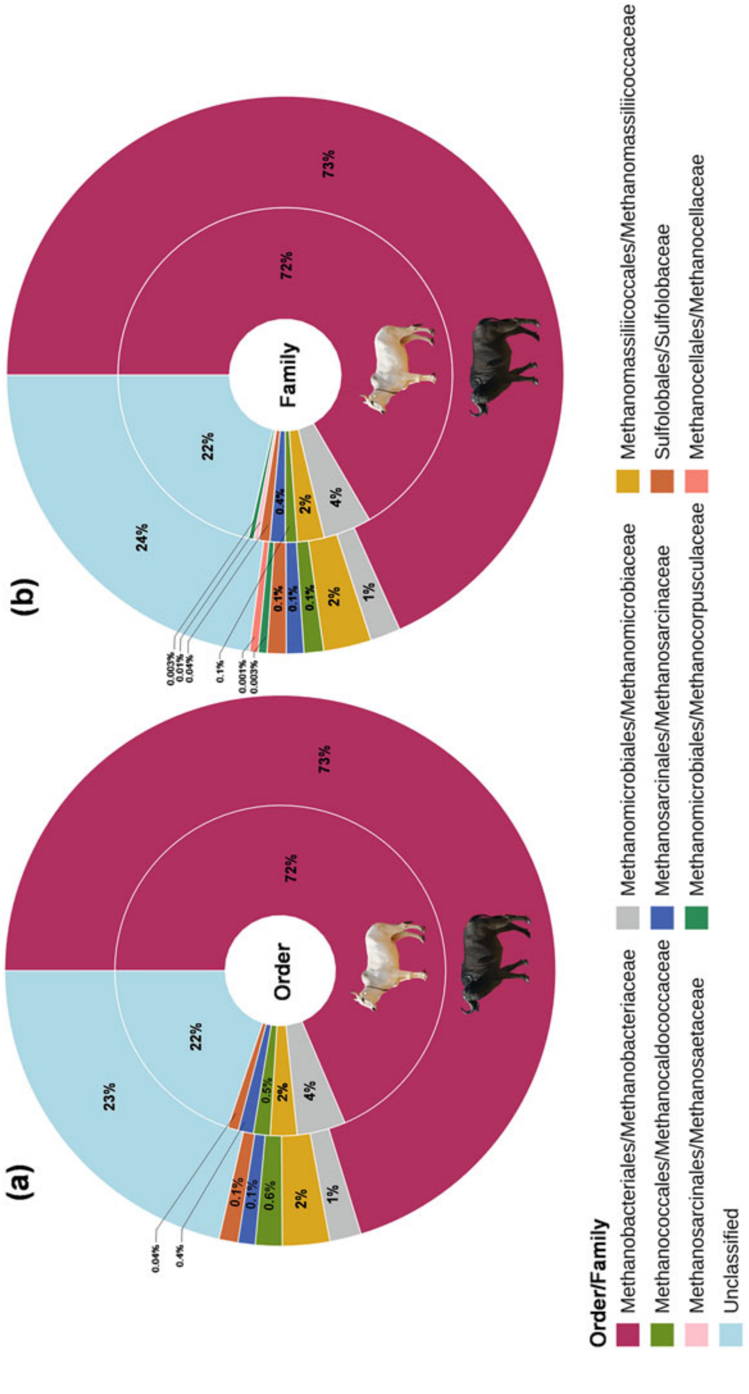

Fig. 19.1 Ruminal archaea community composition in cattle and buffaloes both at (**a**) order level and (**b**) family level (Malik et al. 2021). The inner-circle represents the methanogens distribution in cattle; while the outer circle represents the distribution in buffaloes

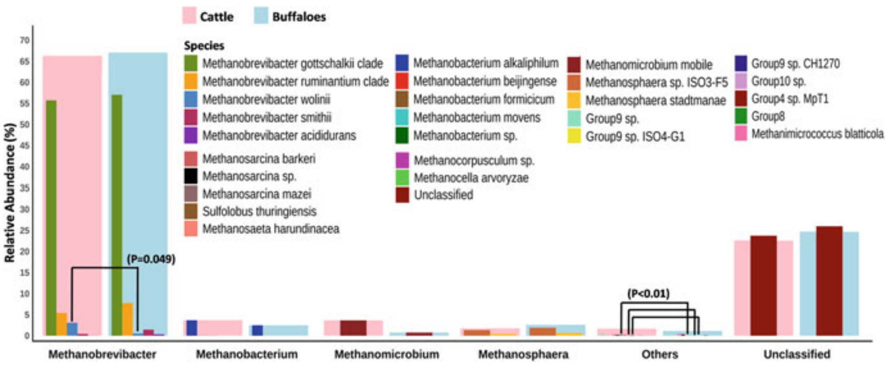

Fig. 19.2 Ruminal archaea community composition at genus and species levels in cattle and buffaloes (Malik et al. 2021). Each genus is represented by larger bars that are underlaid on the smaller bars representing the abundance of all the species affiliated to the corresponding genus

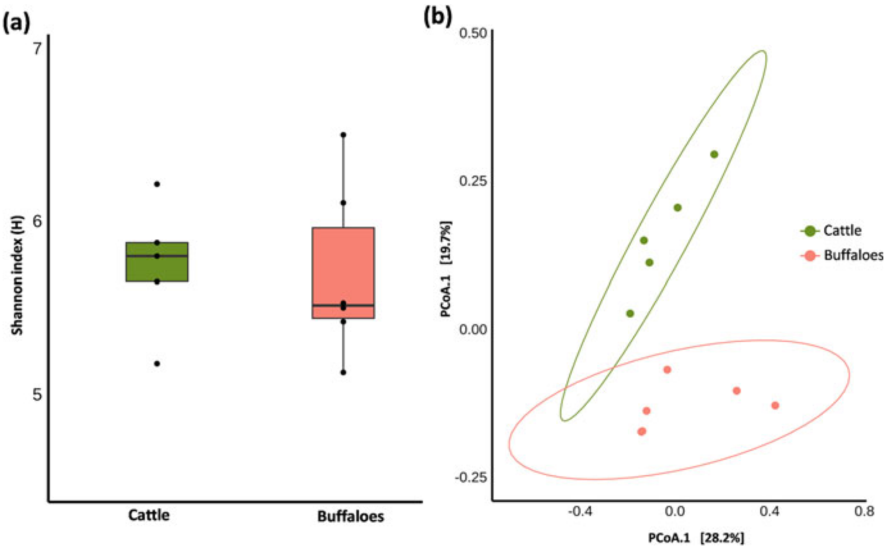

Fig. 19.3 (**a**) Alpha diversity and (**b**) beta diversity of the ruminal methanogens in cattle and buffaloes (Malik et al. 2021)

to be the sole representative of the methanogens' core microbiome in buffaloes (Fig. 19.4d).

The protozoal community was more variable than bacteria and archaea (Henderson et al. 2015), with more than 90% of samples including *Entodinium* and *Epidinium* genera. Buffaloes have a substantially larger protozoal population than cattle (Franzolin and Dehority 2010; Iqbal et al. 2018). The prevalence of *Metadinium* was greater in buffaloes, but the *Entodinium* genus predominated in Jersey cows. *Epidinium* and *Dasytricha* are the dominant genera in Zebu cattle,

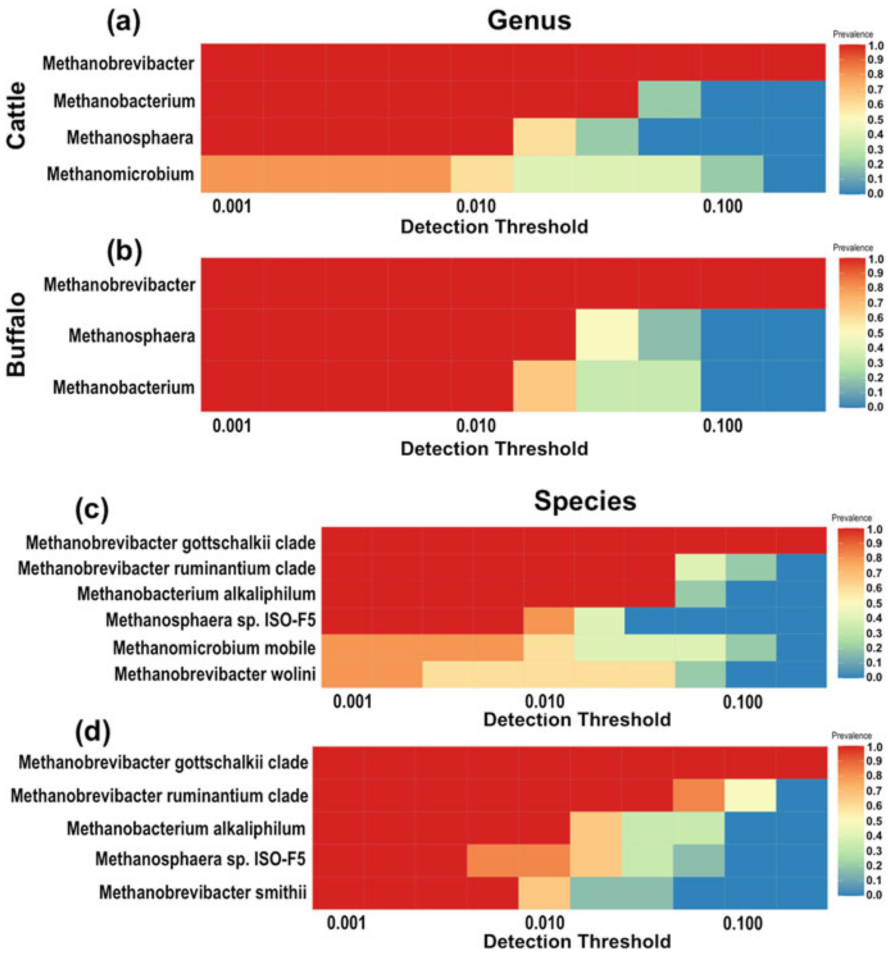

Fig. 19.4 Ruminal archaea representing the core microbiome at 50% minimum prevalence in (**a**) cattle at genus level (**b**) buffalo at genus level (**c**) cattle at species level (**d**) buffaloes at species level (Malik et al. 2021). The colour gradient indicates variability in prevalence

whereas the *Diplodiniinae* subfamily is prevalent in the rumen fluid of buffaloes (González et al. 2007). *Entodinium* are prevalent in cattle fed a variety of diets. Numerous earlier investigations (Kittelmann et al. 2013) established *Entodinium* as the dominating protozoa in cow and water buffalo rumen. Water buffaloes, on the other hand, exhibit a high prevalence of *Metadinium* (73.2%) in their rumen (Iqbal et al. 2018) Strong positive associations between *Entodinium* and *M. gottschalkii* and *Methanomassiliicoccales* suggested a symbiotic relationship within the rumen (Iqbal et al. 2018). Although the *Epidinium* genera (*E. cuadatum and E. ecuadatom*) and *D. crystagali* are not found in cow rumens, they have been observed in buffalo rumens. *Entodinium*, *Epidinium*, *Ophryoscolex*, and *Isotricha* were found in greater abundance in cows than in buffaloes (Jabari et al. 2014), suggesting that water buffaloes have a greater rumen metabolic capacity than Holstein cows.

In the case of anaerobic gut fungi, it has been demonstrated that the host species is critical in defining community composition, with the influence of the host being greater than the effect of diet (Janssen and Kirs 2008). *Lewia*, *Neocallimastix*, and *Phoma* were found to be more abundant in calves on a high grain diet, but *Alternaria*, *Candida Orpinomyces*, and *Piromyces spp*. were found to be less abundant (Ishaq et al. 2017). At the family level, *Neocallimastigaceae* was identified with three major genera (*Caeocomyces*, *Cyllamyces*, and *Orpinomyces*). *Caecomyces* and *Cyllamyces* collectively accounted for 25% of all anaerobic fungus reported in cows (Kumar et al. 2015). *Neocallimastix* has the capacity to physically or enzymatically degrade lignin plant cells, whereas *Orpinomyces* has the ability to dissolve lignocellulose and produce an effective enzyme for hydrolyzing cellulose and hemicellulose.

Cow anaerobic fungal communities were more variable than bacteria (73.2%), archaea (77.9%), and even ciliate protozoa (41.4%) (Kittelmann et al. 2013). About 60% of *Aspergillus*, *Candida*, or *Kluyveromyces* were found in the rumens of all buffaloes. Numerous investigations have detected *Engyodontium* and *Sarocladium* in the rumen of buffaloes; certain members of *Engyodontium* have been reported to possess protease capacity (Zhou et al. 2021). *Sarocladium*, on the other hand, is a well-known phytopathogen that is specialised in secreting enzymes that destroy the cell wall and other tough plant cellular components. The anaerobic fungi isolated from buffalo rumen fluid have the potential to accelerate the decomposition of lignocellulose in diet (Paul et al. 2004).

Learning Outcomes

- Bacterial diversity and Shannon index were much higher in buffalo than cattle demonstrating that buffaloes have a greater rumen microbial diversity than cattle.
- Both cattle and buffaloes have a comparable rumen methanogen community, with *Methanobrevibacter* genus accounting for more than 90% of the total archaeal population.
- Higher populations of bacteria, fungi and protozoa are found in buffalo rumen when compared with cattle which indicates different metagenome profiles exhibited by both the species.

19.4 Conclusions

There are few comparative studies on the rumen microbial ecology of buffaloes and cattle. Numerous earlier researches comparing the microbiome compositions of cattle and buffaloes found that buffaloes are more effective at using food due to their higher rumen fermentation efficiency. Furthermore, buffalo rumen exhibits higher bacterial, fungal and protozoal populations compared to cows, indicating different metagenome profiles. However, studies indicate that the host has a minor effect on the ruminal archaea population in cattle and buffaloes. These studies provided novel and fascinating insights into different aspects of the structure, function and activity of the rumen microbiome. The relationships between the host genotype and the bacterial, fungal, protozoal, and viral populations inside the rumen may be similar or variable.

Future research at molecular level on the rumen microbiome may offer information on the role of host genetics in affecting these microbial communities. Fundamental knowledge of the rumen microbiome on a worldwide geographic scale is critical for developing innovative methods for increasing animal productivity while minimising environmental impact.

19.5 Future Directions

Over the last 10 years, a lot of effort has gone into figuring out what makes up the rumen microbiome and how it influences the host's growth and health. The study of the rumen microbiome has primarily focused on the comprehensive study of rumen bacteria alone, ignoring the other eukaryotic counterparts, which limits our ability to understand the rumen microbiome. Future research focusing on other members of the rumen microbiome might give information on how host genetics shape these microbial communities. Furthermore, studies need to be focused on the influence of species and breed genomics on microbiome composition and its implications on breed specific physiological differences (nutrient digestibility, innate immunity, stress adaptability) as well as product quality (milk meat etc.). Our knowledge of how a stable rumen microbial community forms and what variables influence the makeup and function of rumen microbial communities is limited. With research showing that host genetics influences host immunity, it's tempting to explore how host genetics may alter rumen microbial community composition directly or indirectly in ruminants.

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Metagenomics Application in Understanding Rumen Functions

20

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V. Sejian et al. (eds.), *Climate Change and Livestock Production: Recent Advances and Future Perspectives*, https://doi.org/10.1007/978-981-16-9836-1_20

Abstract

Cattle and other ruminant food production is critical to the health of both humans and the environment. A unique organ in the digestive system known as the "rumen" allows ruminants to transform low-nutrient rich diet into food for humans. As part of the digestion process, the rumen microflora is key to the generation of essential nutrients for the animal. Rumen bacteria are the most researched, however only 15% of the species in the rumen microbiota have been cultivated in a laboratory. Metagenomics offers the ability to address fundamental questions concerning the ecology of microorganisms. In the past, genetic fingerprinting techniques were used to study the structure and dynamics of microbial communities. The advent of whole-genome metagenomics has opened new avenues for studying the makeup of the microbiome and its relationship to the metabolic processes involved in the digestion, absorption, and use of nutrients. The rumen's enormous diversity of metabolic capabilities may hold the key to boosting the efficiency of animal production and industrial fermentation. A better understanding of the animals' microbial communities would allow for the modification of growth and other desired qualities, such as minimizing methane emissions and extracting the most nutrients from their diets.

Keywords

Methane emissions · Metagenomics · Microbiota · Nutrients · Ruminants · Rumen

Abbreviations

16s rRNA	16 sedimentation coefficient rRNA
ARISA	Automated ribosomal intergenic spacer analysis
Bp	Base pair
CH_4	Methane
DGGE	denaturing gradient gel electrophoresis
DNA	Deoxyribonucleic acid
FAOSTAT	Food and Agriculture Organization Corporate Statistical Database
Gbp	Giga base pairs
GC	Guanine and cytosine
ITS	Internal transcribed spacer
Kb	KiloByte
KEGG	Kyoto encyclopedia of genes and genomes
Ml	Milliliter
mRNA	Messenger ribonucleic acid
NGS	Next generation sequencing
OUT	Operational taxonomic unit
PCR	Polymerase chain reaction
pH	Potential of hydrogen
RdRP	Ribonucleic acid polymerase

RFLP	Restriction fragment length polymorphisms
RNA	Ribonucleic acid
rRNA	Ribosomal RNA
SCFA	Short chain fatty acid
SMRT	Single molecule real time
Tbp	Tera base pairs
TGGE	Temperature gradient gel electrophoresis
T-RFLP	Terminal restriction fragment length polymorphism
VFA	Volatile fatty acids
ZMW	Zero-mode waveguides

20.1 Introduction

Hosts and microbes can be thought of as a holobiont organism, with the microbiome occupying various niches and interacting with the host in a symbiotic manner (Hall et al. 2017). Complex features such as body mass index in humans or feed efficiency in farm animals may be accurately predicted by the microbiome (Ross et al. 2013; Camarinha-Silva et al. 2017). There are also associations between host genotype and microbial composition in the gastrointestinal tract, confirming that particular digestive niches are not only influenced by the environment and nutrition, but are also impacted by the host's genetic makeup. Food production from cattle and other ruminants is crucial for human and environmental well-being. Over 296 million beef and 273 million dairy cattle are included in the FAOSTAT database from 2013, as well as an additional 468 million milk-producing ruminants (Stewart et al. 2018). Though it has been studied extensively by both industry and the scientific community, most of the rumen's microbiota is still to be cultured. As a result, research into how ruminants transform their food into energy and, ultimately, milk and muscle protein, is gaining traction among livestock species. We may be able to produce more food with less resources if we can enhance ruminant digestive efficiency, which is an important objective in addressing global food security.

20.2 Anatomy and Physiology of Rumen

The digestion of tough plant tissues, which are particularly high in lignin and cellulose, is a barrier that all herbivores must overcome. Relative to their gut physiology, animals can be divided into three categories: ruminants, monogastrics, and hind gut fermenters. The ruminant stomach is divided into four compartments: the rumen and reticulum (which account for 84% of the total stomach volume), the omasum (12%), and the abomasum (4%), each of which performs a specific role. The stomach of a ruminant takes up nearly 75% of the abdominal cavity.

Rumen Microbial fermentation takes place in the rumen, which acts as a fermentation vat. In the rumen, 50–65% of the starch and soluble sugar eaten is digested. Rumen bacteria absorb cellulose from plant cell walls, digest complex starch, create protein from nonprotein nitrogen, and produce B vitamins and vitamin K. The pH of the rumen is usually between 6.5 and 6.8. The rumen is an anaerobic environment (without oxygen). Carbon dioxide, methane, and hydrogen sulphide are among the gases produced in the rumen.

Abomasum Also known as the "real stomach," the abomasum is a part of the digestive system. This compartment serves the same purpose as the stomachs of other nonruminants, such as humans. The pH of the abomasum is usually between 3.5 and 4.0. The abomasum is where microbial and dietary proteins are digested.

Omasum The omasum is a spherical tissue fold that looks like a leaf and is attached to the reticulum by a small tunnel. The many folds or leaves that mimic pages of a book are referred to as the "many piles" or "butcher's bible." These folds enhance surface area, which improves absorption of nutrients from feed and water. It also serves as a filtration system, allowing only little amounts of fluid and fine particles entering the abomasum.

Reticulum The reticulum gets its name from the honeycomb-like appearance of its lining. Ingesta pass freely between the rumen and the reticulum because the reticulum is not physically separated from the rumen. The reticulum's primary role is to gather smaller digesta particles and transport them to the omasum, while bigger particles are regurgitated and rechewed with more saliva (a process known as rumination).

20.3 Rumen Microbiome

Rumen is a fascinating complex microenvironment that includes a multi-kingdom community of 10^{11} bacteria cells/ml, 10^4–10^6 protozoa cells/ml, 10^3–10^6 fungi cells/ml, 10^6 cells/ml methanogens, and 10^7–10^{10} particles/ml bacteriophages (Morgavi et al. 2013), all of which benefit each other in a symbiotic relationship. These microbes that thrive in a strict anaerobic environment perform an important and necessary activity called anaerobic fermentation, which involves the destruction of complex plant polysaccharides such as cellulose, hemicellulose, lignin, and pectin, which are indigestible to humans. There is a sequential syntropic connection of microflora, comprising bacteria, protozoa, fungi, archaea, and bacteriophages, as well as host, for completing complete feed digestion. Cellulolytic bacteria breakdown polysaccharic plant fibres, anaerobic fungi degrade lignocellulosic components of feed particles, and the resulting substrate is acted upon by other bacteria. As a result of these microbial action, volatile fatty acids (VFAs) such as acetate (used in fat synthesis), propionate (used in glucose production), and butyrate are produced. These VFAs are later used as a source of energy by the animal.

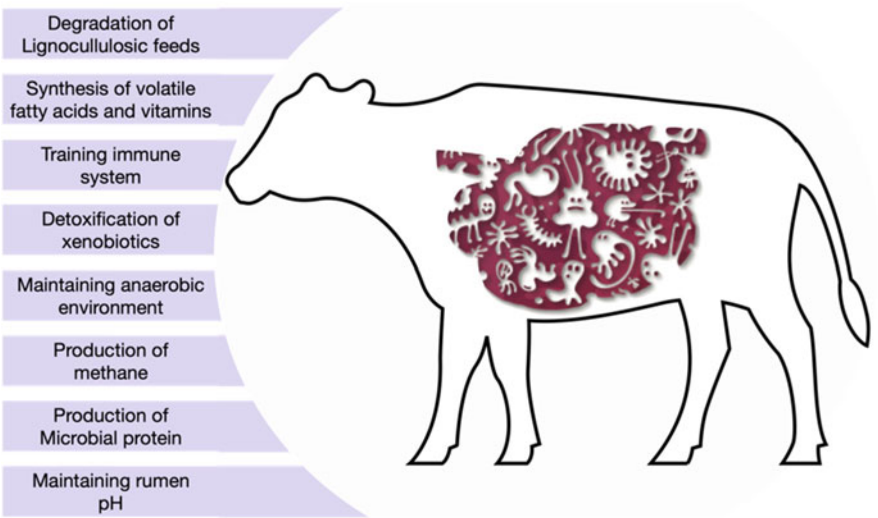

Fig. 20.1 Functions of rumen microbiome

Methane (CH_4), one of the end product gases, is a powerful greenhouse gas. The health of the local communal flora determines overall energy production as well as digestion. Rumen microbiome perform a variety of functions (Fig. 20.1) that benefit the host.

20.4 Why Metagenome?

It was long widely believed that organisms isolated in pure culture from their natural environment represented numerically and functionally important species. The organisms obtained using traditional culture techniques, on the other hand, were seldom dominant. Due to their capacity to thrive on nutrient-rich medium at a moderate temperature and under aerobic circumstances, these organisms have been isolated (Hugenholtz 2002). The culturable species make up less than 1% of the overall diversity in some environments. This phenomenon was previously known as the "great plate count anomaly," and the unculturability could not be confirmed until molecular technologies arrived. To discover uncultured bacteria in the environment, ribosomal RNA and rRNA genes were sequenced. This method was primarily used to reconstruct phylogenies, compare microbial distribution in samples using sequencing or RFLP, and quantify relative abundance of each taxonomic group using hybridization with probes unique to that taxonomic group.

Carl Woese proved in the late 1980s that the 16S ribosomal ribonucleic acid (rRNA) gene could be used to establish prokaryote phylogeny (Woese 1987). A similar version of this gene was found in all species. Woese used the gene sequence to classify living things into three categories: Eukaryote (multicellular), Archaea and

Bacteria (singe celled). Norman Pace demonstrated that the polymerase chain reaction (PCR) may be used to isolate 16S rRNA genes from the environment. Most taxa that underwent culture-independent study were new, indicating that there are thousands of species yet to be identified. However, PCR analysis can only amplify the genes of interest in organisms, leaving the remainder of the genome unaccounted for. Microbiologists need a new technique to study genetics and physiology, which prompted the development of the science of metagenomics (Handelsman 2004). Scientists may use metagenomics to examine the genetic material of whole microbial populations. This area detects and classifies genetic material in environment by combining genetics and molecular biology. The microbial research in many ecological niches have been fueled by the sequencing technology and infrastructure created during the human genome sequencing project.

20.5 Understanding Complex Rumen System

Recent research on rumen/gut microorganisms has demonstrated that the rumen microflora is vital in the degradation of ingested feed as well as the production of key nutrients. The rumen microflora is dynamic, changing with the animal's age and species, responding to feeds and environmental conditions, and the imbalance/dysbiosis has an impact on the animal's nutrition and health. Robert Hungate, the "Father of Rumen Microbiology," pioneered the creation of anaerobic bacterial growth techniques that are still the "gold standard" method across the world (Hungate 1966). However, due to the rumen microorganisms' complicated substrate requirements, adequate cultures could not be grown. As a result, for a long period, a significant portion of the rumen microbial diversity was unknown (Krause et al. 1999). The rumen microbial communities have never been thoroughly explored due to the inherent difficulty in culturing and research of these microbes. Despite the fact that rumen bacteria are the most studied, only 15% of the species in the microbiota of the rumen have been cultured in the lab (Morgavi et al. 2013; Creevey et al. 2014). There are now at least 70 rumen bacterial species accessible in pure culture from public sources (Creevey et al. 2014).

Despite the development of culture-based approaches, cloning and sequencing of the 16S rRNA gene-based identification of rumen bacteria is a more potent tool for identifying and characterising community members in greater depth (Sundset et al. 2007). It is possible to study the rumen microflora at several taxonomic levels thanks to the advent of high-throughput, culture-independent technologies. Understanding the rumen microbiome composition will offer new possibilities for improving production, disease resistance, novel enzyme discoveries, sustaining health, and reducing methane emissions from ruminal fermentation in ruminants. The rumen microbial community's composition and evolution in response to a variety of selection forces have been widely researched (Huws et al. 2018).

20.6 Rumen Metagenomics

Even if steps have been taken to cultivate various ruminal microbiota members, there are still a large number that have not yet been characterised (Seshadri et al. 2018). The rumen microbiome holds approximately 100 times more gene pool as compared to the host (McSweeney and Mackie 2012). Therefore, the total gene pool of the microbiome is also regarded as "Second Genome of the animal" (Bath et al. 2013). In order to get a thorough understanding of the genetic structure, population dynamics, and metabolic processes of these uncultured bacteria in the environment, including the rumen, sophisticated culture-independent approaches must be developed. Because of metagenomics, the rumen microbiota's whole genetic repertoire may be accessed without the time-consuming culture procedure (Glendinning et al. 2021). The metagenomics has the potential to provide answers to basic concerns about microbial ecology. Bacterial and archaea genome sequencing has transformed our understanding of microorganisms' diverse roles.

20.7 Methods for Understanding Rumen Microbiome

Bacteria, archaea, protozoa, fungus, yeast, mycoplasma, and bacteriophages are all part of the rumen's microbial community. Anaerobic fermentation of ingested feeds takes place because of their interaction and participation in the detoxification of many anti-nutritional components as well as some toxic substances. As a result, ruminant nutrition relies heavily on microorganisms. Light and electron microscopy were used to study the microbial members, although only 11% of the overall variety of microbes present in the rumen was known to have been studied (Wolin et al. 1997; Edwards et al. 2007). For the identification and quantification of rumen microbiota, molecular approaches employing the 16/18s rRNA gene and it's associated ITS region were developed. A culture-free approach to microbial community structure and function is provided by metagenomics. A variety of PCR techniques, including clone libraries, terminal restriction fragment length polymorphism (T-RFLP), denaturing gradient gel electrophoresis (DGGE), ARISA (Welkie et al. 2010), amplicon sequencing, and shotgun metagenome sequencing, have been used to study the diversity. The final two strategies rely significantly on genetic sequencing and computational technologies. To better understand the structure and composition of microbial communities, 16s rRNA gene amplicon sequencing can be used. Whole metagenome shotgun sequencing, metatranscriptomics, and metaproteomics can be used to discover the functional capacities of the metagenome. Different approaches for studying metagenomics have been represented in Fig. 20.2.

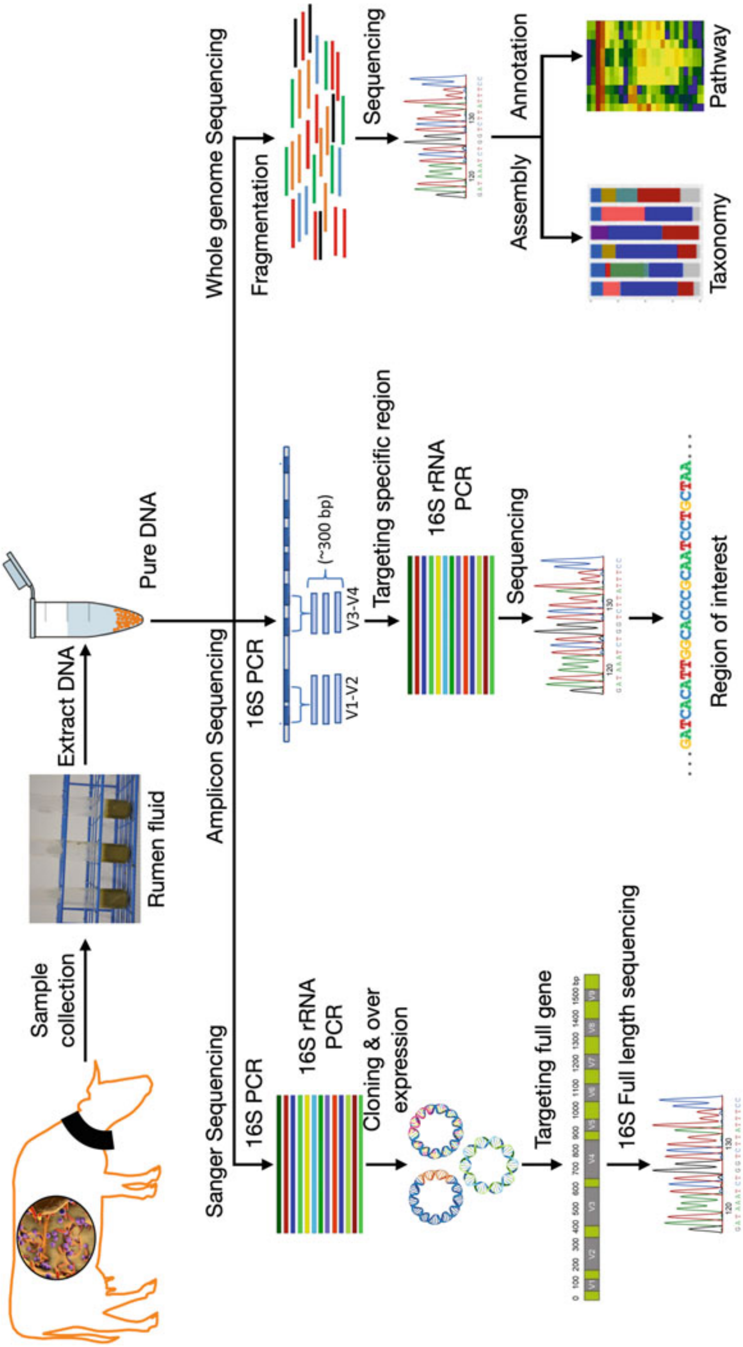

Fig. 20.2 Different approached used for study of rumen microbiome

20.8 Culture Based Methods to Study Rumen Microbes

The creation of the anaerobic glove box for decreasing the exposure of the medium and bacteria during inoculation and subculturing improved the early attempts for cultured the rumen microbes (Aranki et al. 1969). The roll tube method of anaerobic microbe culture was shown to be preferable for researching anaerobic bacteria and was used to examine rumen microbes (Hungate 1960). Using traditional approaches, 10–11% of the entire microbial species might be cultured (Jami et al. 2013; Millen and Romero 2016). Around 200 species of bacteria, 100 species of protozoa, and 100 species of fungus have been identified from the rumen using traditional culture methods (Williams and Coleman 1997; White et al. 1999). Due to the unavailability of universal media that could sustain the growth of the majority of species, these approaches were time consuming and difficult (Kamra 2005). Although genetic identification of rumen bacteria has been widely used to research rumen microbial composition, culture-based investigations will be critical for isolating new species and understanding the physiological and genomic characteristics of specific microbiome members. To extract new rumen bacteria, a liquid culture based on the dilution strategy (also known as extinction culturing method) was devised and successfully proved to grow a high fraction of hitherto unculturable rumen microbiota members (Kenters et al. 2011).

20.9 Culture Independent Approaches for Studying the Rumen Microbes

Due to severe constraints in cultivating microorganisms in anaerobic niches such as the rumen and other biological sites, techniques that do not need growing microbes to identify them were necessary. In many ecosystems, DNA identification of microorganisms has revealed that these bacteria are seldom dominant (Hugenholtz 2002). In some ecological niches, the fraction of cultivable organisms is less than 1–10% of overall microbial diversity (Prakash et al. 2013). The causes for environmental species unculturability are several. The first is a lack of understanding of the exact growth requirements, or they may grow at a much slower rate in the laboratory than we anticipate. The laboratory media could be toxic for the microbes, or they simply cannot exist alone, and may grow only in communities. The uncultivability could not be established until the development of molecular technologies for microbial identification, which was previously known as "the great plate count anomaly." To detect uncultured microorganisms in the environment, sequencing of phylogenetic marker genes was developed. The invention of the polymerase chain reaction (PCR), DNA sequencing technology, and the use of the 16S rRNA gene as a taxonomic identifier (Woese 1987) have made gene-based identification of prokaryotes in various contexts much easier.

Because of the evolutionary conservation of 16S ribosomal RNA, molecular approaches based on 16S rRNA may be used to identify and quantify the abundances of bacteria without the need to culture them. The 16S rRNA approach had revealed the third microbial kingdom 'Archaebacteria' (Woese et al. 1990). Total DNA extracted from ambient samples, as well as amplification, cloning, and sequencing of the 16S rRNA gene using universal degenerate primers (Rappé and Giovannoni 2003), gave a window into the uncultured world. Several reviews and technical guides have published methods for understanding the rumen microbial population (Zoetendal et al. 2003, 2004, 2008; Makkar and McSweeney 2005; McSweeney et al. 2006; Morgavi et al. 2012).

20.10 Phylogenetic Marker Genes in Microbes

In the past, housekeeping genes were employed to detect and categorize microorganisms. However, most of these genes were effective in identifying previously discovered bacteria. The research on the yet-to-be-cultured bacteria necessitated the use of a highly conserved gene or genes that could be targeted using PCR amplification and sequencing. Universality, conserved function across organisms, adequate gene length for discriminating, absence of horizontal transfer, and availability of high-quality sequencing databases are all desired characteristics of the gene. The 16S rRNA gene, out of all the known genes, contains all the necessary characteristics and has been widely employed in microbial molecular phylogeny. Today, different genes are used to identify different classes of microbes, such as 16S rRNA for bacteria and archaea (Rampini et al. 2011; Salipante et al. 2013), 18S rRNA for eukaryotes, ITS regions for fungi (Wagner et al. 2018), and RNA dependent RNA Polymerase (RdRP) for RNA viruses (Culley et al. 2010).

20.11 The 16S rRNA Gene

Ribosomes, which are made up of RNA and proteins, are essential components of all living forms. The ribosome includes the 5S, 16S, and 23S RNA molecules in prokaryotes and archaea. However, 16S rRNA was widely utilized in microbial phylogeny owing to its size advantage (Konstantinidis and Tiedje 2007). The 16S rRNA gene transcript is a component of the small ribosomal subunit, which is responsible for translating messenger RNA (mRNA)-coded information into proteins. Variable regions inside the gene were produced because of the accumulation of evolutionary mutations in regions that did not impact ribosome function. In the late 1980s (Lane et al. 1985), the 16S rRNA gene was originally proposed for phylogenetic study of bacteria, and it has since been the method of choice for molecular identification of isolates as well as characterizing microbial communities (Tringe and Hugenholtz 2008). The 16S rRNA gene is 1500 bases long and has nine hypervariable regions (Fig. 20.3) separated by conserved sections (Baker et al. 2003).

Because of the conserved sections of the gene, universal primers may be designed for PCR amplification of the gene across all bacterial members of the community. Several works have reviewed the design and assessment of primers for different

Fig. 20.3 Schematic diagram of 16S rRNA gene showing the distribution of the conserved and hypervariable regions within the gene

variable areas, as well as the comparison of the region for resolving the taxonomy (Klindworth et al. 2013).

20.12 Molecular Characterization Assays Based on 16s rRNA Gene for Community Analysis

Due to the diversity of ruminal microorganisms, genetic fingerprinting techniques have been utilized in the past to examine population structures and dynamics. Typically, the fingerprinting process begins with the collection of community DNA, followed by PCR amplification of the assay's target gene and gel electrophoresis examination of the PCR results. Comparing almost full 16S rRNA gene sequences has been extensively utilised to identify Bacteria and Archaea and to establish taxonomic relationships among prokaryotic strains (Kim and Chun 2014).

The Terminal Restriction Fragment Length Polymorphism (T-RFLP) approach is used to investigate bacterial diversity. It is based on variation in the 16S rRNA gene, which may be exploited by restriction enzymes to create terminal restriction fragments of varying length. The 16S rRNA gene is amplified using primers that are fluorescently labelled. Tetra cutter restriction enzyme is used to digest the PCR products. Capillary electrophoresis is used to separate the fragments, and the fragment lengths are analyzed to determine the phylotypes. Based on the length of the terminal fragment, a tentative identification can be made by comparing the fragment length to a database of virtual digests of reference 16S rRNA sequences. The 16S rRNA gene was utilized to detect microbial communities in the reticulo-rumen (Fernando 2008; Li et al. 2009). The rumen microorganisms of dairy sheep were examined using TRFLP during sunflower and fish oil supplementation. The approach demonstrated a varied response of *Lachnospiraceae* members to lipid feeding. TRFLP demonstrated that these bacteria do not play a dominating role in the process, although uncultured members may be more significant (Belenguer et al. 2010). Tannin supplementation in lambs resulted in distinct clustering of TRFLP patterns (Vasta et al. 2010). The approach was effectively utilized to investigate the influence of breed on the microbial profiles of sheep guts, revealing that the bacterial populations in the foregut, midgut, and hindgut were significantly different (Douglas et al. 2016).

Denaturing Gradient Gel Electrophoresis (DGGE) and Temperature Gradient Gel Electrophoresis (TGGE) are two types of electrophoresis that employ a temperature or chemical gradient to separate denatured nucleic acids. DGGE/TGGE are primarily based on the principle of amplifying rRNA or functional gene PCR products isolated from community DNA using primers that contain a 50-bp GC-clamp and then separating them on polyacrylamide gels (Rastogi and Sani 2011). DGGE separates identical gene sequences based on their differential denaturing ability, which is dictated by their base pair sequence, whereas TGGE separates nucleic acids based on temperature-dependent changes in structure. Additionally, Denaturing Gradient Gel Electrophoresis (DGGE) and Automated Ribosomal Intergenic Spacer Analysis (ARISA) were utilized to characterize the rumen bacteria (Petri et al. 2012). According to the DGGE profiles, animal to animal variability in the ciliate population was highest in sheep compared to deer and cattle (Kittelmann and Janssen 2011). The microbiome of the sheep mammary gland found that 15 DGGE bands were strongly linked with changes in somatic cell count (Monaghan 2013).

While full-length amplification and sequencing of the 16S rRNA gene is ideal for molecular taxonomic identification of bacteria, restrictions imposed by sequencing methods and universal primers limit its application in community characterization. It is established that the various variable regions have diverse taxonomic resolutions, and the proper variable region selection is dependent on the assay and the source of the samples. Different primers produce distinct fingerprints on DGGE gels (Yu and Morrison 2004). The studies based on amplicon sequencing and primer selection do skew the evaluation of community composition, resulting in under or over representation of specific taxonomic groupings (Yang et al. 2016). Nonetheless, given the cheap cost per sample and the requirement of low template DNA concentrations, 16S rDNA sequencing continues to be one of the most popular high-throughput sequencing technologies (Cao et al. 2017).

20.13 Sequencing Technologies

20.13.1 First Generation Sequencing Approach

The sanger sequencing approach, which is based on capillary electrophoresis, can sequence the whole 16S rRNA gene (1500 bp), but not in a single run and requires the use of two or more primer pairs. While the approach is quite precise, it falls short of providing the depth of coverage necessary for studying microbial communities (Mitreva 2017). This led in difficulty recognizing rare species and insufficient coverage of microbial diversity. Additionally, examining microbial communities with Sanger sequencing is time consuming and expensive in comparison to next generation sequencing platforms (NGS) (Sorek et al. 2007). While next-generation sequencing systems are extremely scalable, cost effective, and capable of concurrently sequencing millions of copies of a gene, they suffer from sequencing length constraints, which limit the identification of bacteria to lower taxonomic ranks. To increase taxonomic resolution, several variable regions of the 16S rRNA gene were sequenced using next-generation sequencing technology, and various primers were described and assessed (Soergel et al. 2012).

20.13.2 Second Generation Sequencing Platforms

In 2005, the 454/Roche sequencing platform was launched. The platform has been extensively utilised in the field of microbial ecology (Tringe and Hugenholtz 2008). The 454 platform use emulsion polymerase chain reaction to clonally amplify DNA fragments that were sequenced in parallel using pyrosequencing technology. Although the platform had limitations in terms of the introduction of artificial replicate sequences and homopolymer sequencing errors that resulted in indels, it provided 10–100 fold greater diversity coverage at a 10–100 fold lower cost than previous methods of cloning and sequencing metagenomic libraries (Tamaki et al. 2011). The FLX+ platform yielded an average sequence length of 600–800 bp, which was adequate to cover more than one variable region in the 16S rRNA gene. Multiplexing of samples was introduced utilizing barcoded primers to increase throughput above the requirement for a single ecological sample. The platform was used to conduct microbiological surveys in various habitats (Dinsdale et al. 2008).

The Illumina/Solexa technology is based on sequencing by synthesis and was released in 2007 with a data output of 1Gbp (Giga base pairs), which was later increased to 1.8 Tbp (Tera base pairs) in the Illumina HiSeq systems. Initially, the platform yielded shorter sequences than the 454/Roche platform, but with better chemistry, the cost of sequencing has decreased significantly (Di Bella et al. 2013), allowing for faster throughput and longer read lengths of up to 250–300 bp. The Illumina platform's primary strength was paired end sequencing, which involved sequencing a bit of DNA from both ends, allowing the platform to cover 600 bases for each piece of DNA. The Illumina MiSeq benchtop sequencer is used to perform the 300 bp paired end chemistry.

With shorter read lengths acquired from Illumina platforms, it is not possible to correctly classify bacteria beyond the genus level using 16S rDNA amplicon sequencing (Claesson et al. 2010). Another drawback of this strategy is the selection of nine hypervariable regions (V1–V9) inside the 16S rDNA gene. Numerous previous investigations have employed various hypervariable areas, including V1/V2/V4 (Sundquist et al. 2007), V2/V3/V4 (Liu et al. 2008) V2/V4 (Wang et al. 2007) and V2/V3 (Chakravorty et al. 2007). PCR amplicon fragments as short as 82 bp targeting the 16S rDNA V5 variable region have been shown to be sufficient for phylum-level bacterial classification (Lazarevic et al. 2009), and a longer amplicon fragment of 100 bp combined with appropriate primer design and downstream analysis could display the same clustering information as a longer 16S rDNA sequence. Even with longer variable regions, pyrosequencing, and increased coverage, amplification of various polymorphic regions resulted in a bias in estimating the microbiome. Despite this, 16S rDNA sequencing remains one of the most common high-throughput sequencing technologies due to its low cost per sample and necessity for low input template DNA concentrations.

20.13.3 Third Generation Sequencing Platforms

One of the most widely utilised third-generation sequencing methods is PacBio's SMRT (Single Molecule Real Time) sequencing. PacBio long-read sequencing, enabled by SMRT Sequencing technology, requires no PCR amplification and has a read length 100 times longer than NGS. A unique flow cell with hundreds of tiny picolitre wells with transparent bottoms—zero-mode waveguides—is used for SMRT sequencing (ZMW). The polymerase is attached to the well's bottom and permits the DNA strand to pass through the ZMW. Real-time imaging of fluorescently tagged nucleotides produced alongside individual DNA template molecules is possible with SMRT sequencing. When the template and polymerase separate, the sequencing process is complete. The PacBio instrument's average read length is about 2 kb, with some readings exceeding 20 kb. Longer reads are very advantageous for de novo genome assembly.

The PacBio method is being used to unravel the puzzle of the complex rumen microbiome. Myer et al. (2016) compared full-length 16S rRNA reads (V1–V8) sequenced with PacBio to shorter V1–V3 reads sequenced using Illumina Mi-Seq to categorize rumen community members in greater depth. While the two platforms revealed similar microbial OTUs, species richness, Good's coverage, and Shannon diversity metrics, the Pac-Bio platform improved taxonomic depth. Another study used the Pacific Biosciences single-molecule real-time (PacBio SMRT) sequencing technique to assess the intestinal microbiota of nine dairy cows during lactation period (Li et al. 2018). *Firmicutes* (83%) was the most common phylum, while *Bacteroides* (6.16%) was the most common genus. All the samples included a lot of microbial diversity, including a lot of short chain fatty acid (SCFA) producers. The research shed new light on the relationship between dairy cow gut microbes and milk production.

Nanopore sequencing is a scalable, yet another technique for sequencing long DNA or RNA segments in real time. It functions by tracking how an electrical current changes when nucleic acids flow through a protein nanopore. The resultant signal is decoded to reveal the DNA or RNA sequence in concern. Flow cells, which include an array of microscopic holes—nanopores—embedded in an electro-resistant membrane, are used in all Oxford Nanopore sequencing equipment. Each nanopore has its own electrode, which is connected to a channel and sensor chip that detects the electric current flowing through it. The current is disturbed when a molecule travels through a nanopore, resulting in a distinctive' squiggle.' The DNA or RNA sequence is then determined in real time by decoding the squiggle using base calling algorithms. MinIONTM, GridIONTM, and PromethIONTM are nanopore systems; MinIONTM is a tiny and portable device introduced in 2014. Nanopore sequencing has the potential to create ultra-long reads, with read lengths exceeding 2 megabytes, allowing for a more comprehensive assessment of genetic variants and the reconstruction of complicated genomes.

A comprehensive assessment of over 6.5 terabytes of sequencing data from 283 cattle's rumen was used to create the rumen genome catalogue, which contains 5845 assembled rumen microbial genomes (Stewart et al. 2019). The study unravelled a metagenomic assembly that included at least three entire bacterial chromosomes as separate contigs demonstrating the ability of long reads for assembling full, entire chromosomes from complicated metagenomes. The nanopore assembly was 268 kb, which was more than 56 times longer than the typical Illumina assembly (4.7 kb). Delgado et al. (2019) used the MinION (Nanopore) and Illumina MiSeq to sequence the rumen content of 12 cows selected for high feed efficiency phenotype. Although Nanopore sequencing produced similar findings as Illumina sequencing, it was able to categorise a greater number of observations at the species level. The findings also suggest that, due to the size of the reads, the same amount of information might be obtained in a shorter duration of time.

20.14 The Hungate1000 Collection

Seshadri et al. (2018) compiled the most comprehensive reference genome library till date, consisting of 410 cultured bacteria and archaea, as well as their reference genomes, representing major rumen-associated archaeal and bacterial families. Illumina or PacBio technology was used to sequence all the Hungate1000 genomes. They investigated polysaccharide breakdown, short-chain fatty acid synthesis, and methanogenesis pathways, as well as attributing functions to specific taxa. 336 species were found in rumen metagenomic data sets, whereas 134 were found in human gut microbiome data sets. 75% of the rumen's genus-level bacterial and archaeal species are represented in the Hungate genome collection. The Hungate Collection serves as the basis for designing rumens to reduce CH_4 emissions, boost productivity, and sustainability (Mcallister et al. 2015), offering insight on what has been termed "the world's biggest commercial fermentation process" (Weimer 1992).

20.15 Metagenomics in Ruminant Research

Pyrosequencing was utilized to examine the bacterial community composition in the rumen of dairy cows and their relationship to physiological factors. According to the study, several physiological indicators, such as milk production and composition, are strongly connected with the prevalence of specific bacteria found in the rumen microbiome. A notable discovery was a substantial link between the proportion of *Firmicutes* to *Bacteroidetes* and milk fat output (Jami and Mizrahi 2012). The combined use of amplicon, shotgun, and metatranscriptome sequencing revealed an enhanced population of sharpea in sheep that produce less methane. The microbiome of high methane emitters was dominated by members of the *Ruminococcaceae* and *Lachnospiraceae* families. The study revealed that the low methane emitter sheep microbiome will produce roughly 24% less methane. The rumen size was lower in low emitters than in high emitters (Kamke et al. 2016). In both high and low methane emitters, deep metagenomic and metatranscriptomic sequencing revealed a similar quantity of methanogens and methanogenesis pathway genes. However, transcription of genes involved in the methanogenesis pathway was significantly elevated in sheep producing large amounts of methane (Shi et al. 2014). The global rumen census employed 16S rRNA sequencing of rumen samples from 742 animals from eight different ruminant species all around the world to try to figure out the core rumen microbiome (Henderson et al. 2015). The predominant rumen bacteria were *Prevotella, Butyrivibrio*, and *Ruminococcus*, as well as unclassified *Lachnospiraceae*, *Ruminococcaceae, Bacteroidales*, and *Clostridiales*, which may represent a core bacterial rumen microbiome. Large collections of rumen microbial genomes have been revealed in two prior investigations. Seshadri et al. (2018) reported 410 reference archaeal and bacterial genomes from the Hungate collection, while Stewart et al. (2018) compiled 913 draft MAGs from the rumens of 43 cattle bred in Scotland. The Hungate genomes are typically of superior value as it contains genomes of isolated rumen microbes those exist in culture collection and thus can be investigated in the lab. Recently a metagenomic investigation of the ruminal contents of cows (*Bos taurus*), sheep (*Ovis aries*), reindeer (*Rangifer tarandus*), and red deer (*Cervus elaphus*) was performed by Glendinning et al. (2021). They constructed 391 metagenome-assembled genomes from 16 microbial phyla and discovered 279 new species by comparing rumen genomes to other publicly accessible microbial genomes. The study also reported substantial changes in the number of microbial taxonomies, carbohydrate-active enzyme genes, and KEGG orthologs in the microbiota of various ruminant species.

20.16 Whole Metagenome Analysis of Rumen

Most of the prior research have used 16S rRNA sequencing to characterize the microbiota. Because readings must be matched against incomplete databases that lack some specific rumen microorganisms, this technique yields minimal information. In addition to culturing and 16S rRNA sequencing, whole genome sequencing and assembly enable a more accurate taxonomic categorization based on several marker genes and avoids PCR biases. Additionally, various taxonomic groupings may perform comparable activities, masking genuine relationship at the gene function level when just the taxonomic makeup is considered. When a DNA molecule is too lengthy to sequence in a single run, it must be sliced into several more manageable "bite-sized" pieces, each of which is copied and sequenced separately with overlapping regions and then reassembled to generate the overall genetic picture. While each DNA cutting, replication, and sequencing step is achievable manually, they are the equivalent of a whole first-generation research using the Sanger or Maxam-Gilbert methods. The research is crucial to create more efficient techniques for perturbing the microbiome.

The era of whole-genome metagenomics has created new opportunities to analyze microbiome composition to assess an individual's feed consumption and its link to metabolic processes involved in nutrient digestion, absorption, and utilization. The ruminant gut microbiota contributes to the heterogeneity in feed consumption efficiency. Delgado et al. (2019) used whole metagenome sequencing to demonstrate a link between the microbiome and feed utilization and consumption levels in a Holstein cow herd. Their investigation discovered that the most effective cows have a greater quantity of Bacteroidetes and *Prevotella* but lacked *Firmicutes*. Additionally, *Methanobacteria* and *Methanobrevibacter* were less prevalent in high-efficiency cows, indicating decreased methane emission. Their findings suggested that there were variations in the microbiota compositions of efficient and inefficient animals at both the taxonomic and gene levels.

Learning Outcomes

- Cloning and sequencing of 16s rRNA gene-based identification of rumen bacteria is a more potent tool for identifying and characterizing rumen microbiome.
- Understanding the rumen microbiome composition offer new possibilities for improving production, disease resistance, novel enzyme discoveries, and reducing enteric methane emissions in ruminants.
- Whole metagenome shotgun sequencing, metatranscriptomics, and metaproteomics can be used to discover the functional capacities of the metagenome.

20.17 Conclusions

The rumen microbiome plays a critical role in ensuring food security and mitigating the effects of climate change. Recent research has resulted in the publication of over 1300 draught and full rumen genomes (Stewart et al. 2019). The vast array of metabolic capabilities seen in the rumen microbiome may hold the key to increasing the efficiency of animal production and industrial fermentation. Nucleic acid-based technology advancements have reshaped our capacity to characterize the rumen microbiome and opened new avenues for investigating the intricate interactions and niches found within the rumen microbial population. The rumen microbiome has been characterized in novel ways using next generation sequencing and other molecular techniques. Pyrosequencing of the 16S ribosomal RNA gene has been used for the taxonomic identification of bacteria and archaea at genus level. Whole genome shotgun sequencing yields genuine metagenomic sequences that can be used to forecast a microbiome's functional capabilities and can also be utilised to synthesized isolated species' genomes. Integrating high-throughput data defining the rumen microbiome with traditional fermentation and animal performance indicators has resulted in significant breakthroughs and opened new research avenues.

To further understand the roles of the rumen microbiome, additional rumen bacteria and archaea must be cultured. Understanding the microbiome and the substrates used by bacteria, as well as how they interact with one another and with their ruminant host to devise reasonable treatments to alter rumen feed conversion or methane emissions, would be necessary. Improved rumen microbiome culture collections and future modification of the rumen microbiome can be better achieved by genome sequencing and microbial genome assemblies.

20.18 New Frontiers

While genome assemblies provide insight into microorganisms' metabolic capabilities, next study will focus on the genes expressed in the microbiome (metatranscriptome) utilizing RNA-seq. Increased data creation rates via high-throughput technologies will necessitate the incorporation of bioinformatics tools for data processing and meaningful (functional) outputs. Considering that diet supplies substrates for the microbiome, fundamental nutrition principles will continue to be crucial for the design of experiments and their interpretation. Further explicating the relationship between the host and microbiome might aid in elucidating a variety of variables impacting microbial community alterations. Knowledge of the animals' microbial communities would allow for the manipulation of growth and other desirable traits, such as minimal methane emissions and maximum nutrition extraction from their diets. Furthermore, the high-throughput microbiome investigations will help us better understand the development of animal systems, immunology, tolerance to biotic and abiotic stress, resistance to infections, metabolic diseases, etc. Using these methods, we want to gain a better understanding of how methane lowering additives affect the rumen microbial population, as well as how methanogenesis works. Safety evaluation of feed additives and rumen manipulation effects could be studied through the profiling of the microbial community analysis. Dysbiosis of the gut microbiome affect the metabolism as well as favor the opportunistic infections to establish, the microbiome dynamics would facilitate the study of such illnesses and remedies. Systems biology approaches to increase animal productivity and welfare might potentially benefit from investigations of the microbiome of the lung, reproductive tract, skin, udder, and genitalia. Understanding the rumen microbiome could help in improving feed efficiency and reducing the carbon footprint of animal products.

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Part VI

Adaptation Strategies and Future Perspectives

Genetic Adaptation of Livestock to Heat Stress Challenges

21

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Abstract

Genetic/genomic selection between and within species and breeds can aid in maintaining production levels in farm animal species under climatic stress. This chapter looks at how genes and animals can be identified and used for this purpose. We also look at over 19,600 genes reported from studies on adaptation cited in the scientific literature for cattle, sheep, goats and horses. Functional analysis revealed pathways involved in developmental and growth processes, regulation (positive and negative) of biological process, regulation of response to stimulus and stress, immune system regulation, function and development, leukocyte activation, oxidoreductase activity, metabolism and behaviour. Future works will look at how we can select for increased tolerance to heat stress and its related traits while maintaining productivity. Solutions may include landscape genomics, genome editing and multi-omics studies. Overall, there is a need to integrate different stakeholders with the development of statistical methodologies

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V. Sejian et al. (eds.), *Climate Change and Livestock Production: Recent Advances and Future Perspectives*, https://doi.org/10.1007/978-981-16-9836-1_21

(including artificial intelligence and machine learning) and a regulatory framework to ensure animal welfare and consumer safety.

Keywords

Gene ontology · GWAS · Landscape · Selection · Sequencing

Abbreviations

B4GALT6	Beta-1,4-galactosyltransferase 6
Cas9	CRISPR associated protein 9
CRISPR	Clustered regularly interspaced short palindromic repeats
DNA	Deoxyribonucleic acid
DSC	Desmocollins
DSG	Desmogleins
EGFR	Epidermal growth factor receptor
FDR	False discovery rate
Fig	Figure
F_{ST}	Fixation index
GEBV	Genomic breeding value
GO	Gene ontology
GWAS	Genowe-wide association study
HS	Heat stress
KEGG	Kyoto Encyclopedia of genes and genomes
LISA	Local indicators of spatial association
MAPK	Mitogen-activated protein kinase
mTOR	mammalian target of rapamycin
PI3k/AKT	phosphatidylinositol 3-kinase/protein kinase B
QTL	Quantitative trait loci
RNA	Ribonucleic acid
SNPs	Single nucleotide polymorphisms
TTR	Transthyretin

21.1 Introduction

There is an increased risk of extreme heat stress (HS) in domestic animals due to climate change, especially in the tropics. Animals undergo stress (internal or external stimuli that disrupt homeostasis) in all types of production systems. Still, how the organism responds to this determines if it remains healthy and productive. This response can be at several levels, including physiological, cellular, molecular or behavioural. Nevertheless, to understand how stress affects an organism, it is necessary to comprehend how response can be affected by several complex and overlapping factors.

Heat stress does not typically occur in isolation. It can be accompanied by a lack of water and feed (Sejian et al. 2013), the need to walk long distances, increased or decreased humidity, and disease/vector prevalence. Input constraints such as forage availability and quality, feed digestion and absorption occur in sequence. Therefore, effects may be directly (thermoregulation, effects on endocrine, metabolism, production, and reproduction systems) or indirectly (environmental effects on food and water supply, pest and pathogen prevalence, and immune system resistance immunological pressures) affect the animal. Under HS, as described above, animals reduce food intake and, thereby, digestive capacity. Consequently, fewer nutrients are absorbed. Reproduction is compromised, followed by production and growth, with energy conserved for vital maintenance functions.

In animal production, selective breeding has improved productivity using modern animal breeding techniques and reproductive technologies. The response is clear with marked increases in efficiency and production levels but with a reduction in fitness, resistance to disease and tolerance to increasing stresses in the environment such as higher temperatures or lower water availability. Pryce and Haile-Mariam (2020) show that heat tolerance has worsened in dairy cattle due to a lack of selection for these traits. Climatic variables determine the choice of livestock species after soils, geography, household characteristics, and country fixed effects have been considered. An individual's susceptibility to heat stress depends on both intrinsic (mainly of genetic origin) and extrinsic (primarily environmental) factors (Gaughan et al. 2002). Also, traits that were not previously deemed relevant, such as methane production, have become important for the livestock industry (Hayes et al. 2013), as well as the need to meet consumer demands for healthier animal products.

Genetic selection is incorporated into breeding programs based on linkage-disequilibrium between polymorphisms in relevant characteristics and markers (usually SNPs – Single Nucleotide Polymorphisms). Genomic breeding values (GEBV) are calculated as the sum of each genetic marker's effects or haplotypes. As these are spread across the entire genome, potentially all quantitative trait loci (QTL) that contribute to variation in the trait of interest are captured. For this, a reference population is used whereby animals are both phenotyped and genotyped for the characteristics of interest. Hayes et al. (2009) showed that linkage disequilibrium varied between breeds. When the prediction equations are to be used across multiple breeds, the definition of the optimal composition of reference populations is necessary.

Genomic selection can aid in selection schemes, as traits related to the response to HS may be challenging to measure or have low heritability. This depends on sufficient phenotyping on reference populations to determine linkages between genotypes and phenotypes. Another option is the identification of genes of large effects, such as the slick hair gene in locally adapted cattle breeds (Olson et al. 2003). Dikmen et al. (2014) showed that introgression of this gene into Holstein cattle improved thermoregulatory ability, with positive effects on milk yield even under conditions whereby housing was modified to reduce HS. More recently, genome editing (especially using CRISPR-Cas9 technology) has been used to introduce beneficial alleles (e.g., heat tolerance, disease resistance) and haplotypes from native locally adapted to commercial populations to improve their productivity under climate change (Singh and Ali 2021).

While mitigation of the effects of climate change on livestock through modification of management conditions may represent a short-term solution to heat stress challenges, genetic changes are more long term. Studies with locally adapted breeds can help identify genes and metabolic pathways of interest in adaptation studies, as they have had sufficient time to become genetically adapted to the environment. These adaptations arise through equilibrium between evolutionary (crossbreeding, artificial selection, genetic drift) and local environment sources.

Collier et al. (2019) identified three responses of the animal to HS: acclimation (phenotypic response to a specific stressor), acclimatisation (coordinated response to more than one stressor) and adaptation (genetic modification as adverse environment persists over a few generations). Therefore, coping involves (McManus et al. 2020) the ability to limit the heat load (resistance) and the ability to limit the harm caused by a specific stressor (tolerance).

The animal's response tries to minimise the effects of HS on cellular functions. It does this through a coordinated gene network involving various cells, tissues and systems. These genes are involved in (McManus et al. 2020) energy production/metabolism, classical heat shock protein genes/chaperones, protein degradation/turnover/DNA repair, genes reducing the impact of oxidative stress/cellular repair, and transcriptional regulation. Gene expression is a crucial component of the cellular response to thermal stress.

21.2 Methods for Identifying Specific Genes or Alleles for Adaptation

Adaptation traits are complex and controlled by many genes control. These genes are commonly grouped based on function or common expression profiles. This grouping attempts to summarise a complex response network into fewer categories. These response categories can then be interpreted due to their perceived roles in re-establishing cellular homeostasis.

Molecular genetic approaches (such as microarray analyses, whole transcriptome analysis, genome-wide association studies and next-generation sequencing) have been used to identify adaptation-related genes. These, in turn, have been used to group the genes associated with thermo-tolerance into different categories. Genome-wide association studies (GWAS) use genetic variations in the genotype and phenotype by scanning the genomes of many different animals and looking for those statistically associated with a specific trait or disease. This method aims to identify common single nucleotide polymorphisms (SNPs) in the animal genome and determine how these polymorphisms are distributed across different populations. Other methods analyse the patterns of genomic diversity within and between populations, as well as the level of admixture in specific genomic regions, to identify adaptive selection signatures. Landscape Genomics use environmental variables as proxies for phenotypes. These approaches require genomic tools to look at individual loci via whole genomic sequence analyses.

HS has been shown to stimulate signal transduction pathways. This stimulus alters gene expression of immune cell mediators, thereby activating the heat shock response, promoting cytokine activity. In sequence, this impairs the cellular immune response by increasing cortisol concentrations, which binds to a specific Transcription Factor that control gene function. Physiological responses are linked to these molecular pathways and processes. Adaptive studies quantify responses through mechanisms such as gene/protein expression, enzyme activity and genome-wide analyses. For these studies to be feasible, there must be sufficient genetic variation within a population.

21.3 Genes Linked to Heat Stress

Vertebrate genomes have millions of single nucleotide polymorphisms (SNPs). Genetic variation can be due to deletions, duplications, copy-number variants, insertions, inversions and translocations, mobile genetic elements, splicing junction heterogeneity, regulatory elements and different sorts of ploidies. On the other hand, crossing over, independent assortment and sexual reproduction are principal mechanisms that maintain genetic diversity within populations. Genotype-environment interactions cause phenotypic variation, which provides the substrate for adaptive mechanisms.

We used three online tools (http://bioinformatics.sdstate.edu/go/; https://tools.dice-database.org/GOnet/ and https://david.ncifcrf.gov/tools.jsp) to analyse over 19,906 genes (Fig. 21.1) found in published papers on the adaptation of cattle, sheep, goats and horses worldwide.

As can be seen, most genes were cited in articles on cattle, and all species showed significant overlaps. The genes found in at least three of the four species were then used in further analyses (1703 genes used for enrichment, KEGG pathways, gene ontology analyses).

Functional analysis revealed pathways involved in developmental and growth processes, regulation (positive and negative) of biological process, response to stimulus and stress, immune system regulation, process and development, leukocyte activation, oxidoreductase activity, metabolism and behaviour (Fig. 21.2). As can be seen, the response to HS involves several response levels. As the papers from which these genes were identified are globally distributed and responses may be region or breed-specific, further studies are needed to improve our understanding of these processes.

Enrichment analysis (Fig. 21.3) shows 19 enriched regions (Bos taurus used as the reference genome for the four species) on chromosomes 3, 7, 8, 13, 18, 19 and 24. For example, the enriched region on chromosome 24 shows genes linked to epidermal, epithelial and mucosal structures (DSC1, DSC2, DSC3, DSG1, DSG2, DSG3, DSG4), thyroxine transport (TTR), glycolipid biosynthesis (B4GALT6), and protein modification (GALNT1), among others.

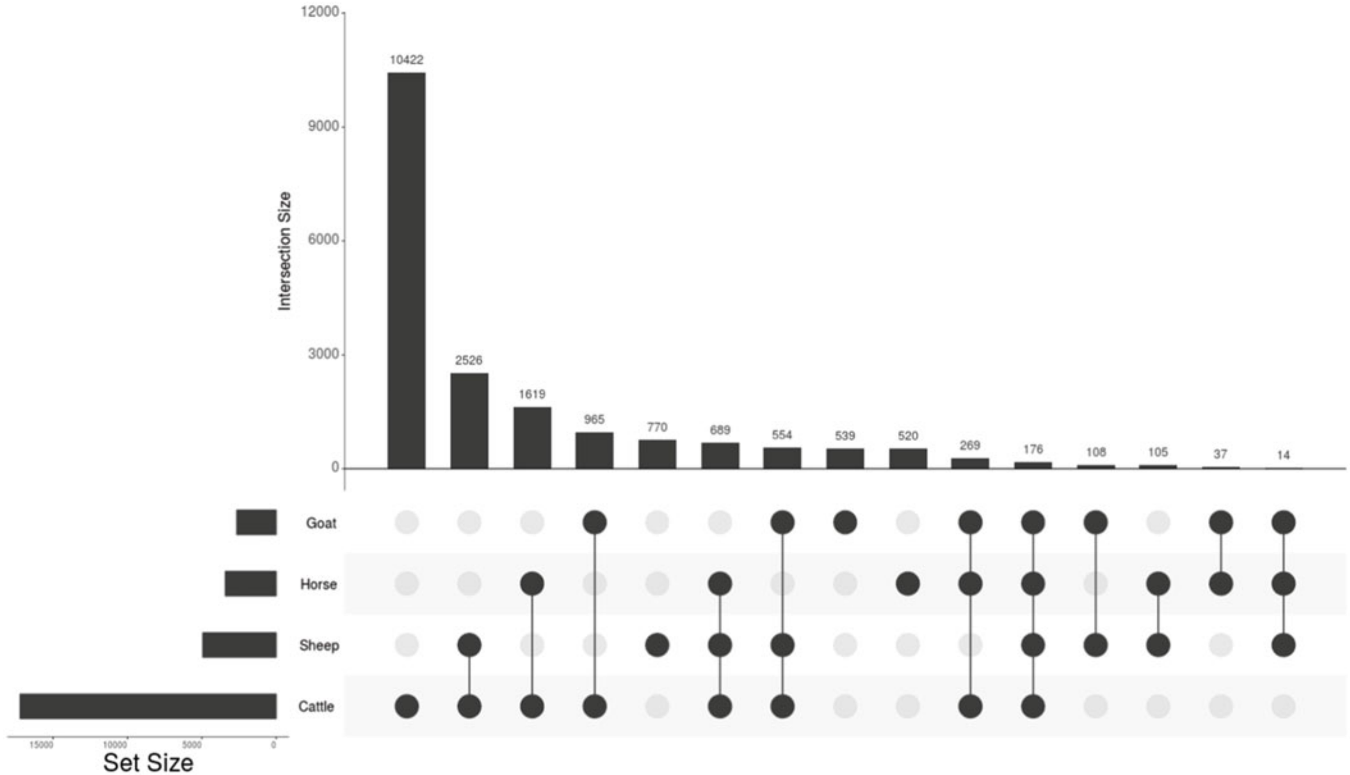

Fig. 21.1 Upset Plot (https://gehlenborglab.shinyapps.io/upsetr/) of the number of genes linked to climatic adaptation cited in scientific papers per species (based on 19,609 genes and 127 scientific papers)

Fig. 21.2 Functional analysis network from 1703 genes related to heat response in cattle, sheep, goats and horses (https://tools.dice-database.org/GOnet/)

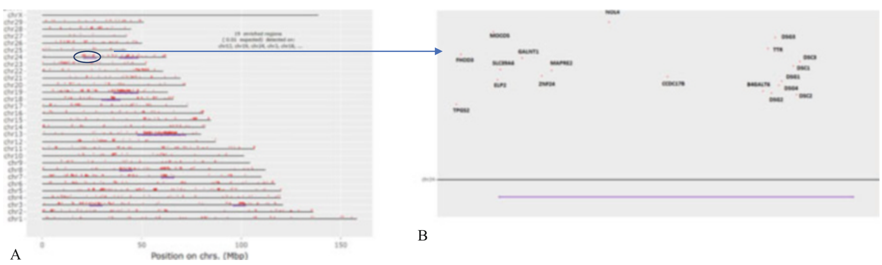

Fig. 21.3 Chromosomal localization of 1703 genes found in published papers on the adaptation of cattle, sheep, goats and horses worldwide (**a**) and a closeup (**b**) of one of the enriched regions based on cattle genomic organization (individual genes in red, enriched regions underlined in purple - these regions were defined by chi-square tests using all 19,906 genes found in the literature. http://bioinformatics.sdstate.edu/go/)

Signalling networks, such as MAPK (immune response, gene expression, cell proliferation, differentiation, apoptosis, and cell motility), mTOR (growth, energy metabolism, ageing), ErbB (cell growth, development and survival, lipid metabolism), and PI3k/AKT (metabolism, proliferation, cell survival, growth and angiogenesis) are also involved in this response (Fig. 21.4 and Table 21.1).

In the reactome (https://reactome.org/) analysis, the importance of the immune response can be seen due to the prevalence of interleukin and MAPK signalling. Here too, the response to heat stress is highlighted. AKT (serine/threonine kinase) is an upstream positive regulator of the mammalian target of rapamycin (mTOR) which controls cell growth. The KEGG analyses (https://www.genome.jp/kegg/pathway.html) highlights some of the same reactions, but also includes melanogenesis and epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor resistance possibly involved in primary defence of the organisms to ultraviolet light. Rap1 regulates the cell's response to external stimuli, and is also involved in cell survival and proliferation. In the Gene Ontology (http://geneontology.org/) for biological processes, development and metabolic processes are emphasised.

Directly annotated Gene Ontology mappings and Tissue expression locations (Fig. 21.5) further enhance the complexity of the heat stress response, as noted in Fig. 21.4 above. The response involves KEGG metabolic pathways in the liver, during foetal development, the digestive system, brain, heart, blood, and several other organs. The liver is an important regulator of growth and metabolism. It controls many physiological processes that are impacted by heat stress (Hubbard et al. 2019). These authors also show that changes in the cell membrane exert important downstream effects on heat stress response genes and metabolites (Fig. 21.5b). Other organs and structures are also important. For example, interleukins (Fig. 21.4a) are expressed in white blood cells and endothelium, while melanocytes (Fig. 21.4b) in the skin, eye, meninges and bones. The thymus (Fig. 21.5b) makes up part of the immune system and is responsible for T cell differentiation and development. Heat stress also affects the digestive and renal systems, heart and blood flow, as well as parts of the brain such as the hippocampus, cerebellum and hypothalamus.

An increase or decrease in the frequency of genetic variants in a population can be caused by natural or artificial selection pressures. Selection signatures are regions of the genome that hold functionally important sequence variants. They, therefore, are or have been under selection (natural or artificial), creating specific patterns of DNA. The reach of such signatures in the genome, up- and downstream of this functional variant, is a consequence of the so-called hitchhiking effect. Therefore, the increase in the prevalence of advantageous alleles under positive directional selection will leave distinctive signatures (patterns of genetic variation) in the DNA sequence.

Local adaptation selection signatures can be studied using whole-genome data. Spatial association with these molecular markers is also possible as candidate loci for adaptation traits can be identified through genome-environment associations (Stucki et al. 2017). Measuring Local Indicators of Spatial Association (LISA) for these candidate loci enables means that we can evaluate how similar genotypes associate spatially (Cesconeto et al. 2017). Paim et al. (2018) described the main statistical

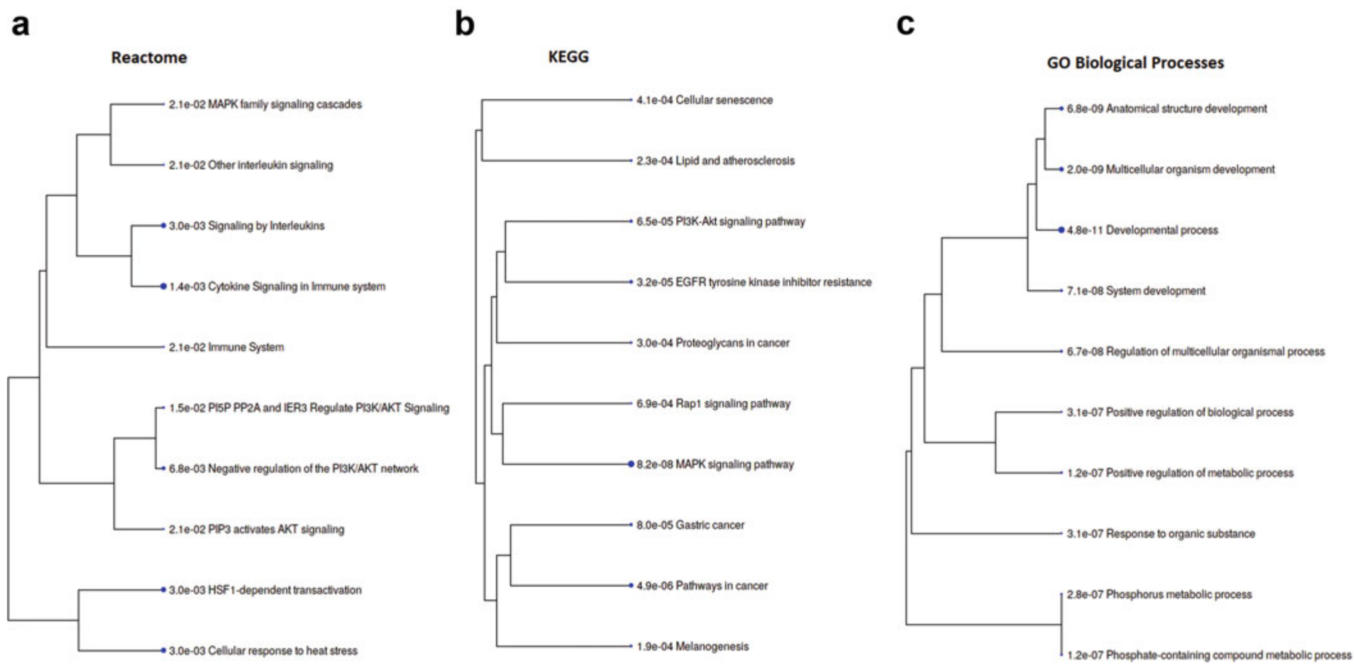

Fig. 21.4 A hierarchical clustering tree summarising the correlation among significant pathways from the Enrichment analysis using Reactome (**a**), KEGG (**b**) and Gene Ontology (GO) analyses (**c**). Pathways with many shared genes are clustered together (http://bioinformatics.sdstate.edu/go/). Numbers are False Discovery Rate (FDR). Bigger blue dots indicate more significant P-values

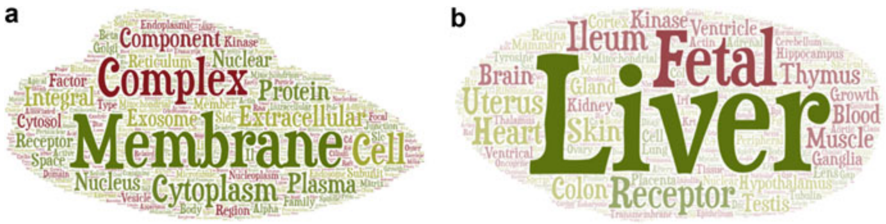

Fig. 21.5 Word clouds for Gene Ontology mappings directly annotated by the source database (**a**) and Tissue expression locations (**b**) (https://david.ncifcrf.gov/tools.jsp)

methods and software used to analyse genomic data and the identification of selection signatures.

The spatial genetic differentiation of loci can vary due to the demographic history of a species (Hoban et al. 2017). Even without selection, all have the same influence of genetic drift and migration. Possible neutral differentiation patterns need to be separated from loci under local selection. Focusing on departures from neutrality for detecting selection is difficult. Random processes affect each locus in different manners. The population structure and demography of the species in question also affect differentiation distribution. This can be problematic when there is a high average level of differentiation. The variance in F_{ST} values (Fixation index) among loci increases with average F_{ST}, even with selective neutrality, making detecting outlier loci difficult for highly differentiated populations.

The genetic bases of species adaptation to geographic conditions or climate change can be carried out using Geographical Information Systems. Manel et al. (2003) described how geographical and environmental features could facilitate genetic variation structure at population and individual levels. This method focuses on fine spatial and temporal scales, such as those with recent farm livestock migration. It has been used to identify genes responsible for adaptive evolution of species at a population level, such as quantifying the influence of spatial environment on genomic divergence and uncovering environmental factors that shape adaptive genetic variation and the genetic basis of adaptive change (Li et al. 2017). The focus in these studies needs to be on phenotypic and genetic variation so as to validate the function and adaptive generality of the detected loci. Therefore, genes involved in the regulation of metabolic pathways, as well as the adaptive phenotypes controlled by these genes, need to be identified. At the same time, Storfer et al. (2018) show that understanding the underlying demographic structure of study populations is essential when selecting genome scan methods. Species adaptability should be used to determine its distribution range, as responding to climate change depends on their landscape adaptability. This is usually determined by the genome adaptive differentiation potential as well as the species/breed's gene dispersal ability.

Learning Outcomes

- Heat stress, when combined with other stresses affects drastically the productivity of farm animals.
- Traits related to the heat stress response have low heritability values for genomic selection.
- Advanced molecular and genetic tools provide greater opportunities to identify potential biomarkers for heat stress.
- Multi-omics approaches involving genome, proteome, transcriptome, epigenome, metabolome, and microbiome could revolutionise genomic selection for heat tolerance in farm animals.

21.4 Future Perspectives

Under controlled management conditions (sufficient feeding, heat mitigation, and controlled parasite and pathogenic environment), selection for heat tolerance within highly productive breeds is likely to offer more opportunity than improving local breeds. On the other hand, crossing local and selected breeds and monitoring of heat tolerance may improve production system productivity where these conditions are not present. Research in functional genomics provides new information on HS impacts on livestock production. Genetically superior animals can be selected by identifying genes that are up- or down-regulated during HS.

Possible candidate genes have been identified in this work that could be associated with adaptation to HS. Stress causes a series of response mechanisms in animal physiology that harm the whole production chain. The identification of major genes positively correlated to heat tolerance can be used as markers in marker-assisted selection, Genomic selection, and gene editing programs. Also, other potential biomarkers on DNA and RNA, including the non-coding ones, have been proposed recently. However, there is still a gap between transforming these markers into tangible tools for breeding programs. Much research is still necessary.

Difficulties in breeding animals for resistance/tolerance to HS (Romero et al. 2015) include the lack of (1) an unambiguous definition of the stress phenotype; (2) a robust (or group of) biomarker to characterise the proposed phenotype; (3) reliable models (theoretical and quantitative) for prediction of how animals react to stressors; (4) a clear understanding of individual variability in responses to stress and transitions between acute and chronic stress. As a whole, these problems limit the ability to assess an individual's physiological status and develop techniques to reverse/control the effects of chronic stress before they become pathological. Essential for the success of breeding programs for improved HS tolerance is the availability of High-throughput phenotyping. Developing low-cost sensors and automated data collection and storage for genotyping reference populations is necessary for the efficient use of genomic breeding values and understanding the biology of the underpinning traits.

Understanding the physiological basis for adaptation requires increased characterisation with high-throughput single nucleotide polymorphism (SNP) assays or genome sequencing (Boettcher et al. 2015). Species-wide HapMap studies and multi-species studies are the first step in understanding the link between the genome and adaptation. Nevertheless, information on more breeds, geographical areas and production system environments are required to acquire a complete picture.

Metagenomics of associated microbiome can also help understand the co-adaptation of Animal Genetic Resources with other organisms in production environments. This can add information on novel biocatalysts, enzymes, genetic linkage, as well as phylogenetic and evolutionary profiles of microorganism's community function and structure and their effects on host phenotypes.

The Multi-omics approach to addressing heat tolerance and methane emissions will become important over the next decade. Examples of a multi-omics data set include some of the following: direct and indirect selection criteria, the metagenome (e.g., rumen, reproductive, and so on), mid-infrared spectral data, in addition to information from the proteome/metabolome (protein/metabolite structure and function) and functional genomic assays (e.g., methylation, transcriptomics). Causal variants can be identified when these approaches are used together with whole-genome sequencing, leading to improved responses to selection. If significant effect variants are found, these could become candidates for gene editing. In a collaborative environment, a multi-omics approach (including genome, proteome, transcriptome, epigenome, metabolome, and microbiome) could revolutionise genomic selection for traits of interest under climate change. This requires the integration of multi-omics data analysis and machine learning. Combined with the fact that mixed models have been applied to GWAS, and can reduce the number of false-positive associations, it is clear that statistical knowledge will play a significant factor in future applications of these technologies.

Genomic selection and advanced biotechnologies such as gene editing can help improve pure- and crossbreeding programmes to include traits linked to adaptation, assuming that phenotypes are available. Therefore, programs for performance recording animals in regions suffering climate change and stressful environments are needed. Integration of geographical and genetic information is also essential and adequate referencing, organisation, storage, and dissemination to stakeholders. This requires new and improved databases and information systems and the capability to link different geographical and production scales in formats that can be analysed.

Regulatory frameworks for the use of advanced technologies such as gene editing will also determine the future of their application as animal health and consumer safety has to be considered. Therefore, cooperation between all stakeholders will be needed to optimise the genetic and genomic resources to adapt livestock to climate change.

Acknowledgements Thanks are due to the *Coordenação* de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for financing.

Conflict of Interest The authors declare no conflict of interest.

Appendix

Table 21.1 Pathway enrichment analysis of 1703 genes (from a total of 19,606) related to heat stress in at least three species of livestock (cattle, sheep, goats or horses)

Enrichment FDR[a]	Number of genes	Pathway Genes	Fold enrichment	Pathways
GO[b] biological processes				
3.10E-07	273	1747	1.4	Response to organic substance
6.70E-08	313	2019	1.4	Regulation of multicellular organismal process
1.20E-07	331	2180	1.4	Phosphate-containing compound metabolic process
2.80E-07	331	2198	1.4	Phosphorus metabolic process
1.20E-07	381	2579	1.3	Positive regulation of metabolic process
2.00E-09	471	3208	1.3	Multicellular organism development
7.10E-08	428	2944	1.3	System development
4.80E-11	558	3841	1.3	Developmental process
6.80E-09	512	3577	1.3	Anatomical structure development
3.10E-07	556	4056	1.2	Positive regulation of biological process
GO molecular function				
1.20E-08	190	1093	1.6	ATP binding
1.20E-08	197	1142	1.6	Adenyl nucleotide binding
1.30E-08	195	1132	1.6	Adenyl ribonucleotide binding
1.30E-08	224	1348	1.5	Purine ribonucleoside triphosphate binding
1.10E-08	234	1413	1.5	Purine nucleotide binding
1.30E-08	232	1410	1.5	Ribonucleotide binding
1.10E-08	261	1609	1.5	Nucleotide binding
1.10E-08	261	1609	1.5	Nucleoside phosphate binding
1.10E-08	293	1862	1.4	Small molecule binding
1.10E-08	320	2065	1.4	Anion binding
KEGG[c]				
3.20E-05	25	76	3	EGFR tyrosine kinase inhibitor resistance
1.90E-04	26	91	2.6	Melanogenesis
8.00E-05	35	136	2.3	Gastric cancer
8.20E-08	65	263	2.2	MAPK signalling pathway
4.10E-04	33	137	2.2	Cellular senescence
2.30E-04	39	168	2.1	Lipid and atherosclerosis
3.00E-04	41	183	2	Proteoglycans in cancer
6.90E-04	40	185	2	Rap1 signalling pathway
6.50E-05	63	307	1.9	PI3K-Akt signalling pathway
4.90E-06	92	471	1.8	Pathways in cancer

(continued)

Table 21.1 (continued)

Enrichment FDR[a]	Number of genes	Pathway Genes	Fold enrichment	Pathways
Panther[d]				
4.30E-02	11	39	2.6	Interleukin signalling pathway
4.20E-02	16	64	2.3	Cadherin signalling pathway
4.30E-02	16	68	2.1	PDGF signalling pathway
2.50E-02	25	113	2	Inflammation mediated by chemokine and cytokine signalling pathway
4.30E-02	20	91	2	Angiogenesis
2.00E-02	33	157	1.9	Wnt signalling pathway
Reactome[e]				
3.00E-03	10	19	4.8	HSF1-dependent transactivation
3.00E-03	21	69	2.8	Cellular response to heat stress
6.80E-03	20	68	2.7	Negative regulation of the PI3K/AKT network
1.50E-02	19	67	2.6	PI5P PP2A and IER3 regulate PI3K/AKT signalling
2.10E-02	28	125	2	PIP3 activates AKT signalling
2.10E-02	33	156	1.9	Other interleukin signalling
2.10E-02	34	161	1.9	MAPK family signalling cascades
3.00E-03	57	285	1.8	Signalling by interleukins
1.40E-03	71	364	1.8	Cytokine signalling in immune system
2.10E-02	170	1188	1.3	Immune system
Wikipathways[f]				
5.30E-04	18	51	3.2	Thyroid Stimulating Hormone (TSH) signalling pathway
1.10E-02	14	45	2.8	Kit receptor signalling pathway
1.30E-02	14	48	2.6	Endochondral ossification
1.30E-02	14	48	2.6	Oncostatin M signalling pathway
1.20E-02	17	63	2.4	Leptin signalling pathway
9.50E-04	30	121	2.2	Integrated breast cancer pathway
9.50E-04	31	127	2.2	EGF/EGFR signalling pathway
2.10E-02	19	80	2.1	WP3231 senescence and autophagy in cancer
2.40E-04	43	182	2.1	MAPK signalling pathway
1.20E-02	25	109	2.1	BDNF signalling pathway
Cellular component				
3.50E-03	9	17	4.8	Desmosome
1.10E-07	346	2302	1.4	Cytosol
3.50E-03	373	2802	1.2	Intracellular non-membrane-bounded organelle
3.50E-03	373	2803	1.2	Non-membrane-bounded organelle
1.60E-03	465	3526	1.2	Protein-containing complex
1.50E-02	338	2571	1.2	Nucleoplasm
3.50E-03	404	3076	1.2	Membrane-enclosed lumen

(continued)

Table 21.1 (continued)

Enrichment FDR[a]	Number of genes	Pathway Genes	Fold enrichment	Pathways
3.50E-03	404	3076	1.2	Organelle lumen
3.50E-03	404	3076	1.2	Intracellular organelle lumen
8.90E-03	374	2856	1.2	Nuclear lumen
All available gene sets				
4.90E-08	234	1413	1.5	Purine nucleotide binding
4.30E-08	261	1609	1.5	Nucleotide binding
4.30E-08	261	1609	1.5	Nucleoside phosphate binding
4.90E-08	293	1862	1.4	Small molecule binding
4.90E-08	313	2019	1.4	Regulation of multicellular organismal process
4.30E-08	320	2065	1.4	Anion binding
3.20E-09	471	3208	1.3	Multicellular organism development
5.30E-08	428	2944	1.3	System development
7.90E-11	558	3841	1.3	Developmental process
1.10E-08	512	3577	1.3	Anatomical structure development

[a]*FDR* false discovery rate
[b]*GO* gene ontology
[c]*KEGG* Kyoto encyclopaedia of genes and genomes—https://www.genome.jp/kegg/pathway.html
[d]*Panther* protein analysis through evolutionary relationships—http://www.pantherdb.org/pathway/
[e]Reactome-database of reactions, pathways and biological processes—https://reactome.org/
[f]https://www.wikipathways.org/index.php/WikiPathways

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Goat as the Ideal Future Climate Resilient Animal Model

22

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V. Sejian et al. (eds.), *Climate Change and Livestock Production: Recent Advances and Future Perspectives*, https://doi.org/10.1007/978-981-16-9836-1_22

Abstract

The negative effects of climate change on livestock production was mediated predominantly through the indirect impacts such as sudden disease outbreaks, less feed and water available as well as shrinking pasture lands. Therefore, it is very essential for the scientific community to identify the best species among the livestock which could cope with the adversities associated climate change. The very few recent research efforts based on modeling approach clearly demonstrated that goat is the only species among the livestock to face very less number of extremely severe heat stress days both by 2050 as well as 2100. Thus it is very important to concentrate on this otherwise neglected species so far because rearing goats could help the livestock farmers to optimize their economy in the changing climate scenario. Goats are predominantly distributed in the tropical countries and they possess the ability to survive in harsh locality due to their high thermo-tolerance, drought tolerance, unique feeding behaviour as well as disease resistance. Most goat breeds recorded are indigenous in nature. Differences in climate resilience were established even among the indigenous breeds. This warrants more research efforts on the indigenous breeds to screen for their climate resilience probably with the intention to identify the best breed which could survive and produce optimally in specific agro-ecological zones. The chapter also highlights the various adaptive strategies that need to be implemented to sustain goat production in the changing climate scenario.

Keywords

Adaptation · Climate resilience · Goat · Heat stress · Thermo-tolerance

Abbreviations

γ	Gamma
%	Abbreviation
DAGT	Diacylglycerol acyltransferase
Fig	Figure
FSHR	Follicle stimulating hormone receptor
GH	Growth hormone
GHR	Growth hormone receptor
HCT	Hematocrit
HGB	Hemoglobin
HSF	Heat shock factor
HSP	Heat shock protein
ICAR	Indian Council of Agricultural Research
IFN	Interferon
IGF-1	Insulin-like growth factor-1
LEP	Leptin
LHR	Luteinizing hormone receptor

MSTN	Myostatin
NIANP	National Institute of Animal Nutrition and Physiology
NOS	Nitrous oxide synthase
NRAMP1	Natural resistance-associated macrophage protein 1
PLR	Prolactin receptor
SOD	Super oxide dismutase
THR	Thyroid hormone receptor
TLR	Toll-like receptor
TNF	Tumour necrosis factor

22.1 Introduction

Livestock farming plays a vital role in ensuring livelihood security especially towards the marginal or economically weaker segments of the society, who lack even the basic resources required to create favorable microclimate for managing the farming system. Global demand for livestock products are predicted to double during the first half of this century as a consequence of the growing human population and their affluence. On the other hand, drastic changes are expected to occur from the global climate change point of view. In the present scenario, climate change is among the most serious challenges faced by farmers and livestock owners around the globe, having a long term effect. The indirect impacts of climate change are proved to significantly impair livestock production. This is even more pronounced in tropical countries which inhabit the major proportion of the livestock population, especially with their indigenous germplasm. Additionally, severe reduction in pasture availability as along with shrinking grazing lands also lead to marked reduction in livestock production. Furthermore, the addition of climate change impact on pasture availability, to the ongoing reduction, would aggravate the issue severely (Sejian et al. 2018). Hence, scientific communities are under enormous pressure to sustain livestock production amidst the chaos and ensure food security from the future perspectives. Therefore, it is very vital to channelize the research efforts to identify potential livestock species that can effectively cope with the adversities associated with climate change and also produce optimally.

With the projected alarming impacts of climate change on the pasture availability, small ruminants and especially goat production gain significance because of their ability to survive on limited pastures. The farming communities are looking to invest in the small ruminant livestock production systems because of their lower feed requirement, lower input cost and better climate resilience than the large ruminants. Among the small ruminants, goats are considered ideal climate animal model due to their better thermo-tolerance, drought tolerance, ability to survive on limited pastures as well as the disease resistance capacity (Reshma Nair et al. 2021). In a recent research effort by Carvajal et al. (2021) and Rahimi et al. (2021) it was clearly established that among the livestock species, goats will experience least number of

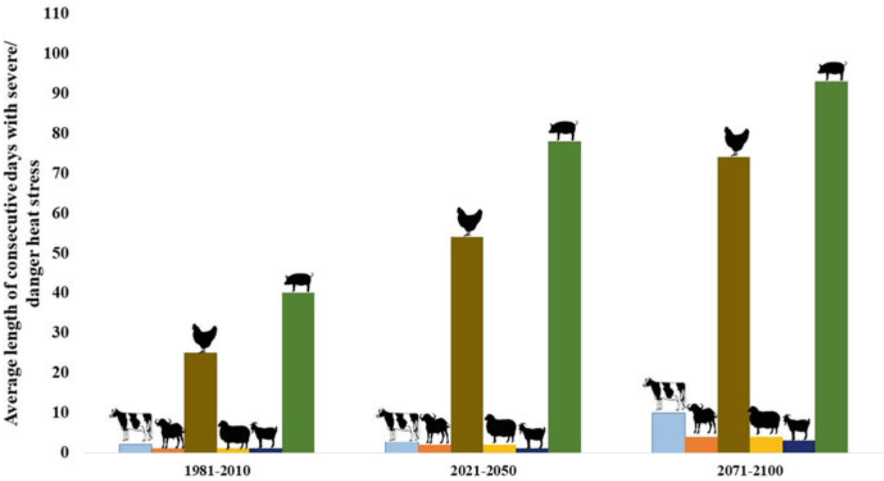

Fig. 22.1 Model projections of extremely severe heat stress to be experienced by different livestock species by 2050 and 2100 in East Africa (source Rahimi et al. 2021; Reshma Nair et al. 2021)

extremely severe stress days both by 2050 as well as by 2100 in the globe as a whole as well as East Africa respectively. Further, rearing goat is considered to be more economical when compared to large ruminants, due to their comparatively lower feed requirement, monetary investment and management (Reshma Nair et al. 2021). Figure 22.1 describes the modeling approach to project the extremely severe heat stress days that major livestock species are going to experience by both by 2050 and 2100 scenario. This compilation is therefore an attempt to collate and synthesis information on this line to project the various advantages associated with goat production to prove them as ideal animal model in the changing climate scenario.

22.2 Goat as the Future Animal from Food Security Perspectives

In order to sustain livestock production under the alarmingly changing climatic conditions, researchers are now channelizing their work towards identification of ideal species that can cater the needs of the growing human population. Several studies have identified goats to be the go-to species, for possessing the ability to efficiently sustain animal agriculture amidst the changing environmental conditions. Pioneers in livestock research had identified goats to be more potential among the other small ruminants, for their better adaptation to a wide range of environmental conditions. Goats being opportunistic feeders, are stated to be least affected due to the ongoing and projected depletion of pasture. Apart from this, the selective feeding behavior exhibited by goats are proved to be advantageous; as these species can consume even the poor quality forages, the nutrients obtained from which are then converted into high quality products. Furthermore, the bipedal stance behavior exhibited by goats gives them an advantage over other livestock species as this gives them access to the tree fodders that are usually inaccessible to other species. Further, the feed efficiency of goats are considered to be better than other ruminant species. Another benefit of goat rearing, especially from economics perspective is their minimal housing requirement. Goats can survive well in any location even with minimum protection from the weather. Additionally, labor costs associated with goat production is minimal as they can be managed efficiently even by using family members, which is the usual practice followed especially by the poor farmers.

The human population across the globe is projected to touch an alarming count of 9.6 billion by 2050. This is a major concern from the food security aspect as animal proteins are the most preferred protein source by majority of the individuals, especially in the developing world. Goats are considered to be the ideal climate animal model that has the ability to perform better than other species amidst the projected climatic adversities and also feed and fodder shortage. As per the model by Ngambi et al. (2013), dairy goats produce approximately 15.2 million tons of milk that constitute 2% of total livestock milk production. Moreover, goat meat and milk are considered to have several health benefits and therapeutic values that further boosts up their demand. In harmony with this, recent reports also suggest that the wide acceptance of goat products across the globe makes the goat enterprises to be of more commercial value. This highlights their pivotal role in meeting the growing humanitarian animal protein needs by the end of this century along with their most important role to financially support poor and marginal farmers around the world. Therefore, it is the need of the hour to gather researchers and policymakers who can efficiently set priorities to design appropriate programs to ensure sustainable livestock production and also satisfy the food demands of the growing human population by 2050.

22.3 Advantages of Goat Farming in Tropical Countries

Goat farming is one of the largest subsectors of agriculture in arid, semi-arid regions of tropical and subtropical countries. It plays a vital role in livelihood and economic wellbeing of marginal, small farmers and landless labourers in both rural and peri-urban areas in developing countries. Goat farming is advantageous over the other livestock farming system since it maintains their production potential under extreme climate conditions of the tropical and subtropical regions. The anatomical and physiological characteristics of goats and economics of goat farming make them more beneficial comparing to the other farm animals.

Goats have been considered as ideal animal to be reared in tropics and subtropical regions. Goats have a capacity to thrive and perform better under harsh environmental conditions because of their superior thermoregulation capacity and ability to withstand during feed and water scarcity. Goats better utilises low quality fodder and shrubs or benefit from feed resources which are not consumed by the other farm animals and convert them into meat and milk. Goats have ability to walk long distance for search of feeds. Comparing to the other domesticated farm animals, goat emits less methane. In addition to their great adaptability to extreme climatic conditions and to different feeds, it require less space (smaller body size), less housing requirement, less maintenance cost, easy to handle, faster growth rate, higher resistance to disease as well as heat stress which make them goats are much better than other ruminant species.

Goat forming requires low investment but gives high rates of return and always available for sales of live goats to generate revenues to meet their immediate needs. Therefore, goats are considered as a poor man's cow since it is an important source of income for rural poor farmers. Thus, goat farming in tropics and subtropical countries stabilize the rural economy.

Goat meat (chevon) is a delicious food for most people across the globe since it is lean and has low cholesterol content. Similarly, goat milk and products demand are increasing and it is most consumed dairy product in the world in recent times. Goat milk are rich in vitamin and mineral content, easily digestible and better taste compared to cow milk. Goat products are widely accepted, no religious taboo and having higher demand across the world. Goat manure are good source natural fertilizers for rural farmers which are good source of nitrogen, phosphorus and potassium that improves the soil fertility and ensure healthy crop growth and productivity.

22.4 Climate Resilient Goat Production

Identification of indigenous breeds that can survive efficiently across varied agro-ecological zones is the key to ensure sustained livestock production. In series of studies conducted at Indian Council of Agricultural Research- National Institute of Animal Nutrition and Physiology (ICAR-NIANP), three different indigenous goat breeds Osmanabadi, Malabari and Salem Black goats from three South Indian states; Karnataka, Kerala and Tamil Nadu, respectively, were compared for their climate resilience capacity. Innumerable phenotypic and genotypic traits associated with heat stress were studied in these goats which thereby led to the conclusive report that Salem Black breed was able to adapt and produce better when compared to Osmanabadi and Malabari breeds upon heat stress exposure (Archana et al. 2018; Angel et al. 2018; Amitha et al. 2019; Rashamol et al. 2019). The significantly lower respiration rate, rectal temperature and heat shock protein 70 (*HSP70*) gene expression in the Salem Black in comparison to the Osmanabadi and Malabari goats during heat stress indicate better thermal resilience of the Salem Black goats (Aleena et al. 2018). Therefore, promoting the Salem Black breed among the local farmers could benefit them and improve their livelihood security. Figure 22.2 depicts the three indigenous goat breeds, Osmanabadi, Malabari and Salem Black.

Fig. 22.2 Three indigenous goat breeds of South India

22.5 Concepts Associated with Climate Resilient Goat Production

The livestock sector in general holds a uniqueness for both being sensitive to climate change and also a contributor to the phenomenon. Though goats are excellent climate resilient species, they too face the deleterious impact of climate change. Amongst the multiple climatic stresses faced by goat, heat stress is of major concern as it destabilizes the production efficiency of the animals. The deleterious effects of heat stress include decline in growth, meat and milk production in goat. Further, climate change leads to the increase and rapid spread of several vector borne diseases in goat which further compromises the immune status of the animals. Nevertheless animals employ some vital adaptive mechanisms through behavioural, physiological, neuro-endocrine, cellular and molecular responses that aid them to efficiently combat the changing climatic condition. Further, climate change can lead to alteration in the rumen function and thereby digestibility in goats. This could aggravate the issue of enteric methane emission apart from the dietary losses incurred. Hence suitable ameliorative strategies need to be enforced to counter the adverse impact of climate change on goat production and reduce its contribution towards global warming. The management strategies can be broadly categorized as housing management, animal management and climate monitoring. Nutritional interventions considering season specific feeding and micronutrient supplementation could also play a efficient role both to ameliorate the climate change impact on goats as well as sustain their production. Adoption of goat specific body condition scoring system may help to optimize economic return by minimising the unnecessary feeding costs. Finally, proper emphasis must be given to develop and enforce appropriate adaptation strategies by active involvement of policy makers. These strategies may include development thermo-tolerant breeds, ensuring water availability, promoting women empowerment, establishing accurate early warning system and effective capacity building programmes for all the stakeholders. These efforts would be vital to ensure sustainable goat production in the changing climate scenario. Figure 22.3 describes the various concepts associated with climate resilient goat production.

Fig. 22.3 Various concepts associated with climate resilient goat production

22.6 Biomarkers for Heat Stress in Goat

22.6.1 Phenotypic Markers

Drinking frequency, respiration rate, and rectal temperature have been reported to be the ideal biological markers for assessing the impact of both heat and nutritional stress in goats. In another study conducted in Osmanabadi goats, Insulin-like growth factor 1 (IGF-1), leptin (*LEP*), growth hormone (GH) and plasma HSP70 were established as biomarkers for assessing nutritional stress (Pragna et al. 2018; Abhijith et al. 2021). Based on the study conducted to comparatively assess the climate resilience of three indigenous goat breeds, Osmanabadi, Malabari and Salem Black breeds, drinking frequency, water intake, haemoglobin, packed cell volume, plasma cortisol, aldosterone, tri-iodo-thyronine and thyroxin were established to be reliable biomarkers for heat stress response in goats cutting across breeds (Shilja et al. 2016). These findings may also be useful for assessing the implications of heat stress from the perspective of animal welfare.

22.6.2 Genotypic Markers

The classical heat stress associated molecular chaperone, *HSP70* gene was identified to act as an ideal biological marker for assessing the impact of combined stress (heat and nutritional stress) in Osmanabadi goats. In the same study, the significantly higher expression of *TLR8* and *TLR10* in heat stressed goats indicated the probable role of these genes as immunological markers of heat stress in goats. In another study conducted in Osmanabadi goats, growth hormone receptor (*GHR*), *HSP70* and *HSP90* were identified as efficient markers for assessing nutritional stress in goats. Further, based on the results obtained from a mega study at ICAR-NIANP a number of biomarkers were reported that were significantly associated with heat stress. These included some of the well established heat stress associated genes like heat shock factor 1 (*HSF1*), *HSP27*, *HSP60*, *HSP70*, *HSP90*, *HSP110*; growth and metabolism associated genes like *GH*, *GHR*, *IGF-1*, *LEP*, leptin receptor (*LEPR*), thyroid hormone receptor (*THR*); genes associated with reproduction, prolactin receptor (*PLR*), follicle stimulating hormone receptor (*FSHR*), luteinizing hormone receptor (*LHR*) (Sejian et al. 2019). It also included a number of immune response linked genes like interleukin 10 (*IL10*), *IL18*, interferon β (*IFNβ*), *IFNγ*, tumour necrosis factor α (*TNFα*), toll-like receptor-1 (*TLR1*), *TLR4*, *TLR5*, Natural resistance-associated macrophage protein 1 (*NRAMP1*), nitrous oxide synthase (*NOS*), superoxide dismutase (*SOD*) (Sophia et al. 2016; Rashamol et al. 2019). Figure 22.4 describes the different biomarkers for quantifying heat stress response in goats.

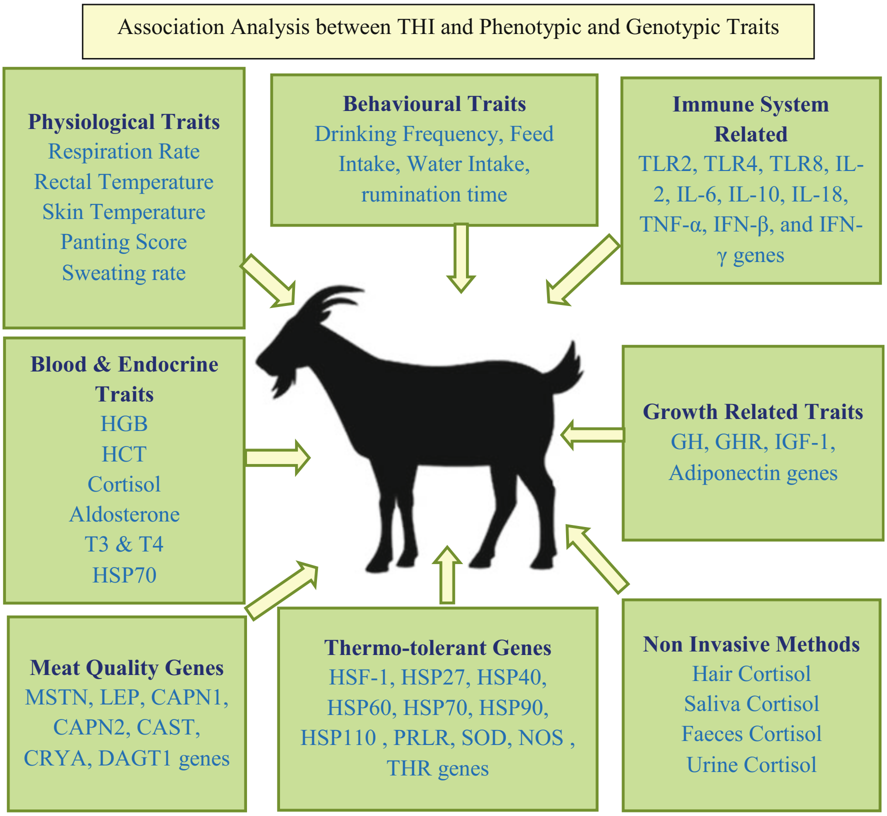

Fig. 22.4 Different biomarkers for quantifying heat stress response in goats

22.7 Adaptation Strategies to Sustain Goat Production Under Changing Climate

Adapting to climate change comprises of the inclusion of accurate measures to reduce the adverse effects of climate change along with enforcing suitable strategies to reduce the contribution of livestock sector to climate change. Development of thermo-tolerant breeds, that not only adapt well but also produce efficiently, using various genomics approach could be one of the most efficient and long term solution. Additionally, reduced methane emission should also be considered as a vital trait while considering the revised livestock breeding program for climate resilient. Further, encouraging the active participation of women is also crucial as they possess rich knowledge and efficient management skills especially for maximizing the use of natural resources. Hence, organizing occasional trainings and participatory research approach into the roles of women can assists to tackle climate change impact from the rural areas point of view. In addition, well-organized and accurate early warning systems can play a pivotal role by avoiding the severe damages that especially occur during unexpected disasters, by providing sufficient time for the policy maker and people to prepare effective response. Another crucial strategy involves development of skilled disease surveillances supported with effective health services. This is often neglected despite the fact that they can effectively control the spread of the diseases especially those that are climate change induced. Furthermore, so as to meet with the water requirements for goat production, especially in tropical regions, improved water resource management should be developed. Another efficient adaptive strategy involves the cultivation of drought tolerant fodder varieties in hot and dry areas as this can ensure feed supply during scarcity period. Finally, the role of extension services to build awareness through capacity building programs among the livestock keepers is vital to improve their adaptive capacities against climate change. Hence, there is an urgent need to improvise the policies and practices so as to ensure cost effective adaptive strategies to combat climate change. Figure 22.5 describes the different adaptation strategies that are vital to sustain goat production in the changing climate scenario.

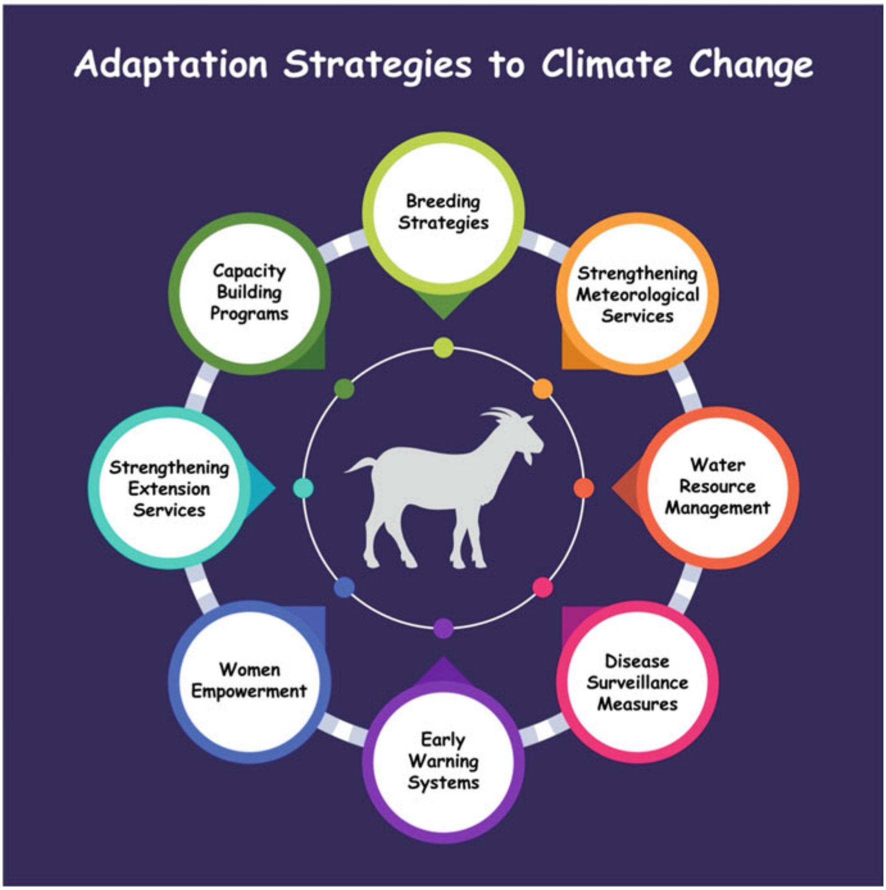

Fig. 22.5 Different adaptation strategies to sustain goat production in the changing climate scenario

22.8 Conclusions

The limited available literature clearly project goat as the ideal climate resilient animal model. As the scientific community are looking forward to identify ways and means to ensure food security by 2050 to feed the expected human population of 9.6 billion, it is very essential to invest on goat promotion. Such approaches can ensure economic security of the weaker sections of poor and marginal farmers. However, there are lot of basic information are lacking in this neglected species till date. Hence research efforts needed to generate baseline information on the indigenous breeds of goats. Such efforts would help the farming community to recommend ideal species specific to specific agro-ecological zone. Generating the baseline information might help to identify the targeting point for intervention in terms of sustaining goat population in the changing climate scenario. Further, appropriate extension system should be in place to transfer these information generated to the ultimate target groups of farmers. Capacity building program should be developed to cater the need of different stakeholders involved in goat production. Research efforts are also needed to develop standard package of practices comprising shelter management, nutritional interventions and disease management for goats. Additionally, genetic approach should be focussed to identify more advanced thermo-tolerant markers with high heritable values to incorporate them in marker assisted selection program to develop more climate resilient goat breeds to disseminate to poor and marginal farmers to ensure their livelihood.

Learning Outcomes

- Goats are considered ideal climate resilient species because of its better thermo-tolerance, drought tolerance, ability to survive on limited pastures as well as the disease resistance capacity in tropical region.
- Differences in climate resilience were achieved even among the indigenous goat breeds. This warrants more such efforts in screening all indigenous goat breeds to identify the best breed for the specific agro-ecological zone.
- Genetic approach to develop more climate resilient goat breeds should focus on identification of more advanced biomarkers with high heritable values for incorporation in marker assisted selection programs.

22.9 Future Perspectives

Substantial evidences are now available to project goat as the ideal climate resilient species and therefore future research must focus on generating baseline information pertaining to adaptive variables in all unexplored indigenous breeds. Such an approach would help the scientific fraternity to identify the best climate resilient indigenous goat breeds which could survive and produce optimally in a given agro-ecological zone. Such identified breed could be disseminated to the poor and marginal farmers for ensuring their livelihoods. Further such identified best climate resilient breeds has to be subjected for testing their survival and production ability in multiple locations. This way it is possible to identify a region specific breeds which has the ability to survive in multiple locations and produce optimally. Such breeds should be subjected for biomarkers identification and such markers could be used in future breeding programs to produce new goat breeds with potential to survive and produce optimally in multiple agro-ecological zones. The policy makers must propagate such breeds for ensuring sustainable goat production in the changing climate scenario by designing appropriate capacity building programs for different stakeholders and providing additional incentives to farmers for rearing such breeds.

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Future Vision for Climate Change Associated Livestock Production

23

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V. Sejian et al. (eds.), *Climate Change and Livestock Production: Recent Advances and Future Perspectives*, https://doi.org/10.1007/978-981-16-9836-1_23

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Abstract

Climate change is inevitable and thus there is an immediate need to plan for the future amelioration and mitigation strategies to sustain livestock production. This chapter is therefore aimed to shed some light on few supreme strategies that need to be considered while planning to tackle future climate change effects in livestock sector. To begin with, improvisation of thermal indices which reveal the impact of stress is necessary. Furthermore, adoption of predicted livestock early warning systems, advanced molecular tools to assess thermo-tolerance and also assess animal welfare, incorporation of nanotechnology in animal production and so on are some of the proposed strategies. Additionally, a lot more consideration should also be given to animal management and livestock farming practices like, improvising the nutritional charts in animals, encouraging climate smart livestock farming keeping a regular check on the soil, climate and livestock harmony. Along with these, approaches to reduce the livestock further contribution to climate change should be considered. Animal breeding is another major division that can play a promising role in future livestock farming in the face of global warming threat. Screening large livestock populations, breeding for climate-resilient livestock, actively incorporating the local indigenous germplasm and further substantiating the target to identify agro-ecological zone specific breeds are some of the vital aspects to be considered. The proposed approaches to effectively combat climate change are of great relevance and hence these concepts should be disseminated across the globe to ensure future sustainability in livestock farming.

Keywords

Food security · Livestock · Policy · Sustainability · Technologies

Abbreviations

%	Percentage
CO_2	Carbon dioxide
CSA	Climate smart agriculture
FAO	Food and Agriculture Organization
GHG	Greenhouse gas
GS	Genomic selection
GWP	Global warming potential

IgG	Immunoglobulin gamma
MAS	Marker assisted selection
NCFR	Non-conventional feed resources
PLEWS	Predictive livestock early warning systems
PLF	Precision livestock farming

23.1 Introduction

The future livestock production in the changing climate scenario requires paradigm shift in the existing policies to develop and propagate new strategies and technologies which could cater the need of growing human population both by 2050 and 2100 (Fig. 23.1). The livestock adaptation strategies developed should be focused and teller made to suit the projected future climatic condition. A wide range of strategies comprising early warning system, advanced thermal indices; identifying more appropriate livestock species; climate smart livestock production; applications of molecular tools; balanced nutrition; low carbon livestock; artificial meat; breeding management; vaccine technologies; advancements to assess animal welfare; vaccine and nanotechnologies applications; promoting indigenous animals; exploiting unconventional feed resources; water resource management and understanding in depth climate change associated livestock disease occurrences. These strategies are discussed in brief in this chapter probably to give an overview to the readers on the possible future technologies which could play a significant role in sustaining livestock production in the future climate change perspectives.

23.2 Thermal Indices with More Applicability

Although there are several thermal indices available to quantify heat stress response, still there are lots of scopes for improvement. Advanced thermal indices incorporating all cardinal weather variables such as temperature, humidity, wind velocity and solar radiation needs to be developed. Such indices should be developed separately for specific agro-ecological zones. Such approach could help to generate useful basic information which may play vital role in determining the appropriate amelioration strategies to sustain livestock production in the changing climate scenario.

23.3 Predictive Livestock Early Warning Systems (PLEWS)

The PLEWS involves a combination of advanced technological and predictive modelling functions that amalgamates the impact of climatic extremities, weather, and can also improve food security concerns especially among the most vulnerable

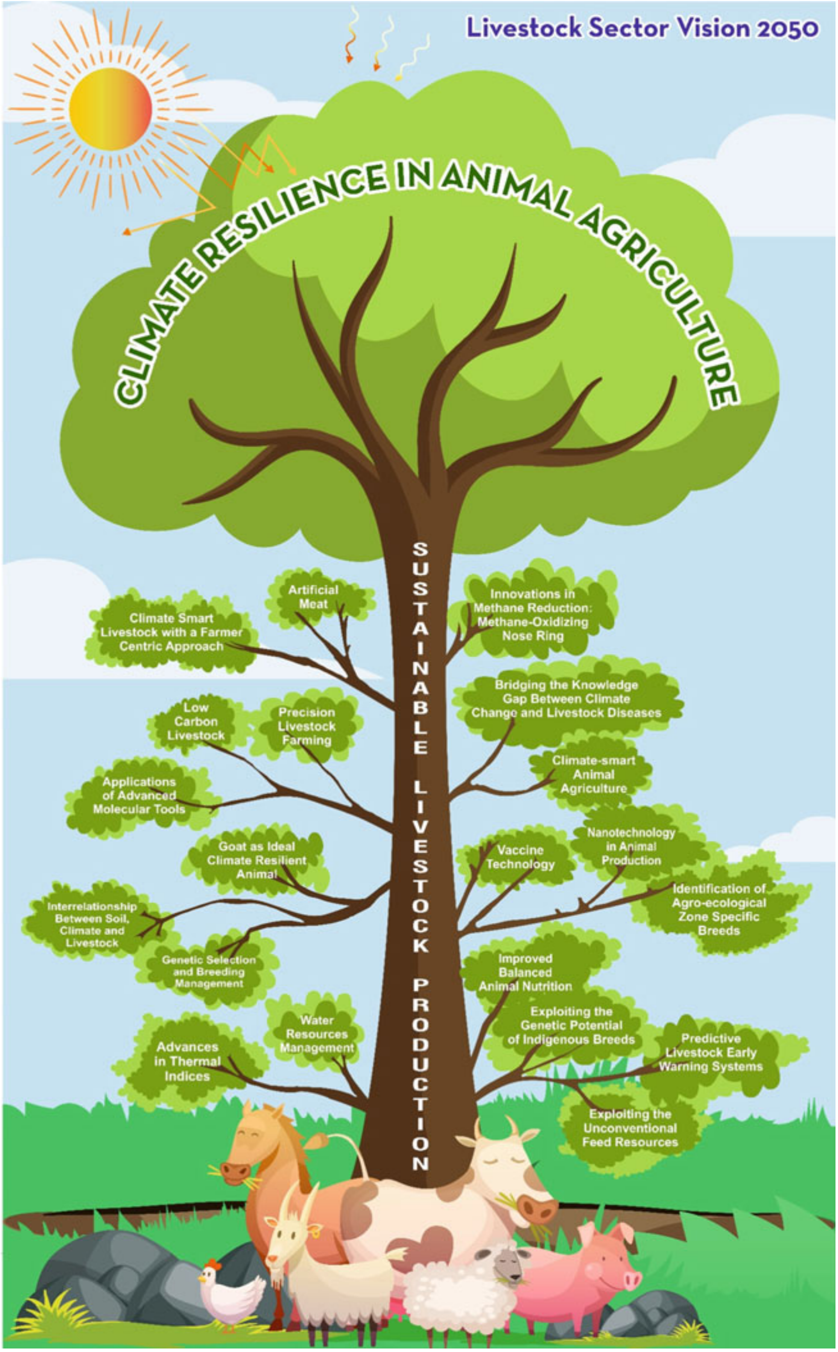

Fig. 23.1 Future strategies to sustain livestock production in the changing climate scenario

populations. Such predictive models eliminates the time gap between a warning and its required intervention thereby providing an anticipatory action rather than the usual combative action. Furthermore, as a consequence of this feature, the PLEWS are also thought to drastically cut down the costs associated with early warning. Additionally, PLEWS has an advantage of strengthening the resilience of populations at-risk, ensure better disaster mitigation strategies and also provide more time to the communities and global actors for preparation, planning and mitigation rather than only response. However, though such advancements promise great possibilities to combat the adversities of climate change, it can be effective only when adopted satisfactorily by the end users. It is equally necessary to educate the potential users about the functions, notifications and all other relevant details associated with such facilities and also their application towards decision making process (Matere et al. 2020).

23.4 Applications of Advanced Molecular Tools for Inducing Thermo-Tolerance

The latest molecular biological tools offer huge scope for improving thermo-tolerance in domestic animals. The latest technologies such as metagenomics, transcriptomics, bisulfite sequencing, genome wide association studies and selection signature may play a greater inroad to understand in depth or may help to elucidate further the hidden intricacies of molecular mechanisms governing livestock adaptation to climate change. Such information may prove very vital in identifying more reliable biomarkers for climate resilience in farm animals. This could revolutionize future animal breeding to evolve more climate resilience breeds which has the potential to survive and produce optimally in a given location.

23.5 More Focus on Goat as Future Food Animals

Recent research findings using modeling approach clearly projects goat as the ideal climate resilient animals (Nair et al. 2021; Carvajal et al. 2021). Their drought tolerance, thermo-tolerance potential, anatomical advantage for browsing, ability to survive on poor quality and limited pastures, and disease resistance potential in tropical regions imparts them the potential to survive and produce optimally in multiple agro-ecological zones. With the projected climate change associated reduction in both green and dry fodders as well as reduction in water resources makes large animal farming very difficult. Based on the above advantages associated with goat and the very low initial investments required for goat farming makes it a go to go species for ensuring food security by 2050 scenario. Hence the focus should shift to investing on goat farming which otherwise remains a neglected species in many parts of the world. Such approach could help to sustain goat production to ensure animal protein for the expected 9.6 billion peoples by 2050.

23.6 Interrelationship Between Soil, Climate and Livestock

Strategies need to be developed for efficient grassland management aimed at optimizing its capacity to serve as a carbon sink. More supports needs to be provided by the government to encourage grazing. Similarly, the land-use change should be brought under strict control, including that related to imported animal feed. Furthermore, the livestock production should have a stronger link to the regional feed data base (Idel et al. 2013).

23.7 Climate Smart Livestock with A Farmer Centric Approach

There are opportunities to transform livestock sector in low and middle income countries through farmer centric approach to make the sector more profitable and sustainable both socially and ecologically. Creating a global livestock climate-smart livelihoods fund could be the way forward approach for creating employment opportunities and building resilient livelihoods of farming households. The crucial factor which could determine this is the global partnership involving the farmers on the ground to the global organisations looking to protect our environment for the next generation. This approach has to be amalgamated with public-private partnerships and this becomes increasingly important for testing innovations, speeding up change and taking success to scale. In this we can build a more sustainable global livestock sector.

23.8 Improved Balanced Animal Nutrition

Ration balancing is a very effective means to sustain livestock production in addition to achieving low emission. This involves (1) formulating balanced feed rations with high-quality feed ingredients; (2) improving feed conversion rates and forage digestibility, through effective silage and hay preservation; (3) better grassland management, e.g. by introducing legumes into grass pasture; (4) selecting better feed resources, including alternative feed products and feed additives; (5) supplementation with small amounts of concentrate feed and mineral/urea/molasses n; and (6) low emission feed. Reducing the amount of feed required per unit of output (e.g. beef, milk) has the potential to reduce greenhouse gas emissions and increase farm profits. Feed efficiency can be increased by developing breeds that grow faster, are hardier, gain weight more quickly, or produce more milk. Rotational grazing is another approach to effectively manage grasslands. This would stop land degradation and increase soil carbon sequestration. Further this will improve the quality of the diet as the animals will be consuming forages in a relatively earlier growth stage. Low emission feed resources is achieved by feeding by-products from agriculture and the agri-food industry or feed that has been produced by using conservation agriculture. Other low emission feed products include insect-based feeds, and specific feed additives (FAO 2021).

23.9 Genetic Selection and Breeding Management

Breeding management and genetic selection has the potential to form the formidable climate smart livestock production comprising both mitigation and adaptation strategies. The future breeding strategies must concentrate on marker assisted selection comprising traits pertaining to adaptation, production and low methane emission. Conservation of local animal genetic resources may play an important role in making available elite animals for the farmers. Involving local breeds in breeding program offers scope for maintaining traits related to thermo-tolerance, coping ability to poor nutrition and disease resistance (FAO 2021).

23.10 Low Carbon Livestock

Low-carbon livestock can help countries achieve a balance whereby animal-source foods feed the hungry and malnourished, yet are produced in a way that is minimizing the overall output of greenhouse gases. FAO (2019) suggests that this could be achieved by five ways: (1) Boosting efficiency of livestock production and resource use; (2) Intensifying recycling efforts and minimizing losses for a circular bioeconomy; (3) Capitalizing on nature-based solutions to ramp up carbon offsets; (4) Striving for healthy, sustainable diets and accounting for protein alternatives; (5) Developing policy measures to drive change.

23.11 Artificial Meat

Cultured meat (also known as artificial, *in vitro* or lab grown meat) promises to be the best alternative to satisfy the growing demand of highly increasing population. It might drastically reduce the need of rearing animals for meat production, though fewer animals have to be grown for the purpose of harvesting cells for production of *in vitro* meat. Muscle cells will be cultured in a culture medium consisting of nutrients, growth factors and hormones, such that the muscle cells proliferates and in turn produces lumpsum amount of meat from minimum number of cells (Baca-González et al. 2020). In spite of owning advantages from animal welfare and human health point of view by producing pathogen free meat, it bears its own disadvantages like usage of hormone and growth factors, palatability issues, etc. Being a new technology, it faces its own critical points such as struggle to fetch public acceptance, huge price, religious debate and so on. Henceforth, with all its pros and cons, still there is long way of research needed in multiple dimensions to overcome all the questions raised and to overcome all the drawbacks.

23.12 Vaccine Technology

Enteric methane production, being the burning topic of interest, its contribution towards GHG pool is quite alarming and the efforts are repeatedly posted towards reducing it. There are various methods suggested in pipeline to mitigate enteric methane emission, among which vaccination against methanogens attracted the attention of many scientists around the globe. Major advantage is that its ability to leave no traces of residues in the product and its potential of universal applicability to all ruminant farm animal species. In a nut shell, it is all about eliciting immune response in rumen against methanogens. Since, rumen lacks any lymphoid tissue, the idea is to use saliva as a immunoglobulin delivering vector into the rumen (Baca-González et al. 2020). This mitigation strategy is at its initial stage of research, with minimal literature available to compare and get a conclusive idea. There are lots of bottleneck hampering the evolution and utilization of this strategy in each step of its development like choice of antigen, selection of appropriate adjuvant, limited IgG transfer from blood to saliva, survival of immunoglobulins in the rumen, determination of booster frequency and finally an exact evaluation method to analyze the evoked immune response. In addition, scarce literature availability majorly holds back the technique from successful implementation in the field. Henceforth, there is vast area of science need to be studied before successful implementation of anti-methanogens vaccination in practice. Therefore, more intensified research efforts are needed to standardise the protocol for a possible universal vaccine against rumen methanogens and such a breakthrough in near future can help to reduce the livestock associated climate change.

23.13 Innovations in Methane Reduction: Methane-Oxidizing Nose Ring

There are few emerging innovative technologies to combat the effect of enteric methane emission (Norris 2018). This technology uses a device in the form of a simple nose ring, to reduce the methane exhaled by ruminants which was produced through the process of enteric methane fermentation. A smart cattle nose-ring is attached to the cattle's nose. Whenever the animal exhales, this device detects the methane in the exhaled air, which activates the micro-oxidation chamber in the device. This is turn leads to the conversion of methane into CO_2 and water vapour. This device is expected to convert upto 80% of exhaled methane. In addition, it also transmits data which shall be stored for the animal's lifetime. Carbon dioxide, being a green house gas with very low global warming potential (GWP) than methane, if studied in detail, this technology could emerge as a promising alternative to reduce the impact of enteric methane emission caused by animals.

23.14 Technologies to Assess Animal Welfare: Precision Livestock Farming

To meet out the blooming demand for meat and milk production, increasing livestock production becomes inevitable. This demand for increase in livestock production should not compromise animal welfare. Henceforth, the need for some innovative technologies which could help to ensure livestock production without compromising animal health and welfare rises. Precision livestock farming (PLF), is one of its kind designed to monitor and control animal productivity, along with animal health and welfare parameters in an automated manner (Schillings et al. 2021). Various technologies such as cameras, sensors or microphones are used to caution the farmers then and there, such that farmers will remain informed about any undesirable conditions of animals at the earliest. PLF technologies have highly encouraging potential to reduce the occurrence of disease and injuries. Any technology will have its own drawbacks. Likewise, PLF might reduce the human animal relationship by reducing the frequency of interaction between animals and farmers, which likely to reduce the opportunity of animals to become acclimatize with people. In addition, the extent to which the PLF technologies could help to improve the animal welfare seems to be limited. Literature and evidences are lacking to prove PLF technologies as welfare indicators. Having said that, integrating such PLF technologies with climate change prediction tools can also aid to assess and predict heat stress or environmental stress events in livestock thereby giving the livestock keepers sufficient time to adopt suitable ameliorative and mitigation strategies.

23.15 Nanotechnology in Animal Production

Nanotechnology, a very novel field of research, which has already been applied in certain areas of animal production, is a scientific approach to study the matter in its atomic and molecular scale, usually in nanometer (nanoparticles). These are very small entities which have large surface-to-volume ratio, which in turn provide them various unique properties. Nanoparticles helps in improving the absorbability, solubility, half-life and bioavailability of various products. Nanomaterials, Nanosensors and microfluidics are few nanotechnology devices which helps in improving animal health, reproduction, production, diagnosis and treatment of diseases. Nanotechnology has the promising potential to improve livestock production system. It is an eco-friendly and economical way to control pathogenic micro organisms (Fesseha et al. 2020). But the major challenge is all about the environmental contamination that might be caused by this very technology. The major risk being, if released into the environment, the threat that might be faced by workers and consumers, pose a serious concern from safety risk perspective. In addition, it is readily accepted that few nanoparticles can evoke toxic and dangerous side effects upon ingestion. Even though, it is one of the major novelties in the current era, it is still in its prime stage of development. There are much more hampering factors from animal and people welfare perspectives which need to be studied in depth. Wide hazard analysis and assessments need to be performed before implementing nanotechnology for livestock production.

23.16 Climate-Smart Animal Agriculture

The FAO has identified Climate Smart Agriculture (CSA) as one among the approaches to address the interlinked challenges of climate change and food security. The FAO has bridged the knowledge gap associated with the terminology of CSA by defining it as ‘the integrated approach to enhance the capacity of the agricultural systems to support food security, incorporating the need for adaptation and the potential for mitigation into sustainable agriculture development strategies. The CSA work with the aim of achieving increased productivity, enhanced resilience and reduced emission from agricultural sector. It involves various components like crops, livestock and trees, arranged in an effective way such that the available resources are effectively utilized and recycled for generating maximum productivity. Although substantial progress has been made in successfully implementing CSA, still such efforts should be more intensified to take up in mass scale throughout the world in the future. Such efforts require development of more innovative technologies to achieve the basic principle of CSA. Implementation of such technologies at farmers’ field requires innovative policies and therefore the policy makers must engage all stakeholders to develop a common policy compromising all aspects of environmental influence on livestock production. The government also must develop reliable financial mechanisms which could help to implement such innovative technologies by the ultimate targeted beneficiaries of farmers.

23.17 Exploiting the Genetic Potential of Local/Indigenous Breeds

It is an unequivocal fact that indigenous germplasm have a higher significance in the current changing climatic scenario. These indigenous livestock however lag behind the established exotic breeds for their lower production ability. Hence it is the need of the hour to conduct extensive studies to assess and improvise the productive performance of indigenous germplasm. Furthermore, with the concept of establishing agro-ecological zone specific livestock breeds (Sejian et al. 2021); it would also be advisable to conduct studies that assess the performance of livestock breeds in a given local environment. The advancements in the field of molecular biotechnology have made it possible to identify several biomarkers for climate resilience. Stimulation studies conducted in climate controlled chambers have made is easier to subject animals to varied environmental conditions across the global by programming the desired temperature and humidity variables. Such experiments thereby enable researcher to subject animals to a diverse combination of environmental stress and with the use of the advanced biotechnological tools can also identify potential markers for climate-resilience. Identification of such markers can pave way towards Marker Assisted Selection (MAS) or Genomic Selection (GS) which can ultimately aid towards breeding of agro-ecological zone specific germplasm.

23.18 Exploiting the Unconventional Feed Resources

In the current scenario, in order to meet out the imperative demand for alternative feed resources, utilization of non-conventional feed resources (NCFR), popularly known as 'new feeds', could be exploited for livestock production (Amata 2014). But this valuable source of feed supply is facing challenges in every step of its development like collection, storage and importantly during detoxification procedures. Major constraint faced by NCFR is its anti-nutritional factors, which can be tackled by utilizing the emerging processing methods which includes physical, chemical and biological processing. Basically, NCFR involves variety of feedstuff such as shrub fodder, tree fodder, agro-industrial by-products, animal by-products, single cell proteins and so on. Among them, agro-industrial by-products, browse foliage and crop residues are gaining importance in the farming community. Furthermore, considering the rapid depletion of fresh water resources and increase in the saline agriculture, use of haplotype plants as NCFR may satisfy major critical points such as food and water demand. Haplotype such as *Acacia* species, are highly saline tolerant and these plants should be fed with energy rich feed supplements like molasses, corn, etc., in order to overcome the effect of its anti-nutritional factors (Abd El-Hack et al. 2018). Practically, from the view of landless and marginal farmers, Cactus (thornless) and Azolla can also be considered as foremost NCFR (Keerthi et al. 2019). Azolla, being easily cultivable and economical, also it serves as the best protein substitute. Cactus is the best plantation in highly water deficit and drought prone areas and it shall be an ideal NCFR when mixed with other fibrous feedstuffs. Apart from the above stated feed/fodder sources, a variety of other NCFR have been fed to livestock like, sugarcane tops, *Moringa oleifera*, mango seed kernel, mango pulp, jackfruit waste, cassava, pineapple waste, tamarind seed hulls and so on (Singh 2018; Onte et al. 2019). NCFR can serve as best feed substitutes, when considerable attention is given along with the involvement of farmers and local extension agencies.

23.19 Identification of Agro-ecological Zone Specific Breeds

Indigenous animals developed the traits to survive in a specific location and they acquire such potential from generation after generation. However, even among indigenous breeds, differences were established in their responses to climatic stresses. Therefore, it is very essential to channelize new research initiatives to identify the most suited indigenous breeds to specific agro-ecological zones. Such efforts can help the farming community to reap maximum by using the most appropriate breeds for rearing. Efforts are also needed to test the potential of indigenous breeds to survive in different location and those breeds which exhibit the potential to survive and produce optimally in different agro-ecological zones must be identified and disseminated apart from using them in breeding programs. These efforts can secure the livelihoods of poor and marginal farmers by helping them to rear the most appropriate breed to generate income.

23.20 Water Resources Management

Water is another vital resource that needs great attention especially in the prevailing climate change scenario. Understanding the water footprint associated with the livestock sector is therefore imperative. Additionally, appropriate methodologies should also be adopted to conserve water resources and sustain fodder production. Broadly, the practical measures to conserve fodder production during water scarcity are categorized into land management, irrigation management, in-situ water harvesting and ex-situ rainwater harvesting. Among all these, the management of irrigation water is considered to be the most harmonizing methodology for water conservation. Land management measures primarily target the reduction of water erosion and loss of water from soil surface. Crop rotation is yet another effective and feasible practice that aids in the reduction of water shortage risk. In-situ land management, a self-explanatory terminology, comprises of strategies that involves conversion of landscapes into permanent and/or temporary structure that conserve water, like terraces, bunds to cover rainwater, thus reducing soil erosion. While on the other hand, ex-situ water conservation practices consists of construction of permanent structures; dams and artificial ponds; that store excess rainwater and also support irrigation during water scarcity. With the projected impact of climate change on the global perspective and also the predicted rise in the global temperature, it is therefore necessary to implement multiple water conservation strategies. Such strategies not only will address the water scarcity concern but can also enhance effective forage production which is also necessary to meet the rising human and livestock demand.

23.21 Bridging the Knowledge Gap Between Climate Change and Livestock Diseases

Interrelating climate change and sudden disease outbreaks is one of the most crucial aspect to reduce animal production. However, not much information on this line is currently available. More intensified research efforts are required to interlink climate change associated disease outbreak in livestock. Particularly the vector-borne diseases are very important as the weather variables directly influence the multiplication of these vectors which acts as the causative agent for the disease. To find solution to the problem such sudden disease outbreaks have to be linked with geographical information system to establish its connection with changing climatic conditions. This has to be followed with more field and laboratory oriented researches. Such attempts should be agro-ecological zone or country specific. Tackling climate change associated disease outbreak in livestock and developing solution to the problem through laboratory research may go in a long way to sustain livestock production. As it is one of the important impact which causes drastic reduction in animal production even among the indigenous breeds.

Learning Outcomes

- The latest technologies such as metagenomics, transcriptomics, bisulfite sequencing, genome-wide association studies, and selection signature may play a greater inroad in understanding the hidden intricacies of molecular mechanisms governing livestock adaptation.
- The future breeding strategies to evolve new climate-resilient breeds must concentrate on marker-assisted selection comprising traits pertaining to adaptation, production, and low methane emission.
- The developed adaptation strategies and designed technologies should be agro-ecological zone specific to cater the needs of the poor and marginal farmers to secure their livelihood.

23.22 Concluding Remarks

Investing in livestock sector in future becomes inevitable probably due to their better climate resilience than other sectors in agriculture. Thus, the livestock sector could play a vital role in ensuring food security to feed the growing human population by 2100. Therefore, strategies needs to be worked out by policy makers to identify most suitable strategies which are cost effective and may play a significant role to ensure sustainable livestock production in the future. All the identified strategies must be concentrated around ensuring optimum production in livestock in the changing climate scenario. A systematic road map needs to be developed keeping in view the anticipated increase in average earth temperature to realistically implement the suitable strategies for augmenting livestock production. A multidisciplinary approach should be in place to effectively utilize the strategies developed to achieve the goal towards sustainable livestock production. Further, the developed strategies and designed technologies should be agro-ecological zone specific to cater the need of the poor and marginal farmers of the location. From the available strategies, only those which are promising should be narrowed down for up scaling by the policy makers for propagating them under field condition. Such systematic approach could help the farming communities to sustain livestock production in the changing climate scenario.

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