The Microbiomes of Humans, Animals, Plants, and the Environment 4

Michael H. Kogut
Glenn Zhang *Editors*

Gut Microbiota, Immunity, and Health in Production Animals

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The Microbiomes of Humans, Animals, Plants, and the Environment

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Managing Intestinal Health in Farm Animals: A Critical View

1

Marcos H. Rostagno

Abstract

As an extremely complex and intricate system, the intestinal tract is still viewed by most as a "black box" with many basic and important gaps of knowledge. Nevertheless, its effective functionality is crucial in determining animal health, well-being, and productive performance. In the increasingly competitive animal production industry, the pressure for more efficient production systems is a constant, and the shift away from using certain technologies, such as antibiotics, has created a need for other options to support and manage intestinal health. In this review, we present a framework for the management of intestinal health applied to farm animals. A multitude of intestinal health management tools is increasingly available, primarily used as feed and water additives. However, a more comprehensive approach is needed, beyond just the use of additives. It is important to further develop and apply nutritional strategies, such as the strategic use of feed ingredients, pay more attention to water availability and quality. Additionally, stress management should be a component of any intestinal health management program. Managing intestinal health in farm animals is a complex challenge, and as such, it requires a broader, holistic approach.

Keywords

Intestinal tract · Intestinal health · Farm animals · Livestock

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1.1 What Is the Intestinal Tract?

In a very simplistic view, the intestinal tract can be defined as a component of the digestive system, structured and intricately regulated to extract and transport nutrients to the circulatory system for distribution to all tissues, and to excrete waste products from the animal's body. Moreover, as it represents the largest mucosal surface continuously exposed to potential aggressors, the intestinal tract is also responsible for protecting the animal, as a complex barrier and home of most of the immune cells.

Accomplishing these vital functions requires the intestinal tract to actively and bidirectionally interact with many other systems in the animal's body, such as the neuroendocrine and immune systems. Unlike other organs, the intestinal tract has a dedicated nervous system, consisting of a network of neurons called the enteric nervous system (ENS). The regulation and coordination of muscular and secretory activity by the ENS are required for effective digestion of feed and absorption of nutrients (Furness 2012; Rao and Gershon 2016). The intestinal tract and the brain are connected through a complex network of signaling pathways collectively termed as the gut–brain axis (GBA) mainly driven by neural, endocrine, immune, and metabolic mediators (Carabotti et al. 2015; Jena et al. 2020). As if this intricate communication network was not complicated enough, the intestinal tract is home for an incredibly large and diverse microbial ecosystem, which includes commensals, symbionts, and pathogens involved in many functional aspects that benefit the animal host (Hollister et al. 2014; Guven-Maiorov et al. 2017; Kho and Lal 2018). In fact, it is viewed by many as another functional body organ, which interacts with the host to promote health and, in some instances, initiate disease (Baquero and Nombela 2012; Kho and Lal 2018). Over the last decade, the role of this microbial ecosystem in the GBA has been intensively investigated, and the term has been extended to gut–brain–microbiota axis, reflecting its importance. This complex neuro-endocrine-immune-microbial axis has been studied using top-down (brain–gut–microbiota) and bottom-up (microbiota–gut–brain) approaches, and whereas the modulation of the intestinal functions by the brain (i.e., top-down) is well established, the modulation of brain functions by the intestinal tract and its microbiota-derived molecules (i.e., bottom-up) is still vastly unknown, but rapidly evolving, making it very difficult to fully unravel this multifaceted interrelationship (recently reviewed by Liu and Zhu 2018; Martin et al. 2018; Cryan et al. 2019; Jena et al. 2020; Megur et al. 2021; Tait and Sayuk 2021).

1.2 Why Do We Need to Manage Intestinal Health?

As an extremely complex and intricate system, the intestinal tract is still viewed by most as a "black box" with many basic and important gaps of knowledge, and therefore, attracting a lot of interest over the past years, quickly becoming a dominant topic in the global animal agriculture, as its effective functionality is crucial in determining animal health, well-being, and productive performance. In

the increasingly competitive animal production industry, the pressure for more efficient production systems is a constant. Considering that feed is by far the main cost of production for any animal production system, it is easy to understand why the health of the intestinal tract becomes a determinant factor for the efficient utilization of the nutrients provided in the diets to the animals. Moreover, the increasing market pressure for competitiveness and quick shift to producing food without the use of antibiotics has created the need for other options to support the intestinal health of the animals. For many years, the ample utilization of antibiotics as common interventions allowed to keep many intestinal challenges under control, which re-emerged during the process of withdrawing this type of additives from the diets. Consequently, an incredibly vast number of new feed additives becomes continuously available in the market with the purpose of managing intestinal health challenges and maintaining production efficiency.

In recent times, the term "intestinal health" (or "gut health") has become increasingly popular, and a very common topic in commercial and scientific events, as well as in the scientific literature, although it is still not very clear what exactly it means and how it can be monitored or measured. Nevertheless, due to the fundamental importance of maximizing the functionality of the intestinal tract to successful farm animal production, there is a lot of interest in manipulating it through a variety of tools and approaches. Therefore, in this review, we attempt to organize and present a critical scientific view of basic concepts and a framework for the management of intestinal tract health applied to farm animals.

1.3 What Does "Intestinal Health" Mean?

It is very challenging to fully understand the multifaceted interrelationship between the intestinal tract and several other systems in the animal's body. Furthermore, it is also very challenging to fully understand the complex neuro-endocrine-immune-microbial interrelationship within the intestinal tract. Consequently, defining or developing a concept of "intestinal health" has been a challenge amongst nutritionists, veterinarians, and scientists, worldwide. Some key components of what could be considered a healthy intestinal tract are:

- Structural or morphological integrity
- Normal neuroendocrine and motor functioning
- Effective digestion of feed and absorption of nutrients
- Effective immune function
- Stable and functional microbiota.

As it can be easily noted, the need to consider all these key components creates a complex obstacle to any attempt of clearly and objectively develop a definition of "intestinal health." Moreover, all these key components interact among themselves by several complex mechanisms and pathways, directly affecting each other. An additional factor to consider in this complex scenario is the variety of external factors commonly present in any animal production system that can affect the intestinal tract

and its components, such as diet (composition, texture/form, quality of ingredients used, and feed management), pathogens, and use of additives (antimicrobials or non-antimicrobials), as well as the occurrence of stress (physical, psychological, or environmental).

In humans, the absence of illness and well-being status are key determinant factors in defining intestinal health (Bischoff 2011). However, it is our view that any intestinal illness would affect one or more of the key components listed above, whereas well-being could only be reached if all five components are not affected, and therefore, are in a state of equilibrium. Moreover, in animal production, interest in intestinal health is not exclusively determined by the need of preventing or controlling diseases, but also by the need of maximizing productive performance by allowing animals to express their genetic potential, under a variety of different conditions. Therefore, while in human medicine intestinal health is often associated with the "absence of clinical disease," this definition cannot be applied to farm animals as it is well known that animal performance can be impaired without any clinical signs of disease. Additionally, differently than humans, any aspect related to health in farm animals must be considered under a populational perspective, as animals are usually kept together in large groups (i.e., herds or flocks), with a broad inter-individual variation.

Kogut and Arsenault (2016) defined intestinal health as "the absence/prevention/avoidance of disease so that the animal is able to perform its physiological functions in order to withstand exogenous and endogenous stressors," whereas Celi et al. (2017) proposed an expanded definition of intestinal health as "a steady state where the microbiome and the intestinal tract exist in symbiotic equilibrium and where the welfare and performance of the animal is not constrained by intestinal dysfunction." These definitions, although also based on the initial five key components listed above, still focus on intestinal disease or dysfunction. Here, we propose to focus on the concept of intestinal health as a homeostasis state to use as a framework or central organizing principle to integrate the intricate relationship between all the complex components and functions of the intestinal tract. This proposed framework aligns with Pluske et al. (2018) stating that "gut health is more general and can be described as a generalized condition of homeostasis in the gastrointestinal tract, with respect to its overall structure and function." According to Billman (2020), homeostasis is a self-regulating process by which biological systems maintain certain stability while responding or adjusting to different conditions. If the concept of a self-regulating system is not understood, then it is not possible to fully comprehend the function of the intestinal tract in health and disease. The disruption of homeostasis is what leads to dysfunction or disease, and therefore, effective interventions must be directed toward supporting/maintaining or reestablishing homeostatic conditions. However, homeostasis is not static, but rather a dynamic self-adjusting process driven by both, feedback and feedforward mechanisms. Homeostasis is the result of the complex interaction and balance between multiple negative and positive feeding systems and provides the basis for regulation, such as in the intestinal tract. Therefore, intestinal health in farm animals can be defined within the homeostasis framework as: The state of resilient equilibrium or homeostasis of the intricate

intestinal neuro-endocrine-immune-microbial systems that allows full functionality of the intestinal tract, overcoming challenges to guarantee animal health, well-being, and productive performance. This concept has important implications regarding how intestinal health intervention strategies are developed and applied in farm animals, as part of a more holistic approach to managing intestinal health.

1.4 Managing Intestinal Health

Attempts to manage intestinal health in farm animals should not be random, but always based on setting a strategy and coordinating interventions to achieve a clear outcome or purpose. If not done properly, there is always a risk of unintended consequences, such as disrupting the homeostasis with potentially detrimental outcomes. In general, the essential goals of managing intestinal health in farm animals consist of prevention or treatment/control. In the first case, the goal is to avoid homeostasis disruption by different types of challenges (infectious or noninfectious) and its consequent negative effects on the performance, health, and welfare of the animals, whereas in the latter, the goal is to reverse a disrupted homeostasis state caused by a challenge, reestablishing intestinal health.

A multitude of intestinal health management tools is increasingly available, primarily used as feed and water additives, including antibiotics, chemicals, probiotics, prebiotics, symbiotics, organic acids, yeast-based products, phytogenics, enzymes, animal plasma, and others, just to list a few. Additionally, micronutrients, such as minerals, vitamins, and amino acids used as supplements (i.e., above basic nutritional requirements) are extensively used to manage intestinal health in farm animals. However, our purpose is not to review, compare or evaluate the efficacy and make recommendations about these intestinal health management tools. Many reviews on these feed additives have been written and are available for readers to consult (Kiarie et al. 2013, 2016, 2019; Pluske 2013; Ruth and Field 2013; Murugesan et al. 2015b; Roto et al. 2015; Zeng et al. 2015; Adhikari and Kim 2017; Gadde et al. 2017; Valenzuela-Grijalva et al. 2017; Bedford and Gong 2018; Liu et al. 2018; Suresh et al. 2018; Adedokun and Olojede 2019; Campbell et al. 2019; Ji et al. 2019; Mou et al. 2019; Bortoluzzi et al. 2020; El-Hack et al. 2020; Ferronato and Prandini 2020; Jackman et al. 2020; Jha et al. 2020; Pearlin et al. 2020; Reddy et al. 2020; Alagawany et al. 2021; Ding et al. 2021; Lauridsen et al. 2021). It is our view that it would be impossible to properly review and assess all the available feed and water additives in a single publication, particularly due to two key factors vastly ignored in the literature, but worth highlighting here:

1. Comparing additives with different modes of action is not straightforward, or even appropriate in many cases. A comparative evaluation of different technologies requires clear rationale, adequate study design, and reasonable expectations. Unfortunately, in most cases, comparisons are done solely based on desired outcomes, without clearly understanding on how they had been achieved or how unknown biases may have affected them. Evaluations of

additives usually target outcomes, such as productive performance parameters, which are influenced by many variables and potential confounders that cannot be standardized across the multitude of studies conducted in different locations and under different conditions.

2. Publication bias is a reality and studies with a successfully proven hypothesis or positive results are often given more importance, with the publication of studies with negative or null results on the verge of extinction. Under-reporting of negative results introduces bias in reviews and meta-analyses leading to distortion of the scientific literature, misleading assessments, conclusions, and ultimately, decision-making. In other words, based on the available published evidence, most additives are effective in managing intestinal health in almost all possible conditions, which is not true, realistic, or even possible.

Nevertheless, feed and water additives are extremely valuable tools for any intestinal health management program in farm animals. However, selecting the additive(s) to use should not be based on a trial-and-error approach or at random as explained above, but instead, it should be based on a well-designed decision-making process, and of course, on solid scientific rationale, such as understanding the challenge, as well as the technology of the intervention to be applied. For instance, results obtained under controlled conditions (e.g., based on academic experiments or studies) are important and do have to be considered as a starting point. However, they must be properly scrutinized and serve as a base to make the decision to take them one step further, closer to the actual conditions of use of the additive(s) on-farm, where challenges to the intestinal health status of animals are very likely to be different.

Although tremendous attention has been dedicated to the use of additives to manage intestinal health in farm animals, other more basic approaches, such as nutritional strategies still have a lot of opportunities to grow. Diet formulation and feeding programs can and should be further explored as opportunities beyond just simply supplying nutrients, but also to manage intestinal health in farm animals. Nutrition and health are interdependent, and therefore, the strategic use of feed and its ingredients to support intestinal health can bring tremendous value to the production of farm animals. The intestinal tract is heavily affected (positively and negatively) by diet composition, feed form, frequency, and amount of consumption, as well as the quality of ingredients. Dietary nutrients are essential for intestinal growth, development, and function, throughout the entire life of the animals (Choct 2009; De Lange et al. 2010; Celi et al. 2017; Adebowale et al. 2019; Adedokun and Olojede 2019; Huting et al. 2021). However, these same nutrients also constitute one of the most important factors affecting intestinal microbiota establishment and composition, including richness, diversity, stability, and functionality. It is important to understand that ingredients used to feed the animals also feed their microbiota, by serving as a substrate that can support or disrupt the intestinal microbial ecosystem. For instance, it is well-known that alteration of intestinal microbiota, through dietary influences (e.g., abrupt compositional changes, withdrawal, etc.), can lead to a state of dysbiosis/dysbacteriosis, which is characterized by disruption of the commensal

microbial ecosystem and subsequent risk for overgrowth of potential pathogens (Brown et al. 2012; Oriach et al. 2016; Klingbeil and de La Serre 2018; Meng et al. 2019). Excess of certain nutrients in the intestinal tract, such as fat, carbohydrates, protein, and fiber, can also disturb the intestinal tract, causing oxidative stress and inflammation, compromising the productive performance, health, and well-being of farm animals (Celi et al. 2017; Klingbeil and de La Serre 2018; Kogut et al. 2018; Jha et al. 2019; Dal Pont et al. 2020). Another very important point to keep in mind when considering how the diet itself can be a useful approach for the nutritional management of intestinal health is that often times, poor-quality feed ingredients are used (e.g., major grains, such as corn, soybean, and wheat) and will negatively impact the health of the intestinal tract of farm animals. For example, it has been extensively shown that the presence of anti-nutritional factors (or anti-nutrients) and mycotoxins have a significant detrimental effect on intestinal health, not only compromising its functionality through oxidative stress and inflammation but also promoting the occurrence of enteric pathogens and diseases (Grenier and Applegate 2013; Murugesan et al. 2015a; Celi et al. 2017; Liew and Mohd-Redzwan 2018; Pan et al. 2018). Diet is an essential regulator/modulator of the overall intestinal tract functionality, and the crosstalk between dietary factors, the immune system, and microbiota is crucial for the maintenance of the intestinal tract homeostasis, and therefore, health (Jansman 2016; Kogut 2017; Leeming et al. 2019; Oviedo-Rondon 2019; Farre et al. 2020; Frame et al. 2020; Huting et al. 2021). Although widely ignored, water is a critical nutrient to all animals, directly affecting the digestive process, availability of certain nutrients, and overall intestinal health. Moreover, water can be a source of toxins and pathogens that can cause severe disruptions to the different systems components of the intestinal tract (Jequier and Constant 2010; Giri et al. 2020; Oviedo-Rondon 2019). Continuous monitoring of water availability and quality should be an integral component of an intestinal health management program.

Often overlooked is the impact of stress on the intestinal health of farm animals. Stress is a biological adaptive response and is a common occurrence in any farm animal. Stress can be caused by a variety of factors and conditions, from simple husbandry or management practices (e.g., crowding, handling, transport, weaning, etc.) to environmental conditions (e.g., cold or heat stress). The intestinal tract is very sensitive and responsive to any type of stress, with a variety of changes resulting, including physiological and immunological responses, as well as impairment of the intestinal integrity and inflammation, and marked alterations in the microbiota (Lee et al. 2016; Moeser et al. 2017; Xiong et al. 2019; Dahl et al. 2020; Osorio 2020; Rostagno 2020; Chauhan et al. 2021). Therefore, stress management (i.e., animal well-being) should also be considered as an important component of intestinal health management programs in farm animals.

With this brief discussion/review, we propose a change or shift in the current way how intestinal health is seen and managed in farm animals, from focusing almost exclusively on diseases and productive performance outcomes to understanding the basics and adopting a more holistic, preventive approach. Managing intestinal health should be part of an overall animal health program, and requires coordination

between nutritionists and veterinarians, as clear planning and actions are essential, and should encompass a multipronged approach, as described here, including, but not limited to: Appropriate use of feed and/or water additives and supplements; strategic nutritional management (from feed ingredients and quality to feeding program, without forgetting about water availability and quality); and stress management (within a good animal welfare program).

It is evident that managing intestinal health is not a simple challenge, with no simple solution or "*magic bullet*" available. Therefore, realistic expectations and multipronged approaches are required. To successfully manage intestinal health, it is critical that we start thinking differently, and more broadly, from better understanding and applying combined health and nutrition concepts and strategies to dedicating more attention to animal management and welfare.

1.5 Final Considerations and Perspectives

Farm animal production in itself is complex and includes an incredible number of variables. On top of that, every and each aspect of intestinal health poses a complex challenge and requires a multifaceted perspective. Further complicating attempts to effectively manage intestinal health is the absence of clearly defined and consistent evaluation or measurement tools. Availability of reliable tools for the assessment of intestinal health status is critical to determine the need for interventions, as well as to measure their effects and impacts. This ability to measure or assess the intestinal health status is crucial, not only at the individual animal level but also at the populational level (i.e., herd, flock, etc.). Great variation exists in what is considered "normal" or "adequate" intestinal tract function and health, particularly in large populations. Whilst it is possible to measure many aspects of the intestinal neuro-endocrine-immune-microbial systems, it is very difficult to interpret the outcomes at the populational level, under a variety of different scenarios or conditions, as wide individual variation is observed within what is considered to be a "normal range" for the different parameters used.

Integration of multidisciplinary knowledge is always a challenge, and it is no different on intestinal health. However, it is urgently needed. Reductionist or fragmented approaches and knowledge (i.e., describing and analyzing complex systems in terms of their individual or fundamental components) has been mostly used to build the foundation of what we know about intestinal health. Granted, it was necessary for several reasons. However, we are approaching a time when greater emphasis must be placed on integrated and more holistic approaches. Unfortunately, the complexity of the intestinal tract, its functioning and health, cannot be fully understood by the view that the whole is merely the sum of its parts. Our knowledge must progress toward a more holistic and integrative stage.

Over the last few years, the application of high-throughput meta-omics methods has provided great progress in improving the knowledge of the intestinal ecosystem and linking its biodiversity to host health conditions, offering complementary support to classical microbiology.

Unfortunately, we do not have all the answers, at the moment. But, we truly believe that good science, and consequent progress, come from the continuous exercise of seeking answers to the multitude of questions that emerge along the way.

References

Adebowale TO, Yao K, Oso AO (2019) Major cereal carbohydrates in relation to intestinal health of monogastric animals: a review. Anim Nutr 5:331–339. https://doi.org/10.1016/j.aninu.2019.001

Adedokun SA, Olojede OC (2019) Optimizing gastrointestinal integrity in poultry: the role of nutrients and feed additives. Front Vet Sci 5:348. https://doi.org/10.3389/fvets.2018.00348

Adhikari PA, Kim WK (2017) Overview of prebiotics and probiotics: focus on performance, gut health and immunity – a review. Ann Anim Sci 17(4):949–966. https://doi.org/10.1515/aoas-2016-0092

Alagawany M, Elnesr SS, Farag MR, Tiwari R, Yatoo MI, Karthik K, Michalak I, Dhama K (2021) Nutritional significance of amino acids, vitamins and minerals as nutraceuticals in poultry production and health – a comprehensive review. Vet Q 41(1):1–29. https://doi.org/10.1080/01652176.2020.1857887

Baquero F, Nombela C (2012) The microbiome as a human organ. Clin Microbiol Infect 18(Suppl. 4):2–4. https://doi.org/10.1111/j.1469-0691.2012.03916.x

Bedford A, Gong J (2018) Implications of butyrate and its derivatives for gut health and animal production. Anim Nutr 4:151–159. https://doi.org/10.1016/j.aninu.2017.08.010

Billman GE (2020) Homeostasis: the underappreciated and far too often ignored central organizing principle of physiology. Front Physiol 11:200. https://doi.org/10.3389/fphys.2020.00200

Bischoff SC (2011) Gut health: a new objective in medicine? BMC Med 9:24. http://www.biomedcentral.com/1741-7015/9/24

Bortoluzzi C, Vieira BS, Applegate TJ (2020) Influence of dietary zinc, copper, and manganese on the intestinal health of broilers under Eimeria challenge. Front Vet Sci 7:13. https://doi.org/10.3389/fvets.2020.00013

Brown K, DeCoffe D, Molcan E, Gibson DL (2012) Diet-induced dysbiosis of the intestinal microbiota and the effects on immunity and disease. Nutrients 4:1095–1119. https://doi.org/10.3390/nu4081095

Campbell JM, Crenshaw JD, Gonzales-Esquerra R, Polo J (2019) Impact of spray-dried plasma on intestinal health and broiler performance. Microorganims 7:219. https://doi.org/10.3390/microorganisms7080219

Carabotti M, Scirocco A, Maselli MA, Severi C (2015) The gut-brain axis: interactions between enteric microbiota, central and enteric nervous systems. Ann Gastroenterol 28:203–209

Celi P, Cowieson AJ, Fru-Nji F, Steinert RE, Kluenter AM, Verlhac V (2017) Gastrointestinal functionality in animal nutrition and health: new opportunities for sustainable animal production. Anim Feed Sci Technol 234:88–100. https://doi.org/10.1016/j.anifeedsci.2017.09.012

Chauhan SS, Rashamol VP, Bagath M, Sejian V, Dunshea FR (2021) Impacts of heat stress on immune responses and oxidative stress in farm animals and nutritional strategies for amelioration. Int J Biometeorol. https://doi.org/10.1007/s00484-021-02083-3

Choct M (2009) Managing gut health through nutrition. Br Poult Sci 50(1):9–15. https://doi.org/10.1080/00071660802538632

Cryan JF, O'Riordan KJ, Cowan CSM, Sandhu KV, Bastiaanssen TFS, Boehme M, Codagnone MG, Cussotto S, Fulling C, Golubeva AV, Guzzetta KE, Jaggar M, Long-Smith CM, Lyte JM, Martin JA, Molinero-Perez A, Moloney G, Morelli E, Morillas E, O'Connor R, Cruz-Pereira JS, Peterson VL, Rea K, Ritz NL, Sherwin E, Spichak S, Teichman EM, van de Wouw M, Ventura-Silva AP, Wallace-Fitzsimons SE, Hyland N, Clarke G, Dinan TG (2019) The microbiota-gut-brain axis. Physiol Rev 99:1877–2013. https://doi.org/10.1152/physrev.00018.2018

Dahl GE, Tao S, Laporta J (2020) Heat stress impacts immune status in cows across the life cycle. Front Vet Sci 7:116. https://doi.org/10.3389/fvets.2020.00116

Dal Pont GC, Farnell M, Farnell Y, Kogut MH (2020) Dietary factors as triggers of low-grade chronic intestinal inflammation in poultry. Microorganisms 8:139. https://doi.org/10.3390/microorganisms8010139

De Lange CFM, Pluske J, Gong J, Nyachoti CM (2010) Strategic use of feed ingredients and feed additives to stimulate gut health and development in young pigs. Livestock Sci 134:124–134. https://doi.org/10.1016/j.livsci.2010.06.117

Ding S, Yan W, Ma Y, Fang J (2021) The impact of probiotics on gut health via alteration of immune status of monogastric animals. Anim Nutr 7:24–30. https://doi.org/10.1016/j.aninu.2020.11.004

El-Hack MEA, El-Saadony MT, Shafi ME, Qattan SYA, Batiha GE, Khafaga AF, Abdel-Moneim AME, Alagawany M (2020) Probiotics in poultry feed: a comprehensive review. J Anim Physiol Anim Nutr 00:1–16. https://doi.org/10.1111/jpn.13454

Farre R, Fiorani M, Rahiman SA, Matteoli G (2020) Intestinal permeability, inflammation and the role of nutrients. Nutrients 12:1185. https://doi.org/10.3390/nu12041185

Ferronato G, Prandini A (2020) Dietary supplementation of inorganic, organic, and fatty acids in pig: a review. Animals 10:1740. https://doi.org/10.3390/ani10101740

Frame LA, Costa E, Jackson SA (2020) Current explorations of nutrition and the gut microbiome: a comprehensive evaluation of the review literature. Nutr Rev 78(10):798–812. https://doi.org/10.1093/nutrit/nuz106

Furness JB (2012) The enteric nervous system and neurogastroenterology. Nat Rev Gastroenterol Hepatol 9:286–294. https://doi.org/10.1038/nrgastro.2012.32

Gadde U, Kim WH, Oh ST, Lillehoj HS (2017) Alternatives to antibiotics for maximizing growth performance and feed efficiency in poultry: a review. Anim Health Res Rev 18(1):26–45. https://doi.org/10.1017/S1466252316000207

Giri A, Bharti VK, Kalia S, Arora A, Balaje SS, Chaurasia OP (2020) A review on water quality and dairy cattle health: a special emphasis on high-altitude region. Appl Water Sci 10:79. https://doi.org/10.1007/s13201-020-1160-0

Grenier B, Applegate TJ (2013) Modulation of intestinal functions following mycotoxin ingestion: meta-analysis of published experiments in animals. Toxins 5:396–430. https://doi.org/10.3390/toxins5020396

Guven-Maiorov E, Tsai CJ, Nussinov R (2017) Structural host-microbiota interaction networks. PLoS Comput Biol 13(10):e1005579. https://doi.org/10.1371/journal.pcbi.1005579

Hollister EB, Gao C, Versalovic J (2014) Compositional and functional features of the gastrointestinal microbiome and their effects on human health. Gastroenterol 146(6):1449–1458. https://doi.org/10.1053/j.gastro.2014.01.052

Huting AMS, Middelkoop A, Guan X, Molist F (2021) Using nutritional strategies to shape the gastro-intestinal tracts of suckling and weaned piglets. Animals 11:402. https://doi.org/10.3390/ani11020402

Jackman JA, Boyd RD, Elrod CC (2020) Medium-chain fatty acids and monoglycerides as feed additives for pig production: towards gut health improvement and feed pathogen mitigation. J Anim Sci Biotechnol 11:44. https://doi.org/10.1186/s40104-020-00446-1

Jansman AJM (2016) Health and functions of the gastrointestinal tract in pigs: effects of functional ingredients and feed ingredient processing. J Anim Sci 94(Suppl 3):12–21. https://doi.org/10.2527/jas.2015-9886

Jena A, Montoya CA, Mullaney JA, Dilger RN, Young W, McNabb WC, Roy NC (2020) Gut-brain axis in the early postnatal years of life: a developmental perspective. Front Integr Neurosci 14:44. https://doi.org/10.3389/fnint.2020.00044

Jequier E, Constant F (2010) Water as an essential nutrient: the physiological basis of hydration. Eur J Clin Nutr 64:115–123. https://doi.org/10.1038/ejcn.2009.111

Jha R, Fouhse JM, Tiwari UP, Li L, Willing BP (2019) Dietary fiber and intestinal health of monogastric animals. Front Vet Sci 6:48. https://doi.org/10.3389/fvets.2019.00048

Jha R, Das R, Oak S, Mishra P (2020) Probiotics (direct-fed microbials) in poultry nutrition and their effects on nutrient utilization, growth and laying performance, and gut health: a systematic review. Animals 10:1863. https://doi.org/10.3390/ani10101863

Ji FJ, Wang LX, Yang HS, Hu A, Yin YL (2019) Review: the roles and functions of glutamine on intestinal health and performance of weaning pigs. Animal 13(11):2727–2735. https://doi.org/10.1017/S1751731119001800

Kho ZY, Lal SK (2018) The human gut microbiome – a potential controller of wellness and disease. Front Microbiol 9:1835. https://doi.org/10.3389/fmicb.2018.01835

Kiarie E, Romero LF, Nyachoti CM (2013) The role of added feed enzymes in promoting gut health in swine and poultry. Nutr Res Rev 26:71–88. https://doi.org/10.1017/S0954422413000048

Kiarie E, Walsh MC, Nyachoti CM (2016) Performance, digestive function, and mucosal responses to selected feed additives for pigs. J Anim Sci 94:169–180. https://doi.org/10.2527/jas2015-9835

Kiarie EG, Leung H, Kakhki RAM, Patterson R, Barta JR (2019) Utility of feed enzymes and yeast derivatives in amerliorating deleterious effects of coccidiosis on intestinal health and function in broiler chickens. Front Vet Sci 6:473. https://doi.org/10.3389/fvets.2019.00473

Klingbeil E, de La Serre CB (2018) Microbiota modulation by eating patterns and diet composition: impact on food intake. Am J Physiol Regul Integr Comp Physiol 315:R1254–R1260. https://doi.org/10.1152/ajpregu.00037.2018

Kogut MH (2017) Issues and consequences of using nutrition to modulate the avian immune response. J Appl Poult Res 26:605–612. https://doi.org/10.3382/japr/pfx028

Kogut MH, Arsenault RJ (2016) Gut health: the new paradigm in food animal production. Front Vet Sci 3:71. https://doi.org/10.3389/fvets.2016.00071

Kogut MH, Genovese KJ, Swaggerty CL, He H, Broom L (2018) Inflammatory phenotypes in the intestine of poultry: not all inflammation is created equal. Poult Sci 97:2339–2346. https://doi.org/10.3382/ps/pey087

Lauridsen C, Matte JJ, Lessard M, Celi P, Litta G (2021) Role of vitamins for gastro-intestinal functionality and health in pigs. Anim Feed Sci Technol 273:114823. https://doi.org/10.1016/j.anifeedsci.2021.114823

Lee IK, Kye YC, Kim G, Kim HW, Gu MJ, Umboh J, Maaruf K, Kim SW, Yun CH (2016) Stress, nutrition, and intestinal immune responses in pigs – a review. Asian Australas. J Anim Sci 29(8):1075–1082. https://doi.org/10.5713/ajas.16.0118

Leeming ER, Johnson AJ, Spector TD, Le Roy CI (2019) Effect of diet on the gut microbiota: rethinking intervention duration. Nutrients 11:2862. https://doi.org/10.3390/nu11122862

Liew WPP, Mohd-Redzwan S (2018) Mycotoxin: its impact on gut health and microbiota. Front Cell Infect Microbiol 8:60. https://doi.org/10.3389/fcimb.2018.00060

Liu L, Zhu G (2018) Gut-brain axis and mood disorder. Front Psychiatry 9:223. https://doi.org/10.3389/fpsyt.2018.00223

Liu Y, Espinosa CD, Abelilla JJ, Casas GA, Lagos LV, Lee SA, Kwon WB, Mathai JK, Navarro DMDL, Jaworski NW, Stein HH (2018) Non-antibiotic feed additives in diets for pigs: a review. Anim Nutr 4:113–125. https://doi.org/10.1016/j.aninu.2018.01.007

Martin CR, Osadchiy V, Kalani A, Mayer EA (2018) The gut-brain-microbiome axis. Cell Mol Gastroenterol Hepatol 6:133–148. https://doi.org/10.1016/j.jcmgh.2018.04.003

Megur A, Baltriukiene D, Bukelskiene V, Burokas A (2021) The microbiota-gut-brain axis and Alzheimer's disease: neuroinflammation is to blame? Nutrients 13:37. https://doi.org/10.3390/nu13010037

Meng X, Zhang G, Cao H, Yu D, Fang X, de Vos WM, Wu H (2019) Gut dysbacteriosis and intestinal disease: mechanism and treatment. J Appl Microbiol 129:787–805. https://doi.org/10.1111/jam.14661

Moeser AJ, Pohl CS, Rajput M (2017) Weaning stress and gastrointestinal barrier development: implications for lifelong gut health in pigs. Anim Nutr 3:313–321. https://doi.org/10.1016/j.aninu.2017.06.003

Mou Q, Yang HS, Yin YL, Huang PF (2019) Amino acids influencing intestinal development and health of the piglets. Animals 9:302. https://doi.org/10.3390/ani9060302

Murugesan GR, Ledoux DR, Naehrer K, Berthiller F, Applegate TJ, Grenier B, Phillips TD, Schatzmayr G (2015a) Prevalence and effects of mycotoxins on poultry health and performance, and recent development in mycotoxin counteracting strategies. Poult Sci 94:1298–1315. https://doi.org/10.3382/ps/pev075

Murugesan GR, Syed B, Haldar S, Pender C (2015b) Phytogenic feed additives as an alternative to antibiotic growth promoters in broiler chickens. Front Vet Sci 2:21. https://doi.org/10.3389/fvets.2015.00021

Oriach CS, Robertson RC, Stanton C, Cryan JF, Dinan TG (2016) Food for thought: the role of nutrition in the microbiota-gut-brain axis. Clin Nutr Exp 6:25–38. https://doi.org/10.1016/j.yclnex.2016.01.003

Osorio JS (2020) Gut health, stress, and immunity in neonatal dairy calves: the host side of host-pathogen interactions. J Anim Sci Biotechnol 11:105. https://doi.org/10.1186/s0104-020-00509-3

Oviedo-Rondon EO (2019) Holistic view of intestinal health in poultry. Anim Feed Sci Technol 250:1–8. https://doi.org/10.1016/j.anifeedsci.2019.01.009

Pan L, Farouk MH, Qin G, Zhao Y, Bao N (2018) The influences of soybean agglutinin and functional oligosaccharides on the intestinal tract of monogastric animals. Int J Mol Sci 19:554. https://doi.org/10.3390/ijms19020554

Pearlin BV, Muthuvel S, Govidasamy P, Villavan M, Alagawany M, Farag MR, Dhama K, Gopi M (2020) Role of acidifiers in livestock nutrition and health: a review. J Anim Physiol Anim Nutr 104:558–569. https://doi.org/10.1111/jpn.13282

Pluske JR (2013) Feed- and feed additives-related aspects of gut health and development in weanling pigs. J Anim Sci Biotechnol 4:1. https://doi.org/10.1186/2049-1891-4-1

Pluske JR, Turpin DL, Kim JC (2018) Gastrointestinal tract (gut) health in the young pig. Anim Nutr 4:187–196. https://doi.org/10.1016/j.aninu.2017.12.004

Rao M, Gershon MD (2016) The bowel and beyond: the enteric nervous system in neurological disorders. Nat Rev Gastroenterol Hepatol 13(9):517–528. https://doi.org/10.1038/nrgastro.2016.107

Reddy PRK, Elghandour MMMY, Salem AZM, Yasaswini D, Reddy PPR, Reddy AN, Hyder I (2020) Plant secondary metabolites as feed additives in calves for antimicrobial stewardship. Anim Feed Sci Technol 264:114469. https://doi.org/10.1016/j.anifeedsci.2020.114469

Rostagno MH (2020) Effects of heat stress on the gut health of poultry. J Anim Sci 98(4). https://doi.org/10.1093/jas/skaa090

Roto SM, Rubinelli PM, Ricke SC (2015) An introduction to the avian gut microbiota and the effects of yeast-based prebiotic-type compounds as potential feed additives. Front Vet Sci 2:28. https://doi.org/10.3389/fvets.2015.00028

Ruth MR, Field CJ (2013) The immune modifying effects of amino acids on gut-associated lymphoid tissue. J Anim Sci Biotechnol 4:27. https://doi.org/10.1186/2049-1891-4-27

Suresh G, Das RK, Brar SK, Rouissi T, Ramirez AA, Chorfi Y, Godbout S (2018) Alternatives to antibiotics in poultry feed: molecular perspectives. Crit Rev Microbiol 44(3):318–335. https://doi.org/10.1080/1040841X.2017.1373062

Tait C, Sayuk GS (2021) The brain-gut-microbial axis: a framework for understanding functional GI illness and their therapeutic interventions. Eur J Int Med 84:1–9. https://doi.org/10.1016/j.ejim.2020.12.023

Valenzuela-Grijalva NV, Pinelli-Saavedra A, Muhlia-Almazan A, Dominguez-Diaz D, Gonzalez-Rios H (2017) Dietary inclusion effects of phytochemicals as growth promoters in animal production. J Anim Sci Technol 59:8. https://doi.org/10.1186/s40781-017-0133-9

Xiong X, Tan B, Song M, Ji P, Kim K, Yin Y, Liu Y (2019) Nutritional intervention for the intestinal development and health of weaned pigs. Front Vet Sci 6:46. https://doi.org/10.3389/fvets.2019.00046

Zeng Z, Zhang S, Wang H, Piao X (2015) Essential oil and aromatic plants as feed additives in non-ruminant nutrition: a review. J Anim Sci Biotechnol 6:7. https://doi.org/10.1186/s40104-015—0004-5

Gastrointestinal Tract Barrier Efficiency: Function and Threats

2

Haitham M. Yakout and Erik Eckhardt

Abstract

The gastrointestinal tract (GIT) serves as a massive interface with the outside environment and physical barriers in the upper part, such as acids in the stomach and antimicrobial factors in saliva and other excreta, it is inevitably colonized by a multitude of micro-organisms which must be prevented from entering the sterile body-proper along with the nutrients. The absorptive intestinal epithelial cells are therefore assisted along the GIT by other epithelial cells that provide mechanical, biochemical, and biological barriers and by various immune cells that distinguish a small number of potential foes from a large number of friends. Both the failure to engage the small number of pathogens and the failure to ignore the large amounts of harmless antigens could lead to detrimental inflammatory responses and loss of health. The GIT and its associated barrier and immune functions evolve with time and can be altered by certain nutrients or other dietary additives. The purpose of this chapter is to give an overview of fundamental properties of the GIT barrier distal of the stomach and its associated immune system, where possible with emphasis on chickens; how these evolve during their lifespan; and how these functions could be affected by functional nutrients.

Keywords

Gut barrier functions · Inflammatory response · Immune system · Functional nutrients

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2.1 Introduction

Modern poultry and swine production integrates ultra-fast-growing breeds with relatively low-quality raw dietary feedstuff, stressful rearing conditions, and significant pathogenic challenges within difficult sanitary conditions. The animals thus need to balance highly efficient nutrient uptake with efficient exclusion of pathogenic challenges while tolerating harmless antigens. The ever-shorter lifespan of production animals, especially broiler chickens, means that there is little time to develop a highly performing gastrointestinal tract (GIT) barrier and a highly efficient immune system.

The consequences of GIT barrier breakdown, whatever the reason may be, can be severe as they may expose the body-proper to large amounts of pro-inflammatory material. As a consequence, the animal (if it survives) needs to divert valuable resources to combat invading micro-organisms, clear all sorts of antigens, and repair damaged tissue. During such inflammatory responses, less resources are available for growth and appetite is generally suppressed. The gut barrier is also an important endocrine organ that regulates insulin sensitivity and food intake, among others, and affects behavior by signaling to the central nervous system with its own signaling molecules and through bacterial metabolites it acquires from the nearby microbiome.

The GIT barrier is both a mechanical and an immunological barrier and some dietary components or additives may strengthen the one and thereby unintentionally weakening the other. For example, dietary immune stimulants may strengthen the antimicrobial activity of intestinal immune cells but in absence of pathogens this would cost energy and perhaps even create a chronic low-grade inflammatory response which could weaken the barrier.

In this chapter, some features of the GIT barrier will be discussed as will some threats to these features and nutritional interventions aimed at alleviating the threats.

2.2 Gut Barrier Function

2.2.1 GIT Barrier Composition

If it were not for a healthy gut barrier, micro-organisms and dietary and microbial antigens (collectively "antigens") would enter the body via gut-draining lymph or gut-draining portal blood. The mesenteric lymph nodes (Mueller and Macpherson 2006) and the liver (Knolle and Gerken 2000) are the ultimate firewalls for these ports of entry and clear most antigens without inflammatory responses. Chicken lymph nodes are not as well defined as their mammalian counterparts, so the chicken's lymphatic firewall (if any) remains to be defined.

Before antigens can enter portal blood or mesenteric lymph, they must first enter the lamina propria of the GIT, a layer of connective tissue upon which the basement membrane and the mucosal epithelial layer are stacked. Here they must escape the many macrophages (Bain and Mowat 2014; Lee et al. 1985) patrolling the tissue. In contrast to most macrophages elsewhere in the body, GIT macrophages are

surprisingly tolerant toward the many antigens they may encounter (Smythies et al. 2005), which prevents uncontrolled inflammatory responses to the inevitably frequently infiltrating antigens. Failure to adopt this phenotype may cause chronic inflammatory bowel diseases (Na et al. 2019). How gut macrophages adopt this important phenotype once they enter the tissue from the bloodstream is not entirely clear but it can be induced by exogenous factors such as butyric acid (Schulthess et al. 2019) or endogenous factors such as Transforming Growth Factor beta 2 (TGFb2) (Maheshwari et al. 2011). Their phagocytic and bacteriocidic capability remains fully intact, however, and they express significant amounts of antimicrobial peptides (AMP) (Schulthess et al. 2019; Sunkara et al. 2011). Intestinal macrophages are also essential for maintenance and repair of the gut barrier, because they clear dead (apoptotic) cells and promote epithelial repair (Birkl et al. 2019; Cosín-Roger et al. 2016).

Dendritic cells, which are closely related to macrophages, also take up antigens in the lamina propria but then move to gut-draining lymph nodes or Gut Associated Lymphoid Tissue ("GALT") such as Peyer's Patches ("PP") where they present the acquired antigens to cognate T-lymphocytes. Signals that the cells had experienced at time of antigen uptake then dictate the type of response they instill into the lymphocytes: When dietary antigens were sampled, for example, signals were likely benign and cognate T-cells should differentiate into regulatory T-cells clones which suppress immune responses to these antigens ("oral tolerance") (Pabst and Mowat 2012). If alarm signals were present during sampling, for example because of tissue damage during an infection, then the T-cells will develop into effector cells hunting for the antigen or into helper cells ("Th cells") that will instruct other immune cells to combat the antigen when it shows up again. Lymphocyte clones "trained" by dendritic cells then either remain in the GALT where they could respond to antigens that cross epithelial "Microfold" cells ("M cells") (Jeurissen et al. 1999) in a rather controlled manner or move into the lamina propria where they could encounter antigens that breach the barrier in an uncontrolled manner. Altogether, these adaptive immune responses enable a more fine-tuned response to antigens than the more aspecific response of macrophages. One such response consists of production of immunoglobulin A (IgA) by lamina propria B-lymphocytes which is then shuttled through epithelial cells into the gut lumen (Mueller and Macpherson 2006; Brandtzaeg 2013). Secretory IgA thus adds another layer to the gut barrier.

Before entering the lamina propria, antigens must first have crossed the intestinal epithelium, a single layer of highly polarized cells that are tightly connected with each other with several extracellular and membrane-anchored proteins. These "Tight Junctions" (TJ) are impermeable for even the smallest molecules (Zihni et al. 2016) and separate a functionally and morphologically distinct apical (lumen-facing) surface from the basolateral surface. Loss of TJ integrity would lead to uncontrolled paracellular leakage (i.e., between epithelial cells) of antigens which could set in motion an inflammatory response. The epithelial TJ is the barrier layer that has perhaps received the most attention.

Before being able to move across or in between epithelial cells, the antigens must first have crossed the mucus on top of the epithelium. This layer consists of a mesh of

mucins, a group of glycoproteins excreted by epithelial Goblet cells. These mucins then get entangled with other mucin proteins which are attached to the apical surface of most epithelial cells. Mucus production requires significant amounts of threonine so it must be regulated to avoid wasting of this precious nutrient (Zhang et al. 2017b; Munasinghe et al. 2017). The mucus layer is hard to penetrate by bacteria unless they express flagella. Numerous antimicrobial factors within the mucus layer, such as Serum Amyloid, are produced by epithelial cells themselves and can further reinforce the mucus layer's barrier effect (Eckhardt et al. 2010), defensins (Ramasamy et al. 2012; Veldhuizen et al. 2008; Wehkamp et al. 2005), REGIIIg (Mukherjee et al. 2014), and cathelicidins (Achanta et al. 2012) while others (such as IgA) are produced elsewhere and are subsequently transcytosed across the epithelial cells into the gut lumen. Intestinal epithelial cells also possess membrane-bound alkaline phosphatase, which readily detoxifies LPS (Bates et al. 2007; Lallès 2014), and these cells thus form another protective layer.

Another feature of intestinal epithelial cells, which is becoming increasingly appreciated, is that they help orchestrate mucosal immune responses. This goes beyond merely releasing potent chemokines (such as Interleukin 8 (Il-8)) during infections to attract immune cells: Depending on the type of threat, epithelial cells release cytokines that may skew the subsequent immune response. Some dietary factors trigger epithelial release of TSLP, IL-25, or IL-33 (Li et al. 2013) which would be optimal for a Th2 response towards parasites. Conversely, segmented filamentous bacteria in the intestine cause epithelial cells to release SAA (serum amyloid A) which induces a Th17 bias (Okumura and Takeda 2017), a preferable response to extracellular bacterial pathogens.

The first barrier, however, that an antigen must breach before it even reaches the mucus is not the least and consists of the gut microbiome. It is composed of hundreds of different species of micro-organisms (mainly bacteria) which help directly or indirectly reduce the risk of infections with pathogenic species. One protective mechanism consists of pathogen exclusion (Callaway et al. 2008; Ceccarelli et al. 2017; Mead 2000), in that a healthy microbiome may occupy pathogens' niches, compete for their nutrients, outright kill them, or prevent access to receptors on the apical surface of epithelial cells. The gut microbiome can be modulated by the diet or by supplements such as pre- and probiotics and such manipulations are attractive strategies to improve GIT barrier function (Fouhse et al. 2016; Sanders et al. 2019; Kogut 2018).

2.2.2 GIT Barrier Assessment

When GIT barrier function is discussed, it is often done so in terms of leakage of material from the gut into the bloodstream, the extent of which can be predicted with a variety of methods. Some are active in the sense that they require oral administration of macromolecular markers and subsequent measurement of these markers in urine or blood. Two markers are usually administered in a certain ratio. Marker A is only absorbable in a paracellular manner across leaky tight junctions (or across an

epithelial wound) whereas marker B is readily absorbed transcellularly. A high ratio of A to B in urine would indicate strong intestinal leakage. In human studies a combination of the non-metabolizable sugars Lactulose (or Rhamnose) and Mannitol usually serves as paracellular and transcellular tracers, respectively. The method has revealed increased intestinal permeability in, for example, Crohn's (Andre et al. 1988) and celiac (Juby et al. 1989) disease and acute intestinal infections (Zhang et al. 2000). The method has also been used in swine to demonstrate increased gut leakiness during weaning (Hu et al. 2012b) or diet-induced dysbiosis (Li et al. 2018a), for example. Its application in chickens has also been described (Gilani et al. 2017) but requires blood sampling. Given this limitation, it may be easier to use markers that are easier to measure in blood such as fluorescein-isothiocyanate-labeled dextran ("FITC"-dextran). However, in absence of a second marker to control for gastric emptying one might miss peak serum levels of orally administered FITC dextran.

"Passive" assessment of intestinal permeability is based on measuring blood levels of markers of epithelial damage, such as enterocyte-restricted Diamine Oxidase (DAO) (Gilani et al. 2017; Hu et al. 2012a; Honzawa et al. 2011) or Fatty-Acid Binding Protein (López-Colom et al. 2019). Blood levels of Zonulin-1, a regulator of TJ function, have also been found to correlate with intestinal permeability (Fasano et al. 2000).

In vitro models are useful to study cellular and molecular aspects of intestinal barrier function and their regulation by antigens, toxins, or cytokines. The most widely used model consists of Caco-2 cells grown on permeable filter supports. Though of colonic origin, after about 2 weeks this human cancer cell line will differentiate into small-intestine-like epithelial cells with strong TJ the strength of which can be assessed by measuring trans-epithelial electrical resistance (TEER) or by measuring leakage of fluorescent FITC dextran or Lucifer yellow. The cells' TJ are sensitive to exogenous cytokines, toxins, or various antigens which enables pre-clinical studies to the efficacy of additives. They can also be co-cultured with macrophage-like cells and other immune cells (Satsu et al. 2006) to study the effect of additives in more complex models (Olejnik et al. 2016). IPEC-J2 cells are comparable porcine cell lines of jejunal origin (Yan and Ajuwon 2017). Chicken in vitro studies mostly rely on freshly isolated primary cells which cannot be maintained in culture very long (Byrne et al. 2007). The recently discovered ability to cultured intestinal organoids has revolutionized research into regulation of cell differentiation during gut development (Wallach and Bayrer 2017) and has been developed for swine (Wellock et al. 2009) and chickens (Cowieson et al. 2004). This technique could accelerate research and could reduce the use of experimental animals.

2.2.3 Gut Barrier Threats

As outlined above, a healthy GIT barrier is essential for good health and performance because it prevents uncontrolled inflammatory responses to the many

antigens that reside in the gut. The GIT barrier is constantly experiencing different threats. When any of these threats lead to GIT barrier rupture, the result may be inflammation in the intestine or in the rest of the body, leading to reduced growth and resilience. Restoration of gut barrier function, regardless of whether it is a cause or consequence of a disease, could improve health. In the following some of the more common stresses in production animals are discussed as are some mitigation strategies involving dietary supplements.

2.2.3.1 Emotional Stress

One important risk factor affecting GIT barrier function in early weaned piglets is emotional stress, of which the gut stress hormone signaling is partially mediating GIT barrier impairment (Moeser et al. 2007, 2017). Some additives or supplements have been shown to improve gut barrier function during weaning stress, such as butyric acid (Huang et al. 2015). This bacterial metabolite is an important energy source for (colonic) epithelial cells and has a variety of beneficial effects on intestinal cells (Bedford and Gong 2018). It is often added to the diet of production animals as a sodium salt or can be coated to mask odor and reduce its absorption in the upper GIT. Coated sodium butyrate improves ileal villous architecture in weaned piglets, a proxy for GIT barrier function (Upadhaya et al. 2020). Another strategy of reduction of upper GIT absorption of butyric acid is by covalently linking to glycerol, which increases exposure of distal parts of the GIT to butyric acid. Other feed additives believed to strengthen weaning piglets' GIT barrier function, such as certain amino acids or plant extracts, may or may not exert their beneficial effect directly on gut epithelial cells (Xiong et al. 2019).

The GIT contains a sizeable nervous system, which communicates with the central nervous system and controls motility and neuroendocrine functions in the gut. The relationship between the central and intestinal nervous systems is complex, as is the relation between the intestinal nervous system and the mucosal immune system (Powell et al. 2017). On top of this, all these functions are affected by metabolites produced by the gut microbiome (Cryan et al. 2019). These components of the so-called microbiota–gut–brain axis can affect each other (Cryan et al. 2019) and perturbations in one can affect the function of all others. One could argue that stimulation of production of beneficial metabolites in the GIT, for example by improving the intestinal microbiome, could help improve well-being of animals and help them cope with emotional stress. This is an active area of research, not in the least in the feed additive industry, but is still in its early stages.

An important emotional stressor for chickens is their high packing density and there are indications that this may affect gut barrier function (Goo et al. 2019). Heat stress is another important source of stress in the poultry industry (Goo et al. 2019; Song et al. 2014; Tabler et al. 2020). Its impact can be blunted to some extent with additives conceivably acting on the GIT barrier itself, such as probiotics (Song et al. 2014), butyric acid (Abdelqader and Al-Fataftah 2016), or additives with a potentially broader target (Shakeri et al. 2019; Zhang et al. 2017a; Wu et al. 2018). When the GIT suffers from chronic oxidative stress, due to an imbalance in production and clearance of reactive oxygen species (ROS), this results in various intestinal

diseases, including enteric infections, inflammatory diseases, and in human's inflammatory bowel disease (Wang et al. 2020). Numerous natural antioxidants in "functional foods" that include fat-soluble (vitamin E, carotenoids), water-soluble (ascorbic acid), proteins, selenium (Se), and phytochemicals have been documented to play a key role in maintaining chicken health through protecting the intestinal health and restoring damage caused by oxidative stress (Surai et al. 2019). Within the body an antioxidant defense system utilizes various antioxidants that work together with Se playing a huge role expressing antioxidants modulatory effects in breeders, newly hatched chicks, and postnatal chickens as well. At the molecular level, most stresses are associated with overproduction of free radicals and oxidative stress (Surai 2016). One main source of free radicals is electron transport chain of mitochondria which can lose up to 3% of oxygen from the energy production process and become free radicals damaging various biological systems including lipids, proteins, and DNA (Surai and Kochish 2019). This is followed by the second most important source of free radicals, phagocyte cells, which produce free radicals to kill pathogens. However, as some of these free radicals escape, they can impose damage to healthy tissues (Surai and Kochish 2019). Furthermore, transition metals (Fe2+ and Cu+), excess polyunsaturated fatty acids, and high oxygen concentrations are part of a wide range of external and internal factors that increase free radical production.

Evolutionally, multiple antioxidants defense systems have been developed and are responsible for the higher eukaryotes survival rates and can be summarized into three main lines. (1) detoxification of superoxidase radical through SOD (Surai 2016), which produces hydroperoxide (H2O2) that is toxic and must be removed from the cell by GSH-Px through conversion to water. Another essential element of the first line of defense is metal-binding proteins that are important to sequester free form transition metals which are involved in free radical formation. These metal-binding proteins are part of mitochondria integrity matrix. (2) chain breaking antioxidants that work in various mechanisms of antioxidants recycling. This includes but not limited to, vitamins E, C, carotenoids, GSH system, selenoproteins, and others. For example, vitamin E is usually oxidized after reacting with free radical and losses the antioxidant protective activity, but the presence of vitamin C helps converting vitamin E again to a rather "reduced" active form. Then, vitamin C is oxidized in turn but is further reduced by thioredoxin (TR). (3) heat shock proteins (HSP), methionine sulfoxide reductase, phospholipase, and DNA repair enzymes work together on preventing damage to biological molecules, lipids, proteins, and DNA.

As mono-gastric farm animals commercial production becomes more integrated, and production is more intensive, multiple stressful conditions require better management systems and regulating antioxidants defense systems in poultry via application of dietary supplements. To maintain poultry optimal productive and reproductive performance, feed rations must provide optimal dietary levels of vitamin E, Se, and carotenoids. Selenium, for example, expresses its unique antioxidant features through involvement in expression and synthesis of 25 selenoproteins, glutathione peroxidase (GSH-Px, TrxR, and SeP) (Sun et al. 2019; Zhao et al. 2017;

Li et al. 2018b; Surai and Kochish 2019). The SeCys from of Se works efficiently as a one main component of multiple selenoproteins that are essential modulators of a stronger antioxidant defense system through its effects on antioxidant enzymatic or non-enzymatic defense mechanisms.

The organic form is the main Se form that is supplied from grains with SeMet representing at least 50% of the total Se. With dietary concentrations vary greatly and most of the time is deficient, Se is incorporated in premixes across all poultry diets at 0.1–0.3 ppm. Dietary Se supplementation in poultry diet is in various forms including inorganic; selenite and selenate, and organic; Se-yeast, SeMet, OH-SeMet (Surai and Fisinin 2014). A majority of Se absorption (~80%) would occur in the small intestine, specifically in duodenum and jejunum regions. Absorption efficacy also varies among different Se forms with most absorbed for organic (SeMet) to medium for Selenate and poor absorption for Selenite. Passive absorption is considered selenite's main route while Na+ mediated absorption mechanisms are utilized by selenate and organic Se (SeMet) are absorbed the same way as pure methionine (Surai and Kochish 2019). After absorption, Se binds to blood proteins and is delivered to the liver which converts all forms of Se to hydrogen selenide (H2Se), an essential molecule for selenoprotein synthesis. Inside the cell selenoprotein expression and synthesis are regulated by Se status and stress level. Selenoproteins that are responsible for maintaining important cellular functions are known as housekeeping selenoproteins and those are usually not significantly affected by Se status of stress levels. Others that are stress responsive usually modulate their expression or synthesis by dietary Se supplementation and surrounding environmental conditions.

2.2.3.2 Mycotoxins

Mycotoxins, toxic metabolites that are produced by mold, yeast, and sometimes bacteria are considered another type "dietary" challenges, mostly presented directly from contaminated feed. These are defined as secondary metabolites of fungi that can cause serious health problems in animals, which would reflect on deleterious effects in farm animals ranging anywhere from lower feed consumption and growth impairment to lower diseases resistance to even death. Recently, fungi have been designated as a greater threat to animal, plant, and ecosystem health than the other taxonomic classes of pathogens (Fisher et al. 2012). The major problem associated with mycotoxins contaminated animal feed is not acute disease episodes, rather it is the ingestion of low levels of toxins over a longer period "chronic" which may cause an array of metabolic, physiologic, and immunologic disturbances (Oswald et al. 2005; Bryden 2012). A substantial progress has been made in mycotoxin research regarding effects on intestinal functions in recent years. By contrast to the limited distribution of mycotoxins into systemic tissues, the GIT is exposed to all the mycotoxins in contaminated feed. This suggests that the intestinal epithelium is the major site for the effects of mycotoxin contaminated material, even low levels of contamination. So, the constant exposure of the intestinal epithelium cells to 100% of contaminated feed is always cited as the first target of these contaminants. These epithelial cells can either be compromised by mycotoxins ingested prior to

absorption in the upper GIT region or throughout the entire intestine by non-absorbed toxins. Non-absorbed mycotoxins are constantly located in the lumen, they negatively affect the gut integrity and epithelial cell's structure and with most mycotoxins quickly appear in blood circulation, this clearly indicates that the proximal part of the GIT is the main site for absorbing majority of the ingested toxin (Cavret and Lecoeur 2006).

As of early 2000, many studies have focused creating in vitro models studying mycotoxins effects on intestinal permeability. Differentiated and polarized intestinal epithelial cells into a polarized monolayer became a particularly useful tool with TEER (Transepithelial electrical resistance) and are utilized as a good feasible indicator of the epithelial barrier integrity. TEER can be significantly reduced by exposure to different concentrations, types of mycotoxins, particularly DON. Tight junctions (TJ), a major component for the epithelial cells' integrity is formed from different proteins including ZO-1 and one or more claudin, and they seal the lumenal end of the intercellular space limiting transport of small hydrophilic molecules by this paracellular route. ZO-1 acts as a bridge organizing transmembrane TJ proteins and attracting signaling molecules to the complex, while claudin binds to ZO-1 and plays a key role in regulating permeability through TJ (McLaughlin et al. 2004). Therefore, in most of the published research studies the claudin protein family are referred to as a key determinant of paracellular permeability. Many studies showed that either by immunofluorescence or immunoblotting, the expression of claudin 4 on the intestinal epithelial cells was either reduced or even removed (McLaughlin et al. 2004; Lambert et al. 2007; van de Walle et al. 2010; Pinton et al. 2010; Diesing et al. 2011; Pinton et al. 2012) as animal/bird was fed DON-contaminated feed. Claudin 4 expression in the jejunum was reduced as pigs were fed lower DON concatenations for five consecutive weeks (Pinton et al. 2009). DON impose a negative effect on total protein synthesis (van de Walle et al. 2010), this was linked to reduce claudin 4 expression.

Fumonisin (FB) is another example, being poorly absorbed in the GIT of monogastric animals (1–6%) indicates that the epithelial layer is at great exposure to a higher toxin ingested. Similarly, the absorption of deoxynivalenol (DON) is considered moderate in pigs (55%) but fairly limited in poultry (5–20%). No data of FB1 on claudin is available, while occludin and ZO-1 show inconsistent results. However, FB1 has a key role in sphingolipids metabolism (Loiseau et al. 2007) which in turn have a significant role in the establishment and maintenance of TJ (Lambert et al. 2007). Therefore, GIT epithelium cells might be a target for FB1 negative effects. This was noted in lower expression of claudin and E-cadherin in the ileum of pigs as they were fed lower FB1 dosage (Bracarense et al. 2012).

Intestinal lesions due to mycotoxin exposure are always dominant in the duodenum and jejunum. Following the ingestion of DON-contaminated feed, proximal intestinal epithelial cells are exposed to high concentrations of DON. In fact, with as extraordinarily little DON being absorbed in poultry upper GIT it still acts as an inhibitor to protein synthesis at the ribosomal level, especially at the high proliferating cells in proximal GIT tissues that are characterized by high protein turnover, including immune system and intestine (Dänicke et al. 2002). Though, it is

not surprising to see significant reduction of duodenal villus height, for example, which is a clear demonstration of DON alteration to mucosal structural following toxin ingestion. The effectiveness of nutrients absorption would be compromised due to reduced villus height (Grenier and Applegate 2013). During the migration along the crypt-villus axis, enterocytes must differentiate to fully express their digestive functions indicated by an increase in enzyme activities including sucrase and maltase (Applegate et al. 2009). However, feeding DON-contaminated feed would always be associated with shorter villus and impaired nutrient absorption because of a smaller number of differentiated epithelial cells (Grenier and Applegate 2013). Another example of DON assault to proximal intestinal cells is through modulation of intestinal paracellular transport which leads to an increased passage of macromolecules and bacteria (Pinton et al. 2009). As a result, the intestinal barrier integrity and function would be negatively affected leading to increased permeability or "leaky gut." The reduction in epithelial integrity will then contribute to an increased protein availability in the intestinal lumen due to plasma proteins and amino acids leakage into the gut, which creates a favorable environment of massive overgrowth of pathogens including C. perfringens. Indeed, Antonissen et al. reported an increase in duodenal total protein levels (Antonissen et al. 2014). Nutrient's malabsorption could be the reason, due to the negative effect of DON on nutrients digestion or increased plasma amino acids and proteins leakage into the intestine because of altered intestinal barrier integrity. Reduced duodenal villus height has also been reported to be associated with nutrients maldigestion and malabsorption. Multiple studies have shown different intestinal transporter proteins for different nutrients being selectively modulated by DON. This would negatively affect sodium associated amino acids co-transport of serine and proline resulting in an increased lumenal contents of such amino acids (Awad et al. 2004; Dietrich et al. 2012; Huang et al. 2015; Bedford and Gong 2018; Upadhaya et al. 2020; Xiong et al. 2019).

2.2.3.3 Infectious Agents

Infectious bacteria or viruses are a major threat for GIT barrier function and often directly target intestinal epithelial cells. Some pathogens thrive within these cells (such as *Lawsonia intracellularis* in swine) and attempt to subvert the immune system to avoid their destruction (Vannucci and Gebhart 2014). This is also an apparent strategy of parasites such as Eimeria spp., the causative agent of cocciodioses, which induces production of anti-inflammatory IL-10 in the host to subvert immune responses (Abdul Rasheed et al. 2020). Additives like probiotics or microbial components could act as immune stimulators, which could circumvent these immune-invasion strategies (Ohashi and USHIDA 2009; Tiwari et al. 2020). However, when no pathogens are encountered during the life cycle, chronic stimulation of immunity may come at a metabolic cost or cause low-grade chronic inflammation (Arsenault et al. 2017).

Another strategy to prevent GIT barrier damage due to infectious agents would be to prevent infections altogether. Some probiotics affect viability of pathogens or achieve this indirectly by promoting a healthy microflora. Other additives, such as butyric acid or medium-chain fatty acids, may affect virulence of pathogens such as

Salmonella spp. (van Immerseel et al. 2006) or may reduce viability of certain viruses (Thormar et al. 1987). Some supplements have been claimed to block access of pathogens to epithelial cells by interfering with pathogen-binding to host cells (Fernandez et al. 2000). Access to epithelial cells could further be restricted by increasing mucin production, which can be increased by butyrate (Gaudier et al. 2004; Sikandar et al. 2017). This short-chain fatty acid is often added to the diet or it is raised by increasing the number of butyrate-producing bacteria in the gut, for example by adding certain prebiotics or probiotics. Pre-biotics can also be produced through enzymatic breakdown of NSP (Non-Starch Polysaccharides) (Yacoubi et al. 2017), possibly even within the gut from plant NSP upon exogenous enzyme administration. Mucin production can also be enhanced with other supplements, such as amino acids (Zhang et al. 2019; Dong et al. 2017). Host-defense peptides produced by epithelial cells may further limit the viability of infectious agents, and their production can also be stimulated with butyrate (Zeng et al. 2013) or probiotics (Wang et al. 2019) and possibly other supplements. The effect of butyrate likely also extends to stimulation of host-defense peptide production by intestinal macrophages (Sunkara et al. 2012; Schulthess et al. 2019).

If infection cannot be prevented, it is then essential to at least limit harmful effects of collateral damage which occurs mainly in two ways: Inflammation and oxidative stress. Unless a pathogen manages to subvert the host cell, the infected cell will send alarm signals to attract immune cells. These signals typically elicit an inflammatory response which helps clear the pathogen, but it may also impair gut barrier integrity by weakening TJ function. Many feed additives can effectively exert anti-inflammatory effects on intestinal epithelial cells and conceivably also nearby immune cells. Examples are several strains of Bacillus that can dampen inflammatory signaling in epithelial cells by interfering with NFkB signaling (Rhayat et al. 2019) or in macrophages by inducing an M2 phenotype in these cells, which is anti-inflammatory (Paynich et al. 2017). Butyrate also exerts anti-inflammatory effects in the gut (Segain et al. 2000; Jiang et al. 2015; Wang et al. 2018) although this is likely dose- and location dependent.

A second consequence of infection occurs mainly when phagocytes are newly recruited into the gut and help clear invading pathogens. These cells (mainly macrophages and neutrophils, or “heterophils” in birds) have not yet adopted the tolerant phenotype and besides significant amounts of pro-inflammatory cytokines often produce reactive nitrogen species (mainly Nitric Oxide) or superoxide radicals to kill the invaders. Excessive production of these oxidants can further flame inflammatory responses and may directly damage intestinal tissue (Kruidenier et al. 2003) or even weaken tight junctions in epithelial cells (Rao 2008). Oxidative stress responses can also be provoked in epithelial cells themselves, for example when they are exposed to mycotoxins (Del Regno et al. 2015; Mahfoud et al. 2002). Supplementation of poultry or swine feed with Tocopherols (vitamin E) and Selenium may reduce oxidative stress responses in challenged conditions. Tocopherol scavenges reactive oxygen species (ROS) while Selenium gets incorporated into the catalytic unit of enzymes (as selenocysteine), thereby enhancing the removal of ROS. Together, both supplements can help maintain intestinal barrier function and

reduce oxidative stress in swine experiencing heat stress (Liu et al. 2016). Plant-derived polyphenols may also help reduce oxidative stress in the intestine and may thereby stop the vicious cycle that is sustained by a leaky gut (Gessner et al. 2017; Prakash and Srinivasan 2010).

As much as a healthy gut microbiome plays a key role in maintenance of GIT barrier function, among others by controlling pathogens, strengthening immunity and providing functional and nutritional metabolites (e.g. butyrate), an unhealthy microbiome can have a very detrimental effect. It is generally accepted that a healthy microbiome is characterized by a strong diversity in its composition at every level (from domain level via phylum to species and strains). In some cases, however, this balance is disturbed leading to a flourish of a limited set of species that will overwhelm the host-microbiome equilibrium. This state of "dysbiosis," which is still poorly defined, may lead to massive production of harmful metabolites and toxins which can cause significant breakdown of gut barrier function. Dysbiosis can be caused by poorly digestible diets (Ducatelle et al. 2018; Gresse et al. 2017) or by antibiotics treatments (Guevarra et al. 2019) and can be reversed by several additives, including pre- and probiotics (McFarland 2014; Ducatelle et al. 2015).

Some dietary factors not only threaten GIT barrier function by inducing microbial dysbiosis. Certain anti-nutritional factors commonly present in many food staples may cause intestinal epithelial damage, such as soybean agglutinins (Zhao et al. 2011), while others such as soy galactomannans may indirectly damage the barrier by activating intestinal immune cells (Arsenault et al. 2017; Pont et al. 2020). Human and rodent studies had shown that high-fat, high energy diets cause a breakdown of gut barrier function with translocation of LPS into the circulation in the postprandial state (Cani et al. 2007). This is problematic for animal production because current chicken and swine breeds ingest incredible amounts of food and are almost permanently in a postprandial state. While reduction of feed intake thus is not an option, it is clear that solutions are needed to prevent GIT barrier breakdown and maintain health and performance.

2.3 Concluding Remarks

The GIT is literally at the center of health and well-being and disturbances in GIT barrier function are either a cause or consequence of a variety of disorders. Therefore, nutritional support of GIT barrier function may be a promising strategy to support health and growth of modern production animals.

References

Abdelqader A, Al-Fataftah A-R (2016) Effect of dietary butyric acid on performance, intestinal morphology, microflora composition and intestinal recovery of heat-stressed broilers. Livest Sci 183:78–83. https://doi.org/10.1016/j.livsci.2015.11.026

Abdul Rasheed MS, Tiwari UP, Oelschlager ML, Smith BN, Jespersen JC, Escobar J et al (2020) Dietary supplementation with anti-IL-10 antibody during a severe Eimeria challenge in broiler chickens. Poult Sci 99(12):6493–6502. https://doi.org/10.1016/j.psj.2020.09.012

Achanta M, Sunkara LT, Dai G, Bommineni YR, Jiang W, Zhang G (2012) Tissue expression and developmental regulation of chicken cathelicidin antimicrobial peptides. J Anim Sci Biotechnol 3(1):15. https://doi.org/10.1186/2049-1891-3-15

Andre F, Andre C, Emery Y, Forichon J, Descos L, Minaire Y (1988) Assessment of the lactulose-mannitol test in Crohn's disease. Gut 29(4):511–515. https://doi.org/10.1136/gut.29.4.511

Antonissen G, Van Immerseel F, Pasmans F, Ducatell R, Haesebrouck F et al (2014) The mycotoxin deoxynivalenol predisposes for the development of clostridium perfringens-induced necrotic enteritis in broiler chickens. PLoS One 9(9):e108775

Applegate TJ, Schatzmayr G, Pricket K, Troche C, Jiang Z (2009) Effect of aflatoxin culture on intestinal function and nutrient loss in laying hens. Poult Sci 88:1235–1241. https://doi.org/10.3382/ps.2008-00494

Arsenault RJ, Lee JT, Latham R, Carter B, Kogut MH (2017) Changes in immune and metabolic gut response in broilers fed β-mannanase in β-mannan-containing diets. Poult Sci. https://doi.org/10.3382/ps/pex246

Awad WA, Bohm J, Razzazi-Fazeli E, Hulan HW, Zentek J (2004) Effects of deoxynivalenol on general performance and electrophysiological properties of intestinal mucosa of broiler chickens. Poult Sci 83:1964–1972. https://doi.org/10.1093/ps/83.12.1964

Bain CC, Mowat AMI (2014) Macrophages in intestinal homeostasis and inflammation. Immunol Rev 260(1):102–117. https://doi.org/10.1111/imr.12192

Bates JM, Akerlund J, Mittge E, Guillemin K (2007) Intestinal alkaline phosphatase detoxifies lipopolysaccharide and prevents inflammation in zebrafish in response to the gut microbiota. Cell Host Microbe 2(6):371–382. https://doi.org/10.1016/j.chom.2007.10.010

Bedford A, Gong J (2018) Implications of butyrate and its derivatives for gut health and animal production. Anim Nutr 4(2):151–159. https://doi.org/10.1016/j.aninu.2017.08.010

Birkl D, O'Leary MN, Quiros M, Azcutia V, Schaller M, Reed M et al (2019) Formyl peptide receptor 2 regulates monocyte recruitment to promote intestinal mucosal wound repair. Feder Am Soc Exp Biol J 33(12):13632–13643. https://doi.org/10.1096/fj.201901163R

Bracarense APFL, Lucioli J, Grenier B, Drociunas Pacheco G, Moll WD, Schatzmayr G, Oswald IP (2012) Chronic ingestion of deoxynivalenol and fumonisin, alone or in interaction, induces morphological and immunological changes in the intestine of piglets. Br J Nutr 107:1776–1786

Brandtzaeg P (2013) Secretory IgA. Designed for anti-microbial defense. Front Immunol 4:222. https://doi.org/10.3389/fimmu.2013.00222

Bryden WL (2012) Mycotoxin contamination of the feed supply chain: implications for animal. Anim Feed Sci Technol 173:134–158

Byrne CM, Clyne M, Bourke B (2007) Campylobacter jejuni adhere to and invade chicken intestinal epithelial cells in vitro. Microbiology 153(Pt 2):561–569. https://doi.org/10.1099/mic.0.2006/000711-0

Callaway TR, Edrington TS, Anderson RC, Harvey RB, Genovese KJ, Kennedy CN et al (2008) Probiotics, prebiotics and competitive exclusion for prophylaxis against bacterial disease. Anim Health Res Rev 9(2):217–225. https://doi.org/10.1017/S1466252308001540

Cani PD, Amar J, Iglesias MA, Poggi M, Knauf C, Bastelica D et al (2007) Metabolic endotoxemia initiates obesity and insulin resistance. Diabetes 56(7):1761–1772. https://doi.org/10.2337/db06-1491

Cavret S, Lecoeur S (2006) Fusariotoxin transfer in animal. Food Chem Toxicol 44:444–453

Ceccarelli D, van Essen-Zandbergen A, Smid B, Veldman KT, Boender GJ, Fischer EAJ et al (2017) Competitive exclusion reduces transmission and excretion of extended-spectrum-β-lactamase-producing Escherichia coli in broilers. Appl Environ Microbiol 83(11). https://doi.org/10.1128/AEM.03439-16

Cosín-Roger J, Ortiz-Masiá D, Calatayud S, Hernández C, Esplugues JV, Barrachina MD (2016) The activation of Wnt signaling by a STAT6-dependent macrophage phenotype promotes

mucosal repair in murine IBD. Mucosal Immunol 9(4):986–998. https://doi.org/10.1038/mi.2015.123

Cowieson AJ, Acamovic T, Bedford MR (2004) The effects of supplementation of maize-based diets with exogenous phytase on amino acid digestibility and nitrogen retention by young broiler chicks. Br Poult Sci 45(S1):S5–S6

Cryan JF, O'Riordan KJ, Cowan CSM, Sandhu KV, Bastiaanssen TFS, Boehme M et al (2019) The microbiota-gut-brain axis. Physiol Rev 99(4):1877–2013. https://doi.org/10.1152/physrev.00018.2018

Dänicke S, Ueberschär KH, Halle I, Matthes S, Valenta H, Flachowsky G (2002) Effect of addition of a detoxifying agent to laying hen diets containing uncontaminated or Fusarium toxin-contaminated maize on performance of hens and on carryover of zearalenone. Poult Sci 81 (11):1671–1680. https://doi.org/10.1093/ps/81.11.1671

Diesing AK, Nossol C, Panther P, Walk N, Post A, Kluess J, Kreutzmann P, Danicke S, Rothkotter HJ, Kahlert S (2011) Mycotoxin deoxynivalenol (DON) mediates biphasic cellular response in intestinal porcine epithelial cell lines IPEC-1 and IPEC-J2. Toxicol Lett 200:8–18

Dietrich B, Neuenschwander S, Bucher B, Wenk C (2012) Fusarium mycotoxin-contaminated wheat containing deoxynivalenol alters the gene expression in the liver and the jejunum of broilers. Animal 6:278–291

Dong XY, Azzam MMM, Zou XT (2017) Effects of dietary threonine supplementation on intestinal barrier function and gut microbiota of laying hens. Poult Sci 96(10):3654–3663. https://doi.org/10.3382/ps/pex185

Ducatelle R, Eeckhaut V, Haesebrouck F, van Immerseel F (2015) A review on prebiotics and probiotics for the control of dysbiosis. Present status and future perspectives. Animal 9 (1):43–48. https://doi.org/10.1017/S1751731114002584

Ducatelle R, Goossens E, de Meyer F, Eeckhaut V, Antonissen G, Haesebrouck F, van Immerseel F (2018) Biomarkers for monitoring intestinal health in poultry. Present status and future perspectives. Vet Res 49(1):43. https://doi.org/10.1186/s13567-018-0538-6

Eckhardt ERM, Witta J, Zhong J, Arsenescu R, Arsenescu V, Wang Y et al (2010) Intestinal epithelial serum amyloid A modulates bacterial growth in vitro and pro-inflammatory responses in mouse experimental colitis. BMC Gastroenterol 10:133. https://doi.org/10.1186/1471-230X-10-133

Fasano A, Not T, Wang W, Uzzau S, Berti I, Tommasini A, Goldblum SE (2000) Zonulin, a newly discovered modulator of intestinal permeability, and its expression in coeliac disease. Lancet 355(9214):1518–1519. https://doi.org/10.1016/S0140-6736(00)02169-3

Fernandez F, Hinton M, van Gils B (2000) Evaluation of the effect of mannan-oligosaccharides on the competitive exclusion of Salmonella Enteritidis colonization in broiler chicks. Avian Pathol 29(6):575–581. https://doi.org/10.1080/03079450020016823

Fisher MC, Henk DA, Madoff LC, Brownstein JS, Briggs CJ, McCraw SL (2012) Emerging fungal threats to animal, plant and ecosystem health. Nature 484:186–194

Fouhse JM, Zijlstra RT, Willing BP (2016) The role of gut microbiota in the health and disease of pigs. Anim Front 6(3):30–36. https://doi.org/10.2527/af.2016-0031

Gaudier E, Jarry A, Blottière HM, de Coppet P, Buisine MP, Aubert JP et al (2004) Butyrate specifically modulates MUC gene expression in intestinal epithelial goblet cells deprived of glucose. Am J Physiol-Gastrointest Liver Physiol 287(6):G1168–G1174. https://doi.org/10.1152/ajpgi.00219.2004

Gessner DK, Ringseis R, Eder K (2017) Potential of plant polyphenols to combat oxidative stress and inflammatory processes in farm animals. J Anim Physiol Anim Nutr 101(4):605–628. https://doi.org/10.1111/jpn.12579

Gilani S, Howarth GS, Kitessa SM, Tran CD, Forder REA, Hughes RJ (2017) Intestinal permeability induced by lipopolysaccharide and measured by lactulose, rhamnose and mannitol sugars in chickens. Animal 11(7):1174–1179

Goo D, Kim JH, Park GH, Reyes D, Badillo J, Kil DY (2019) Effect of heat stress and stocking density on growth performance, breast meat quality, and intestinal barrier function in broiler chickens. Animals 9(3):107. https://doi.org/10.3390/ani9030107

Grenier B, Applegate TJ (2013) Modulation of intestinal functions following mycotoxin ingestion: meta-analysis of published experiments in animals. Toxins 5:397–430

Gresse R, Chaucheyras-Durand F, Fleury MA, van de Wiele T, Forano E, Blanquet-Diot S (2017) Gut microbiota dysbiosis in postweaning piglets. Understanding the keys to health. Trends Microbiol 25(10):851–873. https://doi.org/10.1016/j.tim.2017.05.004

Guevarra RB, Lee JH, Lee SH, Seok M-J, Kim DW, Kang BN et al (2019) Piglet gut microbial shifts early in life: causes and effects. J Anim Sci Biotechnol 10:1. https://doi.org/10.1186/s40104-018-0308-3

Honzawa Y, Nakase H, Matsuura M, Chiba T (2011) Clinical significance of serum diamine oxidase activity in inflammatory bowel disease. Importance of evaluation of small intestinal permeability. Inflamm Bowel Dis 17(2):E23–E25. https://doi.org/10.1002/ibd.21588

Hu CH, Gu LY, Luan ZS, Song J, Zhu K (2012a) Effects of montmorillonite–zinc oxide hybrid on performance, diarrhea, intestinal permeability and morphology of weanling pigs. Anim Feed Sci Technol 177(1–2):108–115. https://doi.org/10.1016/j.anifeedsci.2012.07.028

Hu C, Song J, You Z, Luan Z, Li W (2012b) Zinc oxide-montmorillonite hybrid influences diarrhea, intestinal mucosal integrity, and digestive enzyme activity in weaned pigs. Biol Trace Elem Res 149(2):190–196. https://doi.org/10.1007/s12011-012-9422-9

Huang C, Song P, Fan P, Hou C, Thacker P, Ma X (2015) Dietary sodium butyrate decreases postweaning diarrhea by modulating intestinal permeability and changing the bacterial communities in weaned piglets. J Nutr 145(12):2774–2780. https://doi.org/10.3945/jn.115.217406

Jeurissen SH, Wagenaar F, Janse EM (1999) Further characterization of M cells in gut-associated lymphoid tissues of the chicken. Poult Sci 78(7):965–972. https://doi.org/10.1093/ps/78.7.965

Jiang Y, Zhang W, Gao F, Zhou G (2015) Effect of sodium butyrate on intestinal inflammatory response to lipopolysaccharide in broiler chickens. Can J Anim Sci 95(3):389–395. https://doi.org/10.4141/cjas-2014-183

Juby LD, Rothwell J, Axon ATR (1989) Lactulose/mannitol test. An ideal screen for celiac disease. Gastroenterology 96(1):79–85. https://doi.org/10.1016/0016-5085(89)90767-1

Knolle PA, Gerken G (2000) Local control of the immune response in the liver. Immunol Rev 174:21–34. https://doi.org/10.1034/j.1600-0528.2002.017408.x

Kogut MH (2018) The effect of microbiome modulation on the intestinal health of poultry. Anim Feed Sci Technol. https://doi.org/10.1016/j.anifeedsci.2018.10.008

Kruidenier L, Kuiper I, Lamers CBHW, Verspaget HW (2003) Intestinal oxidative damage in inflammatory bowel disease. Semi-quantification, localization, and association with mucosal antioxidants. J Pathol 201(1):28–36. https://doi.org/10.1002/path.1409

Lallès J-P (2014) Intestinal alkaline phosphatase. Novel functions and protective effects. Nutr Rev 72(2):82–94. https://doi.org/10.1111/nure.12082

Lambert D, Padfield PJ, McLaughlin J, Cannell S, O'Neill CA (2007) Ochratoxin A displaces claudins from detergent resistant membrane microdomains. Biochem Biophys Res Commun 358:632–636

Lee SH, Starkey PM, Gordon S (1985) Quantitative analysis of total macrophage content in adult mouse tissues. Immunochemical studies with monoclonal antibody F4/80. J Exp Med 161(3):475–489. https://doi.org/10.1084/jem.161.3.475

Li J, Wang Y, Tang L, de Villiers WJS, Cohen D, Woodward J et al (2013) Dietary medium-chain triglycerides promote oral allergic sensitization and orally induced anaphylaxis to peanut protein in mice. J Allergy Clin Immunol 131(2):442–450. https://doi.org/10.1016/j.jaci.2012.10.011

Li Q, Gabler NK, Loving CL, Gould SA, Patience JF (2018a) A dietary carbohydrase blend improved intestinal barrier function and growth rate in weaned pigs fed higher fiber diets. J Anim Sci 96(12):5233–5243. https://doi.org/10.1093/jas/sky383

Li S, Gao F, Huang J, Wu Y, Wu S, Lei XG (2018b) Regulation and function of avian selenogenome. In: Biochimica et biophysica acta. Gen Subj 1862(11):2473–2479. https://doi.org/10.1016/j.bbagen.2018.03.029

Liu F, Cottrell JJ, Furness JB, Rivera LR, Kelly FW, Wijesiriwardana U et al (2016) Selenium and vitamin E together improve intestinal epithelial barrier function and alleviate oxidative stress in heat stressed pigs. Exp Physiol 101(7):801–810. https://doi.org/10.1113/EP085746

Loiseau N, Debrauwer L, Sambou T, Bouhet S, Miller JD, Martin PG, Viadere JL, Pinton P, Puel O, Pineau T (2007) Fumonisin B-1 exposure and its selective effect on porcine jejunal segment: sphingolipids, glycolipids and trans-epithelial passage disturbance. Biochem Pharmacol 74: 144–152

López-Colom P, Yu K, Barba-Vidal E, Saco Y, Martín-Orúe SM, Castillejos L et al (2019) I-FABP, Pig-MAP and TNF-α as biomarkers for monitoring gut-wall integrity in front of salmonella typhimurium and ETEC K88 infection in a weaned piglet model. Res Vet Sci 124:426–432. https://doi.org/10.1016/j.rvsc.2019.05.004

Maheshwari A, Kelly DR, Nicola T, Ambalavanan N, Jain SK, Murphy-Ullrich J et al (2011) TGF-β2 suppresses macrophage cytokine production and mucosal inflammatory responses in the developing intestine. Gastroenterology 140(1):242–253. https://doi.org/10.1053/j.gastro.2010.09.043

Mahfoud R, Maresca M, Garmy N, Fantini J (2002) The mycotoxin patulin alters the barrier function of the intestinal epithelium. Mechanism of action of the toxin and protective effects of glutathione. Toxicol Appl Pharmacol 181(3):209–218. https://doi.org/10.1006/taap.2002.9417

McFarland LV (2014) Use of probiotics to correct dysbiosis of normal microbiota following disease or disruptive events. A systematic review. BMJ Open 4(8):e005047. https://doi.org/10.1136/bmjopen-2014-005047

McLaughlin J, Padfield PJ, Burt JPH, O'Neill CA (2004) Ochratoxin A increases permeability through tight junctions by removal of specific claudin isoforms. Am J Physiol Cell Physiol 287: C1412–C1417

Mead GC (2000) Prospects for 'competitive exclusion' treatment to control salmonellas and other foodborne pathogens in poultry. Vet J (Lond, Engl 1997) 159(2):111–123. https://doi.org/10.1053/tvjl.1999.0423

Moeser AJ, Klok CV, Ryan KA, Wooten JG, Little D, Cook VL, Blikslager AT (2007) Stress signaling pathways activated by weaning mediate intestinal dysfunction in the pig. Am J Physiol-Gastrointest Liver Physiol 292(1):G173–G181. https://doi.org/10.1152/ajpgi.00197.2006

Moeser AJ, Pohl CS, Rajput M (2017) Weaning stress and gastrointestinal barrier development. Implications for lifelong gut health in pigs. Anim Nutr. https://doi.org/10.1016/j.aninu.2017.06.003

Mueller C, Macpherson AJ (2006) Layers of mutualism with commensal bacteria protect us from intestinal inflammation. Gut 55(2):276–284. https://doi.org/10.1136/gut.2004.054098

Mukherjee S, Zheng H, Derebe MG, Callenberg KM, Partch CL, Rollins D et al (2014) Antibacterial membrane attack by a pore-forming intestinal C-type lectin. Nature 505 (7481):103–107. https://doi.org/10.1038/nature12729

Munasinghe LL, Robinson JL, Harding SV, Brunton JA, Bertolo RF (2017) Protein synthesis in mucin-producing tissues is conserved when dietary threonine is limiting in piglets. J Nutr 147 (2):202–210. https://doi.org/10.3945/jn.116.236786

Na YR, Stakenborg M, Seok SH, Matteoli G (2019) Macrophages in intestinal inflammation and resolution. A potential therapeutic target in IBD. Nat Rev Gastroenterol Hepatol 16(9):531–543. https://doi.org/10.1038/s41575-019-0172-4

Ohashi Y, Ushida K (2009) Health-beneficial effects of probiotics. Its mode of action. Anim Sci J = Nihon chikusan Gakkaiho 80(4):361–371. https://doi.org/10.1111/j.1740-0929.2009.00645.x

Okumura R, Takeda K (2017) Roles of intestinal epithelial cells in the maintenance of gut homeostasis. Exp Mol Med 49(5):e338. https://doi.org/10.1038/emm.2017.20

Olejnik A, Kowalska K, Olkowicz M, Juzwa W, Dembczyński R, Schmidt M (2016) A gastrointestinally digested Ribes nigrum L. fruit extract inhibits inflammatory response in a co-culture model of intestinal Caco-2 cells and RAW264.7 macrophages. J Agric Food Chem 64 (41):7710–7721. https://doi.org/10.1021/acs.jafc.6b02776

Oswald IP, Marin DE, Bouhet S, Pinton P, Taranu I, Accensi F (2005) Immunotoxicological risk of mycotoxins for domestic animals. Food Addit Contam 22:354–360

Pabst O, Mowat AM (2012) Oral tolerance to food protein. Mucosal Immunol 5(3):232–239. https://doi.org/10.1038/mi.2012.4

Paynich ML, Jones-Burrage SE, Knight KL (2017) Exopolysaccharide from Bacillus subtilis induces anti-inflammatory M2 macrophages that prevent T cell–mediated disease. J Immunol (Baltim, MD 1950) 198(7):2689–2698. https://doi.org/10.4049/jimmunol.1601641

Pinton P, Nougayrède JP, del Rio JC, Moreno C, Marin DE, Ferrier L, Bracarense AP, Kolf-Clauw M, Oswald IP (2009) The food contaminant deoxynivalenol, decreases intestinal barrier permeability and reduces claudin expression. Toxicol Appl Pharmacol 237:41–48

Pinton P, Braicu C, Nougayrede JP, Laffitte J, Taranu I, Oswald IP (2010) Deoxynivalenol impairs porcine intestinal barrier function and decreases the protein expression of claudin-4 through a mitogen activated protein kinase dependent mechanism. J Nutr 140:1956–1962

Pinton P, Tsybulskyy D, Lucioli J, Laffitte J, Callu P, Lyazhri F, Grosjean F, Bracarense AP, Kolf-Clauw M, Oswald IP (2012) Toxicity of deoxynivalenol and its acetylated derivatives on the intestine: differential effects on morphology, barrier function, tight junctions proteins and MAPKinases. Toxicol Sci 130:180–190

Pont CD, Gabriela, Farnell M, Farnell Y, Kogut MH (2020) Dietary factors as triggers of low-grade chronic intestinal inflammation in poultry. Microorganisms 8(1). https://doi.org/10.3390/microorganisms8010139

Powell N, Walker MM, Talley NJ (2017) The mucosal immune system. Master regulator of bidirectional gut-brain communications. Nat Rev Gastroenterol Hepatol 14(3):143–159. https://doi.org/10.1038/nrgastro.2016.191

Prakash UNS, Srinivasan K (2010) Gastrointestinal protective effect of dietary spices during ethanol-induced oxidant stress in experimental rats. Appl Physiol Nutr Metab = Physiologie appliquee, nutrition et metabolisme 35(2):134–141. https://doi.org/10.1139/H09-133

Ramasamy KT, Verma P, Reddy MR (2012) Differential gene expression of antimicrobial peptides β defensins in the gastrointestinal tract of Salmonella serovar Pullorum infected broiler chickens. Vet Res Commun 36(1):57–62. https://doi.org/10.1007/s11259-011-9512-8

Rao R (2008) Oxidative stress-induced disruption of epithelial and endothelial tight junctions. Front Biosci 13:7210–7226. https://doi.org/10.2741/3223

Regno D, Marisanta, Adesso S, Popolo A, Quaroni A, Autore G, Severino L, Marzocco S (2015) Nivalenol induces oxidative stress and increases deoxynivalenol pro-oxidant effect in intestinal epithelial cells. Toxicol Appl Pharmacol 285(2):118–127. https://doi.org/10.1016/j.taap.2015.04.002

Rhayat L, Maresca M, Nicoletti C, Perrier J, Brinch KS, Christian S et al (2019) Effect of Bacillus subtilis strains on intestinal barrier function and inflammatory response. Front Immunol 10:564. https://doi.org/10.3389/fimmu.2019.00564

Sanders ME, Merenstein DJ, Reid G, Gibson GR, Rastall RA (2019) Probiotics and prebiotics in intestinal health and disease. From biology to the clinic. Nat Rev Gastroenterol Hepatol 16 (10):605–616. https://doi.org/10.1038/s41575-019-0173-3

Satsu H, Ishimoto Y, Nakano T, Mochizuki T, Iwanaga T, Shimizu M (2006) Induction by activated macrophage-like THP-1 cells of apoptotic and necrotic cell death in intestinal epithelial Caco-2 monolayers via tumor necrosis factor-alpha. Exp Cell Res 312(19):3909–3919. https://doi.org/10.1016/j.yexcr.2006.08.018

Schulthess J, Pandey S, Capitani M, Rue-Albrecht KC, Arnold I, Franchini F et al (2019) The short chain fatty acid butyrate imprints an antimicrobial program in macrophages. Immunity 50 (2):432–445.e7. https://doi.org/10.1016/j.immuni.2018.12.018

Segain JP, La Raingeard de Blétière D, Bourreille A, Leray V, Gervois N, Rosales C et al (2000) Butyrate inhibits inflammatory responses through NFkappaB inhibition. Implications for Crohn's disease. Gut 47(3):397–403. https://doi.org/10.1136/gut.47.3.397

Shakeri M, Cottrell JJ, Wilkinson S, Zhao W, Le HH, McQuade R et al (2019) Dietary betaine improves intestinal barrier function and ameliorates the impact of heat stress in multiple vital organs as measured by Evans blue dye in broiler chickens. Animals 10(1). https://doi.org/10.3390/ani10010038

Sikandar A, Zaneb H, Younus M, Masood S, Aslam A, Khattak F et al (2017) Effect of sodium butyrate on performance, immune status, microarchitecture of small intestinal mucosa and lymphoid organs in broiler chickens. Asian Australas J Anim Sci 30(5):690–699. https://doi.org/10.5713/ajas.16.0824

Smythies LE, Sellers M, Clements RH, Mosteller-Barnum M, Meng G, Benjamin WH et al (2005) Human intestinal macrophages display profound inflammatory anergy despite avid phagocytic and bacteriocidal activity. J Clin Invest 115(1):66–75. https://doi.org/10.1172/JCI19229

Song J, Xiao K, Ke YL, Jiao LF, Hu CH, Diao QY et al (2014) Effect of a probiotic mixture on intestinal microflora, morphology, and barrier integrity of broilers subjected to heat stress. Poult Sci 93(3):581–588. https://doi.org/10.3382/ps.2013-03455

Sun L-H, Huang J-Q, Deng J, Lei XG (2019) Avian selenogenome. Response to dietary Se and vitamin E deficiency and supplementation. Poult Sci 98(10):4247–4254. https://doi.org/10.3382/ps/pey408

Sunkara LT, Achanta M, Schreiber NB, Bommineni YR, Dai G, Jiang W et al (2011) Butyrate enhances disease resistance of chickens by inducing antimicrobial host defense peptide gene expression. PLoS One 6(11):e27225. https://doi.org/10.1371/journal.pone.0027225

Sunkara LT, Jiang W, Zhang G (2012) Modulation of antimicrobial host defense peptide gene expression by free fatty acids. PLoS One 7(11):e49558. https://doi.org/10.1371/journal.pone.0049558

Surai PF (2016) Antioxidant systems in poultry biology: superoxide dismutase. J Anim Res Nutr 1 (1):1–17

Surai PF, Fisinin VI (2014) Selenium in poultry breeder nutrition: an update. Anim Feed Sci Technol 191:1–15

Surai PF, Kochish II (2019) Nutritional modulation of the antioxidant capacities in poultry: the case of selenium. Poult Sci 98:4231–4239

Surai PF, Kochish II, Romanov MN, Griffin DK (2019) Nutritional modulation of the antioxidant capacities in poultry: the case of vitamin E. Poult Sci 98(9):4030–4041. https://doi.org/10.3382/ps/pez072

Tabler TW, Greene ES, Orlowski SK, Hiltz JZ, Anthony NB, Dridi S (2020) Intestinal barrier integrity in heat-stressed modern broilers and their ancestor wild jungle fowl. Front Vet Sci. https://doi.org/10.3389/fvets.2020.00249

Thormar H, Isaacs CE, Brown HR, Barshatzky MR, Pessolano T (1987) Inactivation of enveloped viruses and killing of cells by fatty acids and monoglycerides. Antimicrob Agents Chemother 31 (1):27–31. https://doi.org/10.1128/AAC.31.1.27

Tiwari UP, Fleming SA, Rasheed A, Muhammed S, Jha R, Dilger RN (2020) The role of oligosaccharides and polysaccharides of xylan and mannan in gut health of monogastric animals. J Nutr Sci 9:e21. https://doi.org/10.1017/jns.2020.14

Upadhaya SD, Jiao Y, Kim YM, Lee KY, Kim IH (2020) Coated sodium butyrate supplementation to a reduced nutrient diet enhanced the performance and positively impacted villus height and faecal and digesta bacterial composition in weaner pigs. Anim Feed Sci Technol 265:114534. https://doi.org/10.1016/j.anifeedsci.2020.114534

van de Walle J, Sergent T, Piront N, Toussaint O, Schneider YJ, Larondelle Y (2010) Deoxynivalenol affects in vitro intestinal epithelial cell barrier integrity through inhibition of protein synthesis. Toxicol Appl Pharmacol 245:291–298

van Immerseel F, Russell JB, Flythe MD, Gantois I, Timbermont L, Pasmans F et al (2006) The use of organic acids to combat Salmonella in poultry: a mechanistic explanation of the efficacy. Avian Pathol 35(3):182–188. https://doi.org/10.1080/03079450600711045

Vannucci FA, Gebhart CJ (2014) Recent advances in understanding the pathogenesis of *Lawsonia intracellularis* infections. Vet Pathol 51(2):465–477. https://doi.org/10.1177/0300985813520249

Veldhuizen EJA, Rijnders M, Claassen EA, van Dijk A, Haagsman HP (2008) Porcine beta-defensin 2 displays broad antimicrobial activity against pathogenic intestinal bacteria. Mol Immunol 45(2):386–394. https://doi.org/10.1016/j.molimm.2007.06.001

Wallach TE, Bayrer JR (2017) Intestinal organoids. New frontiers in the study of intestinal disease and physiology. J Pediatr Gastroenterol Nutr 64(2):180–185. https://doi.org/10.1097/MPG.0000000000001411

Wang CC, Wu H, Lin FH, Gong R, Xie F, Peng Y et al (2018) Sodium butyrate enhances intestinal integrity, inhibits mast cell activation, inflammatory mediator production and JNK signaling pathway in weaned pigs. Innate Immun 24(1):40–46. https://doi.org/10.1177/1753425917741970

Wang J, Zhang W, Wang S, Liu H, Zhang D, Wang Y, Ji H (2019) Swine-derived probiotic lactobacillus plantarum modulates porcine intestinal endogenous host defense peptide synthesis through TLR2/MAPK/AP-1 signaling pathway. Front Immunol 10:2691. https://doi.org/10.3389/fimmu.2019.02691

Wang Y, Chen Y, Zhang X, Lu Y, Chen H (2020) New insights in intestinal oxidative stress damage and the health intervention effects of nutrients: a review. J Funct Foods 75:104248

Wehkamp J, Salzman NH, Porter E, Nuding S, Weichenthal M, Petras RE et al (2005) Reduced Paneth cell alpha-defensins in ileal Crohn's disease. Proc Natl Acad Sci U S A 102(50):18129–18134. https://doi.org/10.1073/pnas.0505256102

Wellock IJ, Houdijk JGM, Miller AC, Gill BP, Kyriazakis I (2009) The effect of weaner diet protein content and diet quality on the long-term performance of pigs to slaughter. J Anim Sci 87(4):1261–1269. https://doi.org/10.2527/jas.2008-1098

Wu QJ, Liu N, Wu XH, Wang GY, Lin L (2018) Glutamine alleviates heat stress-induced impairment of intestinal morphology, intestinal inflammatory response, and barrier integrity in broilers. Poult Sci 97(8):2675–2683. https://doi.org/10.3382/ps/pey123

Xiong X, Tan B, Song M, Ji P, Kim K, Yin Y, Liu Y (2019) Nutritional intervention for the intestinal development and health of weaned pigs. Front Vet Sci 6:46. https://doi.org/10.3389/fvets.2019.00046

Yacoubi N, Saulnier L, Bonnin E, Devillard E, Eeckhaut V, Rhayat L et al (2017) Short-chain arabinoxylans prepared from enzymatically treated wheat grain exert prebiotic effects during the broiler starter period. Poult Sci 97(2):412–424. https://doi.org/10.3382/ps/pex297

Yan H, Ajuwon KM (2017) Butyrate modifies intestinal barrier function in IPEC-J2 cells through a selective upregulation of tight junction proteins and activation of the Akt signaling pathway. PLoS One 12(6):e0179586. https://doi.org/10.1371/journal.pone.0179586

Zeng X, Sunkara LT, Jiang W, Bible M, Carter S, Ma X et al (2013) Induction of porcine host defense peptide gene expression by short-chain fatty acids and their analogs. PLoS One 8(8):e72922. https://doi.org/10.1371/journal.pone.0072922

Zhang Y, Lee B, Thompson M, Glass R, Cama RI, Figueroa D et al (2000) Lactulose-mannitol intestinal permeability test in children with diarrhea caused by rotavirus and cryptosporidium. Diarrhea Working Group, Peru. J Pediatr Gastroenterol Nutr 31(1):16–21. https://doi.org/10.1097/00005176-200007000-00006

Zhang C, Zhao XH, Yang L, Chen XY, Jiang RS, Jin SH, Geng ZY (2017a) Resveratrol alleviates heat stress-induced impairment of intestinal morphology, microflora, and barrier integrity in broilers. Poult Sci 96(12):4325–4332. https://doi.org/10.3382/ps/pex266

Zhang Q, Chen X, Eicher SD, Ajuwon KM, Applegate TJ (2017b) Effect of threonine on secretory immune system using a chicken intestinal ex vivo model with lipopolysaccharide challenge. Poult Sci 96(9):3043–3051. https://doi.org/10.3382/ps/pex111

Zhang H, Chen Y, Li Y, Zhang T, Ying Z, Su W et al (2019) l-Threonine improves intestinal mucin synthesis and immune function of intrauterine growth-retarded weanling piglets. Nutrition 59: 182–187. https://doi.org/10.1016/j.nut.2018.07.114

Zhao Y, Qin G, Sun Z, Che D, Bao N, Zhang X (2011) Effects of soybean agglutinin on intestinal barrier permeability and tight junction protein expression in weaned piglets. Int J Mol Sci 12 (12):8502–8512. https://doi.org/10.3390/ijms12128502

Zhao L, Sun LH, Huang JQ, Briens M, Qi DS, Xu SW, Lei XG (2017) A novel organic selenium compound exerts unique regulation of selenium speciation, selenogenome, and selenoproteins in broiler chicks. J Nutr 147:789–797

Zihni C, Mills C, Matter K, Balda MS (2016) Tight junctions. From simple barriers to multifunctional molecular gates. Nat Rev Mol Cell Biol 17(9):564–580. https://doi.org/10.1038/nrm.2016.80

Enzymes and Gut Health in Monogastric Animals: Effects Beyond Digestibility

3

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Abstract

Enzymes have an important role in animal nutrition due to their effects on increasing diet digestibility and animal performance. However, the impact of enzymes is more complex than only increasing digestion and absorption. In the last two decades, it has been shown that diet-supplemented enzymes influence host intestinal microbiota, physiology, immunity, and integrity. Diets supplemented with enzymes are an option to modulate animal's intestinal physiology since their enzymatic products can act as prebiotics, shift the site of digestion and absorption, reduce pathogenic bacteria, diminish the inflammatory response, and improve the quality of the epithelium. However, the outcome of the use of enzymes depends on the diet composition, environmental conditions, age and health status of the animal. Therefore, the current chapter aims to summarize the influence of enzymes on intestinal health and to provide current insights into the mechanisms behind its effects and the influential factors that are produced. Furthermore, we compare enzyme effects on the intestinal physiology in conjunction with performance parameters to support good decision-making process of adding enzymes to the monogastric diet.

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Keywords

Carbohydrase · Enzyme · Gut health · Lysozyme · Lipase · Protease

3.1 Introduction

Since the 1950s, when exogenous enzymes were first added in the diets of farm animals, exogenous feed enzymes (EFE) have been on focus of animal nutrition research (Adeola and Cowieson 2011). In the past 20 years, the enzyme market expanded for an estimated market of $1.3 billion in sales in 2018, with the estimated sales of $2.3 billion by 2026 (Ahuja and Rawat 2019). The increase in enzyme market has been due to the cost-benefit that enzymes provide in diet digestibility and animal performance (Walk and Bedford 2020).

Initially enzyme research concentrated on digestibility and animal growth performance. The main hypothesis was that the performance enhancement was only due to the action of enzymes breaking down complex molecules into digestible components for use by the animal. However, unexpected results were observed in feeding EFE where improved performance was found in animals provided with diets with low available substrates (Cowieson et al. 2010). Further, no effects on digesta viscosity were found in animals fed wheat diets supplemented with xylanase, but the animals exhibited better overall performance (Choct et al. 2004). These and more intriguing results raised two primary questions in the minds of nutritionists: "How are enzymes improving the growth performance of the animals? How are exogenous feed enzymes interacting with diet, microbiota, and the host?"

Therefore, in the past decade, research has been focused on identifying EFE functional activities in vivo, as well as their effects on intestinal physiology that benefits animal production. Currently, several mechanisms have been proposed for the beneficial effects of exogenous enzymes on animal gut health and the microbiota including: (1) shifting of digestion site in the intestine, (2) the production of prebiotics (Bedford 2000); (3) the improvement of intestinal mucosal integrity, (4) reducing intestinal inflammation (Bedford and Cowieson 2012); and (5) the reduction of undigested content in the caudal gut and improvement of tight junction integrity (Cowieson and Roos 2013).

Gut health has been the focus of animal scientists for years (Cummings et al. 2004; Kogut et al. 2016); yet, there is no clear agreement on a definition of "gut health." According to Kogut (2019) such a definition should combine a proper functioning of all the physiological roles of the organ, such as host metabolism and energy generation, stable microbiome, a good mucus layer, barrier function, proper immune response, and obviously, nutrient digestion and absorption. Currently, it is known that the intestine influences animal health and performance not just due to its primary function, but also because its neuroendocrine activity, the production of secondary messengers, and the production of microbiota metabolites that regulate several host physiological functions (Cani and Knauf 2016; Neuman et al. 2015; Weber 2010). Therefore, gut homeostasis and a functional diverse

microbiota are vital to the animals' health and welfare and to achieve good productivity parameters.

Correlations between exogenous enzymes and intestinal benefits have been observed for at least one decade of research. To the best of our knowledge, only few publications have focused on compiling the effects of EFE on gut health, but no publication has provided a holistic discussion of the effects of exogenous enzymes in intestine homeostasis. Therefore, the current review is aimed to compile results published with monogastric animals and to suggest how the use of enzymes may improve and regulate overall intestinal health.

3.2 Exogenous Feed Enzymes and Gut Health

The feed offered to animals in the field often contains several components that challenge gut homeostasis, stimulates the immune system, and impairs the performance of the animals (Dal Pont et al. 2020). Examples of these constituents are mycotoxins, rancid oils, anti-nutritional factors such as enzyme inhibitors and phytate, and non-digestible components such as keratin and fiber. Thus, the presence of these factors in the diet can trigger inflammation (Dal Pont et al. 2020). Ideally, the reduction of the quantity of these components in the diet would avoid unnecessary inflammation. However, ingredients with high non-desirable components are used due to their cost and availability; thus, strategies such as dietary supplementation of EFE which can reduce the amount of those components would be financially valuable.

The hydrolysis of macromolecules by certain enzymes changes the site where nutrients are released, impacting microbiota and its fermentation activity (Cowieson and Roos 2013). Other enzymes are correlated directly with antimicrobial effects, such as lysozymes (Wells et al. 2015; Nyachoti et al. 2012; Ellison III 2012). Therefore, the responses of EFE are directly dependent upon the substrate specificity of the enzyme and the composition of the feed. Moreover, different effects can be promoted with a combination of enzymes, and reports of additional and synergistic effects between enzymes are available in the literature (Woyengo et al. 2010; Selle et al. 2009; Zeller et al. 2015).

Due to the complexity of this topic, we will divide the current review into different categories of enzymes, according to their substrate, aiming to better describe their effects on the gut health. In addition to the several effects on the gut health and physiology of animals, we believe, like other scientists, that the ultimate judge of the efficacy and usage of enzymes should be performance parameters (Aftab and Bedford 2018; Walk and Bedford 2020). Thus, to facilitate assessment and technical judgment, we summarized the current literature on performance and gut health effects of enzymes in Table 3.1.

Table 3.1 Effect of exogenous feed enzyme supplementation on animal performance and intestinal health

Enzyme	Substrate	Performance effect	Gut effect	References
Lysozyme (egg source)	Peptidoglycan (gram – cell wall)	*Weaned pigs*: ≈ antibiotics, ↑BW, ↓conversion	*Weaned pigs*: ↑ villi height:crypt depth ↑ villus height ↓ *Campylobacter spp* ↓ serum pro-inflammatory molecules (challenge animals) ≈ antibiotics	(Oliver and Wells 2015; Wells et al. 2015; Oliver and Wells 2013; Oliver et al. 2014; Nyachoti et al. 2012)
Muramidase	Peptidoglycan (gram – cell wall)	*Pigs and broilers*: ↓FCR	*Broilers*: ↑ villi height: crypt depth ↓ ileal CD45 cells ↑ goblet cells ↓ leaky gut Alteration of microbiota	(Schliffka et al. 2019; Goodarzi Boroojeni et al. 2019; Sais et al. 2019; Goes et al. not published)
Xylanase	Xylans	*Broilers*: ↓FCR, ↑BW, and no effects *Pigs*: no effects	*Broilers*: ↑ cecal fermentation ↑ cecal SCFA ↑ villus height *Pigs*: no effects	(Choct et al. 1999; Cowieson and Masey O'Neill 2013; Taylor et al. 2018; Luo et al. 2009; Passos et al. 2015; Li et al. 2018a)
Xylanase + β-glucanase	Hemicellulose (xylans + β-glucans)	*Boilers*: ↓FCR *Weaned pigs*: no effects	*Broilers*: ↑ villi height: crypt depth ↑ cecal SCFA ↑ cecal microbiota concentration *Weaned pigs*: ↑ villus height ↓ fecal Coliforms ↓ intestinal mucosal macrophages	(Roofchaei et al. 2019; Jiang et al. 2015; Craig et al. 2020; Morgan et al. 2019a)

Cellulase + xylanase + β-glucanase	Cellulose + hemicellulose	*Weaned pigs*: ↑BW, ↑ADG	*Weaned pigs*: ↑ intestinal barrier ↓ IL-22 (blood)	(Li et al. 2018a)
β-Mannanase	β-Mannans (galactomannan, galactoglucomannan, and mannans)	*Broilers (challenged w/ Eimeria sp.)*: ↓FI, ↑FCR *Weaned pigs*: no effects	*Broilers (challenged w/ Eimeria sp.)*: ↓ crypt depth ↑ villi height:crypt depth ↑ intestinal and cecal microbiota diversity ↑ beneficial bacteria (*Lactobacillus, Ruminococcaceae,* and *Akkermansia*) ↓ feed-induced immune response *Weaned pigs*: ↑ intestinal integrity ↓ cecal *E. coli*	(Scapini et al. 2019; Bortoluzzi et al. 2019; Arsenault et al. 2017; Jang et al. 2020)
Protease	Proteins	*Broilers (challenged w/ Eimeria sp.)*: ↑BWG *Weaned pigs*: ↑BW, ↑ADG, and N/E	*Broilers (challenged w/ Eimeria sp.)*: ↑ mucus layer N/E coccidia lesions and oocysts shed *Weaned pigs*: ↓ diarrhea ↑ villi height: crypt depth ↑ intestinal digestive enzymes ↓ anaerobic bacteria in stomach and foregut ↓ immune response against soybean antigenic proteins	(Zuo et al. 2015b; Peek et al. 2009b; Dierick et al. 2004)

(continued)

Table 3.1 (continued)

Enzyme	Substrate	Performance effect	Gut effect	References
			↓ intraepithelial lymphocytes ↓ enterotoxigenic *E. coli* intestinal attachment	
Phytase	Phytate	*Broilers*: ↑BW; ↓FCR	*Weanling piglets*: ↑ SGLT1 (jejunum) N/E on small intestinal histomorphology	(Woyengo et al. 2011; Amerah et al. 2014; Akter et al. 2018)
Phytase—**high dose**	Phytate	*Broilers*: ↑ BW; ↓FCR *Weaned pigs*: ↑ BW; ↑ ADG; ↑ ADFI; ↑ feed efficiency	*Broilers*: ↑ cecal fermentation products ↑ GIT inositol concentration ↑ SMIT2 and MUC2 ↑ intestinal alkaline phosphatase ↓ IL-1β microbiota modulation *Weaned pigs*: ↑ GLUT4 (muscle)	(Ptak et al. 2015; Adedokun and Adeola 2016; Gautier et al. 2018; Jiang et al. 2018; Walk et al. 2018; Lu et al. 2019; Ajuwon et al. 2020; Zanu et al. 2020)

Lipase	Triglycerides	*Broilers*: ↑BW; ↓FCR *Weaned pigs*: no effects *Fish*: ↑final body weight; ↑% weight gain; ↑feed efficiency	*Broilers*: ↑ villus height ↑ villus height: crypt depth *Weaned pigs*: N/E on small intestinal histomorphology Bacterial suppression *Fish*: ↓ TNF-a, IL-1b, IFN-g2, and IL-8 ↑ LEAP-2, hepcidin, IL-10, TGF-b1 ↑ claudin b, claudin c and claudin 3, ↑ ZO-1 and occludin ↑ leukocytes phagocytosis activity	(Dierick et al. 2002; Liu et al. 2016; Fei et al. 2018; Hu et al. 2018; Liu et al. 2018)

For the table, only manuscripts that evaluate performance and intestinal parameters together were used
Symbols: ↑ increases/improve; ↓ decrease/reduction/worse; ≈ similar
Abbreviation: *ADFI* average daily feed intake, *ADG* Average daily gain, *BW* body weight, *FC* feed conversion, *FI* feed intake, *GIT* gastrointestinal tract, *GLUT4* glucose transporter type 4, *N/E* no effects observed, *LEAP-2* Liver-expressed antimicrobial peptide 2, *SCFA* short-chain fatty acids, *SMIT2* sodium-dependent myo-inositol transporter, *SGLT* sodium/glucose cotransporter; *w/* with, *MUC2* mucin 2, *ZO-1* zonula occludens-1

3.3 Carbohydrases

3.3.1 Non-starch Polysaccharide Enzymes

Plants carbohydrates are divided into starch and non-starch polysaccharides (NSP), also called fiber. Non-ruminant animals cannot digest NSP which have negative effects on the gut function and health in these animals (Józefiak et al. 2005). Some undesirable effects of the excess of soluble NSP (mainly arabinoxylans and β-glucans) include increased gut permeability (Latorre et al. 2015; Tellez et al. 2015), reduction of the beneficial microbiota, and increase of pathogenic bacteria in the gut (Langhout 2000). On the other hand, insoluble fiber, mainly present in the plant cell wall, shelters the cellular content producing a "cage effect" which reduces feed digestibility (Bedford and Partridge 2010). β-mannans reduce fat emulsification, decreasing the use of dietary fat (Campbell et al. 1983). Moreover, some NSP can be recognized by host pattern recognition receptors (PRRs), activating innate immune pathways. For example, the major NSP in soybean meal, β-galactomannan, is recognized by the host mannose receptor (Hsiao et al. 2006). The activation of PRRs can lead to chronic feed-induced inflammation that results in the diversion of energy from animal performance to the highly metabolic innate immune response (Kogut et al. 2018; Humphrey and Klasing 2004). Consequently, studies have been conducted to try to understand the mechanisms which NSP exogenous feed enzymes (NSPenz) improves animal performance.

Ingredients commonly used as substitutes for corn in animals feed such as wheat, barley, and sunflower meal (Waititu et al. 2018; Teymouri et al. 2018; Al-Harthi 2017) are rich in NSP (Raza et al. 2019). To reduce the negative effects of fibers to non-ruminants, NSPenz have been widely used by animal nutritionists. Usually, β-glucanases are recommended to be used in barley and oat-based diets and xylanases for wheat-based diets (Bedford and Partridge 2010). The inclusion of enzymes in high NSP diets can improve nutrients availability and digestibility (Saleh et al. 2018; Zhou et al. 2009). Supplementation of xylanases alone or in combination with β-glucanases reduces intestinal viscosity in diets with dried grains with solubles (DDGS) (Yildiz et al. 2018) and wheat/barley diets (Juanpere et al. 2005; Mathlouthi et al. 2002; Wu et al. 2004) which has resulted in an increase in the digestibility of other components of the diet.

However, the argument that NSP enzymes improve performance only due to the decrease of the digesta viscosity has been questioned. The questioning started after observations that the improvement of performance using different xylanases in a wheat-based diet has not always been consistent with the reduction in intestinal content viscosity (Choct et al. 2004). Moreover, research has been concentrated on providing an explanation for the improvement of performance observed when a corn-based diet, which has a small proportion of arabinoxylan, was supplemented with NSPenz (Aftab 2012). Thus, recent findings have claimed that the central role of the NSPenz is the modulation of the gut microbiota (Aftab and Bedford 2018). Józefiak et al. (2005) observed that β-glucanase inclusion in viscous broilers diet increased the concentration of acetate as well as the production of total short-chain

fatty acids (SCFA) in the ceca of chickens, indicating a correlation of the EFE with fermentative bacteria. Xylanase, for example, was correlated with reduced fermentation in the ileum and increased in the ceca when added to wheat-based broiler diets (Choct et al. 1999; Cowieson and Masey O'Neill 2013). Further, studies have shown that xylanases may produce prebiotic-like compounds that modulate the enteric microbiota such as short-chain xylans and xylo-oligosaccharides (Morgan et al. 2019b; Collins et al. 2005). This partial hydrolysis is already effective to reduce diet viscosity (Bedford and Partridge 2010). Additionally, these formed oligosaccharides are used as a substrate for *Lactobacillus* and *Bifidobacterium* species, increasing their population and reducing pathogenic bacteria such as *Clostridium perfringens* (Sun et al. 2015; Thammarutwasik et al. 2009). Thus, NSPenz fiber hydrolysis in the gut of animals reduces the viscosity of the lumen content, increases digestibility, and produces prebiotics that enhance the microbiota. Endorsing the "prebiotic formation" theory, further studies have shown that supplementation of prebiotic or NSPenz to broiler diets results in similar effects on production of cecal SCFA (Craig et al. 2020; Morgan et al. 2019a).

NSPenz supplementation may also be able to mitigate the negative effects of intestinal pathogens. Bortoluzzi et al. (2019) observed that β-mannanase supplementation increased the microbiota diversity indices in broilers regardless of the presence of a coccidiosis challenge. They also observed that β-mannanase supplementation reduced the frequency of *Faecalibacterium* on d 21 and increased the genus *Akkermansia* spp. on d 42 (Bortoluzzi et al. 2019). The *Akkermansia* spp. genus has been suggested as a biomarker for a healthy intestine (Png et al. 2010; Swidsinski et al. 2011). Moreover, supplementation of xylanase and β-glucanase decreased ileal *E. coli* and increased villus length in chickens on a wheat-based feed (Roofchaei et al. 2019). Implications and future paths of developing NSPenz with higher prebiotic-like action have been discussed by Aftab and Bedford (2018).

Due to the effects of NSPenz on microbiota modulation and gut integrity, swine studies have focused on piglets since post-weaning pigs are vulnerable to intestinal disorders and infections (Jiang et al. 2015). In weaning piglets, dietary β-mannanase supplementation had positive effects on intestinal integrity, reduced the counts of *E. coli* in the cecum, and increased fat digestibility by 8%, even though no effect on growth performance, immune response, and oxidative stress was observed (Jang et al. 2020). However, data is suggestive that an additive or synergistic effect of NSPenz combinations with a blend of enzymes improved gut health in pigs. In this context, studies have failed to show positive effects of xylanase alone on the intestine of pigs (Li et al. 2018a; Taylor et al. 2018; Passos et al. 2015). However, when Li and collaborators (2018a) compared xylanase with a carbohydrase blend (cellulase, β-glucanase, and xylanase), they observed that only the blend produced positive outcomes on weaned piglets fed a high fiber diet. The authors detected an improvement in the small intestinal barrier function with an increase in ileal claudin-3 mRNA expression, and reduction of immune activation expressed by the decline of ileal IL-22 gene expression and of urinary lactulose:mannitol recovery. Moreover, xylanase and β-glucanase supplementation can modulate the intestinal microbiota of piglets by decreasing *E. coli* and fecal coliform counts and can influence intestinal

tissue by increasing ileal villus height:crypt depth ratio (V:C ratio) and reducing mucosal macrophages (Jiang et al. 2015). Therefore, the combination of different carbohydrases might be able to effectively break the fiber that activates PRRs and modulate intestinal microbiota. Thus, the reduction of immune response and the presence of a healthier microbiota may also impact the mucosa morphology.

The reduction of the feed-induced immune response fiber triggers using NSPenz was also proved in chickens. Using the kinome array analysis, Arsenault et al. (2017) showed that β-mannanase eliminated most of the immune-related signaling promoted by β-galactomannan in the jejunum of chickens. Furthermore, the authors observed that β-mannanase as an EFE significantly altered several intestinal metabolic/growth processes as well as gut integrity-related pathways.

Therefore, NSPenz can be used to enhance intestinal health by modulating the microbiota, increasing SCFA production, and reducing pathogenic bacteria and inflammation on the intestine. Also, NSPenz can reduce the immune stimulation that fiber components can trigger in the gut, improving epithelial barrier function.

3.3.2 Amylase

Studies with amylase supplementation alone to evaluate intestinal effects besides digestibility are scarce which prevents a proper discussion and makes a definitive conclusion tentative, at best. Yin and collaborators (2018) evaluated the inclusion of amylase and glucoamylase in a diet formulated with newly harvested corn to broilers. The inclusion of the carbohydrases induced a partial reduction of the negative effects caused by corn in the intestine, and a higher villus height and V:C ratio in the broilers fed the diets containing the enzymes. The animals in the same group also showed an increase in the family Lactobacillaceae, specifically with abundance of *Lactobacillus*. This study showed beneficial results regarding the use of this enzyme; however, the dietary inclusion of the enzymes was a tentative to reduce the defective effects newly harvested corn produces, thus perhaps the same effects will not be replicated with different diets.

3.3.3 Lysozyme and Muramidase

The usage of enzymes targeting elements naturally present in the gut instead of components present in the feed has been tried in animal diets over the past decade. Specifically, lysozyme, an enzyme naturally found in body secretions which cleaves peptidoglycans (PNG) (Oliver and Wells 2015). Lysozyme is a 1,4-β-*N*-acetylmuramidase with antimicrobial effects due to its ability to break PNG present in bacterial cell walls thus promoting cell death (Ellison III 2012).

In the past, lysozyme supplementation in the diets of animals was made through transgenic vectors that deliver the enzyme. For example, with the supplementation of transgenic goat milk, the enzyme was added to pigs diet which produced changes in metabolites profile, intestinal microbiota, and morphology (Brundige et al. 2010;

Maga et al. 2006; Brundige et al. 2008). Also, transgenic rice expressing lysozyme was used in chicken feed and produced antibiotic-like effects on the performance and intestine of chicks (Humphrey et al. 2002).

The production of a granulated lysozyme sourced from chicken eggs has made it easier to study lysozyme as an EFE and the results have been promising. Because the sow milk is very low in lysozyme (Oliver and Wells 2015) and piglets frequently have health issues due to dysbiosis and intestinal infection, studies had tested the lysozyme inclusion in nursery pigs. Oliver and Wells (2013) observed that supplementation of granulated lysozyme to weaned pigs improved growth rate and increased jejunum villi height, and V:C ratio, that were comparable to the groups provided with antibiotic supplementation. Egg lysozyme might reduce the bacterial load in the intestine reducing dysbiosis that normally occurs in pigs after weaning and be transferred to the nursery. Wells et al. (2015) detected a decrease in *Campylobacter spp.* and a tendency to reduce enterohemorrhagic *E. coli* shed with the enzyme supplementation to nursery piglets. Weaned pigs challenged with enterotoxigenic *E. coli* (ETEC) had lower ETEC counts when lysozyme was included in the diet (Nyachoti et al. 2012). Additionally, the study showed that the enzyme groups had several parameters comparable to the antibiotic-treated group (chlortetracycline, sulfamethazine, and penicillin), including increased small intestinal weight, longer villus, and reduced pro-inflammatory cytokines. Moreover, piglets exposed to dirty nursery conditions benefited from lysozyme supplementation showing the same growth rates and lower serum TNF-α, haptoglobin and C-reactive protein as the pigs fed an antibiotic diet (Oliver et al. 2014).

An alternative to egg sourced lysozyme, a novel lysozyme called muramidase 007, produced by a *Trichoderma reesei* strain, has emerged. The enzyme has been tested and passed toxicological and tolerance tests in broilers and pigs (Lichtenberg et al. 2017; Schliffka et al. 2019). Studies demonstrated an improvement in feed conversion ratio in broilers and pigs (Table 3.1), but inconsistent results in gut parameters. Goodarzi et al. evaluated muramidase supplementation in broiler diets and observed a linear increase in V:C ratio and decrease in ileal immune cells (CD45 type) (Goodarzi Boroojeni et al. 2019). Sais and collaborators (2019) observed an alteration in the cecal microbiota composition with an increase in *Lactobacillus* genus, an increase of goblet cells and intraepithelial lymphocytes in chickens, but no differences in V:C ratio. Moreover, chickens supplemented with muramidase showed lower intestinal permeability (FITC-d) and occurrence of footpad dermatitis (Goes and Dal Pont, data not published).

Thus, lysozyme supplementation indicates important benefits in gut health especially to young pigs due to its antimicrobial effects. In chickens, results are suggestive of an improvement in the intestinal morphology and barrier function. However, further research with the novel lysozyme sources is necessary to understand their effects on animal performance and gut health under different sanitary and nutritional conditions.

3.4 Proteases

High protein diets, ingredients with low digestibility or physiologic factors, as the low-protein digestibility in piglets, can increase the quantity of intact protein that arrives in the intestine. A high quantity of protein in the lower gut enhances putrefactive fermentation (Silvester and Cummings 1995) which produces cytotoxic, genotoxic, and carcinogenic compounds (Hughes et al. 2000; Toden et al. 2005). Thus, protein concentration in the diet and its source impacts the gut microbiota and intestinal morphology of animals (Drew et al. 2004; Dahiya et al. 2006; Wilkie et al. 2005). Reduction of crude protein in the diet can have positive outcomes, as for example decreased aerobic mesophilic bacteria and *E. coli* counts in broiler excreta (Laudadio et al. 2012).

Therefore, enzymes that hydrolyze protein can be used as EFE to reduce these negative impacts on the animal intestine. The correlation between protease supplementation in animal feed and improvement in gut health has been established. In broilers, protease inclusion in a corn/wheat/soy-based diet reduced the effects of coccidiosis infection, increased intestinal mucus layer, and improved weight gain (Peek et al. 2009a). These enzymes have also been associated with epithelial integrity, by upregulation of claudin-1 gene expression in broilers (Cowieson et al. 2017a) and increasing villus height and decreasing in crypt depth in broilers and weaned pigs (Zuo et al. 2015a; Wang et al. 2008). Moreover, after weaning, piglets suffer from transient hypersensitivity to soybean protein (Dierick et al. 2004) and a dramatic decrease in stomach and pancreatic enzyme activity (Hedemann and Jensen 2004). Consequently, proteases can be a valuable strategy to reduce some frequent intestinal problems in the nursery. Supplementation of microbial protease to weaned pigs reduced the immune response against soy protein (intestinal and serum level) (Dierick et al. 2004) and attenuated the damage on intestinal morphology caused by the vegetable protein source (Zuo et al. 2015b). The same studies showed a reduction in anaerobic bacteria levels in the stomach and foregut and reduction of diarrhea with protease supplementation.

The specific manner by which proteases act on the intestine to induce such results in gut health and animal performance is still unclear. Current results indicate the shift in protein digestion (Liu et al. 2013) allied with reduction of the protein fermentation and its putrefactive products (Windey et al. 2012), which may modulate microbiota to a better profile and reduce harmful products that challenge the GIT. The lysis of proteinaceous antinutrients and antigenic proteins (Ghazi et al. 2002; Rooke et al. 1998) can be another factor involving the optimizations of gut health with proteases supplementation. Moreover, protease usage as EFE may support barrier function of the intestinal mucosa. Studies have shown improvements in the tight junctions with protease supplementation (Cowieson et al. 2017a), which may be correlated with the increase in amino acids availability for tight junction protein synthesis and mucin production (Cowieson and Roos 2013).

3.5 Phytase

Phosphorus is closely related to the animals' metabolism since it is involved in the biochemical functions of cells and metabolic processes such as energy use and bone mineralization (Oster et al. 2016). However, in plant-based feed ingredients, a large amount of the mineral (60–70%) is complexed with the phytic acid molecule, and non-ruminant animals do not have effective endogenous phytase to hydrolyze the phytic acid (Woyengo and Nyachoti 2013). Phytic acid, commonly known as phytate, consists of a ring of myo-inositol associated with six phosphate anions (IP6) which are capable of complex with other minerals in the GIT of animals, as well as with protein and lipids (Singh and Satyanarayana 2015). This complex can interfere with the activity of endogenous enzymes affecting digestion and utilization of nutrients, which may be considered an anti-nutritional factor. Thus, the dephosphorylation of the phytate by exogenous phytase releases the P for absorption as well as the nutrients that were complexed to the phytate molecule. Moreover, a complete dephosphorylation of the phytic acid (IP6) will produce one molecule of myo-inositol and six radicals of inorganic P (Selle and Ravindran 2007). The complete dephosphorylation is associated with high doses of phytase ($>$1500 FTU kg^{-1}), that may originate P from IP2 or IP3 esters, which have a lower chelating capacity (Cowieson et al. 2017b) and myo-inositol (Walk et al. 2018).

The higher availability of P due to the phytase supplementation has been correlated with beneficial changes in the intestinal microbiota of animals (Ptak et al. 2015) and with upregulated expression of intestinal alkaline phosphatase in chickens, complementing the phytate degradation activity by exogenous phytase (Palacios et al. 2008) and providing better performance and health of the animals. Tilocca et al. (2016) identified changes in microbiome functions in the GIT of broilers by metaproteomics analysis and correlated with the variation of phosphorus accessibility. According to the authors, mineral or enzyme supplementation increased the relative abundance of the microbiota and improved its profile.

Additionally, high doses of phytase have been associated with improvements in animal performance and health due to the so-called extra-phosphoric effects of phytase. These effects include maintaining intestinal integrity in broilers due to a better mucus layer, by increased gene expression of mucin 2 (MUC2), one of the genes related to mucin synthesis (Ajuwon et al. 2020). Jiang and collaborators (2018) observed, in addition to the upregulated MUC2 expression, a reduction in IL-1β expression and correlated the best performance of chickens fed with high doses of phytase with the reduction of the intestinal inflammatory process. In addition, birds with a GIT compromised by *Eimeria* sp. or *Clostridium perfringens* can benefit from higher doses of phytase due to the reduction of nutrients available to the microorganisms (Adedokun and Adeola 2016; Zanu et al. 2020).

Moreover, the presence of the myo-inositol molecule in the intestine has gained relevance in physiology and gut health studies mainly because of its biological importance in several metabolic functions, including bone mineralization, lipid metabolism, nervous system development, and reproduction (Gonzalez-Uarquin

et al. 2020). It has been demonstrated that dietary myo-inositol deficiency can be responsible for damaging the physical and immune intestinal barrier in grass carp (Li et al. 2017). According to the authors, the deficiency can decrease antioxidant capacity, upregulated genes involving in cell apoptosis, and downregulated the expression of genes responsible for cell proliferation. In addition, according to Li and collaborators (2018b) myo-inositol deficiency may lead to a reduction in the resistance of pathogens of grass carp by the upregulation of pro-inflammatory cytokines (IL-1β, TNF-α, and IL-8) and downregulation of anti-inflammatory cytokines (IL-10, IL-11, IL4-13B, TGF-β1, and TGF-β2). Also, an increased concentration of myo-inositol in the GIT of animals due to high phytate doses may cause an upregulation of nutrient transporters, since this molecule is a precursor to the phospholipid phosphatidylinositol present in cell membranes (Ajuwon et al. 2020). It has been reported that the insulin-like effects of myo-inositol may increase the use of glucose by animals (Lee and Bedford 2016) via intracellular glucose transporter type 4 (GLUT4) in the muscle of weanling piglets (Lu et al. 2019), and via sodium/glucose cotransporter 1 (SGLT1) in the jejunum of piglets (Woyengo et al. 2011). Cowieson et al. (2016) reported a relationship between the presence of low esters of inositol and free myo-inositol and diverse biochemical pathways, including those responsible for muscle deposition.

Therefore, the compiled information herein suggests that besides the beneficial effects of phytase on the release of P and other minerals, the enzyme may support microbiota modulation and exert anti-inflammatory effects by the action of myo-inositol. All these mechanisms of phytase action might exert a key role in the improvement of gut health and growth performance of the animals.

3.6 Lipase

The inclusion of high levels of fats and oils and their fatty acid profile can cause digestive and metabolic implications (Rodriguez-Sanchez et al. 2019) especially in young broilers due to the low level of natural lipase production (Al-Marzooqi and Leeson 2000). Studies in mice have suggested that a high-fat diet alters the gut microbiota, increases intestinal permeability, and leads to gut inflammation (Cani et al. 2008; Laugerette et al. 2012). According to Kim and colleagues (2012) the alteration of the gut microbiota by high-fat diets in mice increases the concentration of pro-inflammatory endotoxins in the lumen increasing the intestinal permeability and inducing inflammation of the adipose tissue. Zheng and collaborators (2020) found that the negative effect of a diet with high lipids on the immune performance of fish could be correlated with the incomplete hydrolysis of these components in the intestine due to limited endogenous lipase activities. Therefore, the dietary supplementation of exogenous lipase has been investigated to optimize the use of the lipid sources to avoid triggering undesirable immune response.

Although the dietary inclusion of exogenous lipase is not a common practice currently by the livestock industry, studies have reported benefits of using this additive. Besides the positive effects of lipase supplementation on growth

performance, nutrient digestibility, and intestinal morphology of broilers and weaning pigs (Wang et al. 2018; Hu et al. 2018; Liu et al. 2018), recent studies have concentrated efforts to identify the potential molecular mechanisms of the enzyme on the gut health. Liu et al. (2016) reported that exogenous lipase supplementation in high-fat and low-protein diets improved in a dose-dependent way the intestinal functionality and health status of grass carp by downregulating mRNA expression of pro-inflammatory cytokines (TNF-a, IL-1b, IFN-g2, and IL-8), and upregulating antimicrobial peptides (LEAP-2 and hepcidin), anti-inflammatory cytokines (IL-10 and TGF-β1), and intestinal tight junction proteins (claudin b, claudin c and claudin 3, ZO-1, and occludin) gene expression. Fei and collaborators (2018) found that the supplementation of *Yarrowia lipolytica* lipase 2 in *Hybrid sturgeon* diets improves the physical barrier of the intestine and the immune function. In this study, the enzyme improved lysozyme activity (skin and serum), serum peroxidase and alternative complement pathway activity, reactive oxygen species level, and phagocytosis activity of leukocytes. In addition, studies evaluating medium-chain triglycerides as feed additive showed that the exogenous lipase can hydrolyze the triglycerides releasing biologically active medium-chain fatty acids (MCFA) supporting the animal's performance and health (Dierick et al. 2002). According to the authors, medium-chain fatty acids released in the GIT may produce bacterial suppression. As MCFA disrupts the phospholipid membrane of the bacteria, they have antimicrobial activity (Jackman et al. 2020). Therefore, it can be hypothesized that one of the beneficial effects of lipase on the intestinal health of animals is related to the release of MCFA from lipid sources, which would particularly benefit young chicks due to their low endogenous lipase activity and lipid emulsification.

Thus, even the studies about exogenous lipase are still scarce, the previous research data indicate that the supplementation of exogenous lipase can exert beneficial effects on the gut health of animals, being a precedent to further studies on the supplementation of this additive in animals' diets.

3.7 Conclusions

Exogenous feed enzymes can have innumerous impacts on the intestine and if applied correctly can be used to ameliorate gut health. Their main effects that influence intestine are the modulation of microbiota and reducing harmful components in the gut lumen. Non-starch polysaccharide enzymes, for example, can reduce fiber that activates the immune system and, additionally, produce prebiotics that is used by the beneficial microbiota. Lysozymes may reduce the bacterial load in the lumen and pathogens, improving intestinal health. Proteases act reducing protein available for undesirable bacterial in the lower gut, as well as decreasing the amount of antigenic protein, especially for piglets. On the other hand, phytase makes available several nutrients chelated in the phytate and, besides releasing phosphorus, might liberate myo-inositol in higher dosages, which seems to have a role in various physiologic pathways. Moreover, lipases seem to show a

beneficial effect on intestinal morphology and possible bactericidal secondary effects. Although the action of enzymes on different substrates can vary broadly due to the diet composition and formulation, EFE seems to beneficially alter the intestinal physiology, immunology, and microbiology of monogastric animals. Thus, exogenous enzymes supplementation should be planned according to the diet profile as well as flock age and history to bring the desirable effects on gut health.

References

Adedokun SA, Adeola O (2016) The response in jejunal and ileal nutrient and energy digestibility and the expression of markers of intestinal inflammation in broiler chickens to coccidial vaccine challenge and phytase supplementation. Can J Anim Sci 97(2):258–267, 210

Adeola O, Cowieson AJ (2011) Board-invited review: opportunities and challenges in using exogenous enzymes to improve nonruminant animal production. J Anim Sci 89(10):3189–3218. https://doi.org/10.2527/jas.2010-3715

Aftab U (2012) Exogenous carbohydrase in corn-soy diets for broilers. Worlds Poult Sci J 68(3):447–464. https://doi.org/10.1017/S0043933912000566

Aftab U, Bedford MR (2018) The use of NSP enzymes in poultry nutrition: myths and realities. Worlds Poult Sci J 74(2):277–286. https://doi.org/10.1017/S0043933918000272

Ahuja K, Rawat A (2019) Animal Feed Enzymes Market Size By Form (Dry, Liquid), By Product (Phytase, Carbohydrase, Protease, Non-Starch Polysaccharide), By Livestock (Poultry, Swine, Aquaculture, Ruminant) Industry Analysis Report, Regional Outlook, Growth Potential, Price Trend, Competitive Market Share & Forecast, 2019–2026. Global Marker insights. https://www.gminsights.com/industryanalysis/animal-feed-enzymes-market. Accessed Jun 16 2020

Ajuwon KM, Sommerfeld V, Paul V, Däuber M, Schollenberger M, Kühn I, Adeola O, Rodehutscord M (2020) Phytase dosing affects phytate degradation and Muc2 transporter gene expression in broiler starters. Poult Sci 99(2):981–991

Akter MM, Graham H, Iji PA (2018) Influence of different levels of calcium, non-phytate phosphorus and phytase on apparent metabolizable energy, nutrient utilization, plasma mineral concentration and digestive enzyme activities of broiler chickens. J Appl Anim Res 46(1):278–286

Al-Harthi M (2017) The effect of olive cake, with or without enzymes supplementation, on growth performance, carcass characteristics, lymphoid organs and lipid metabolism of broiler chickens. Braz J Poult Sci 19(SPE):83–90

Al-Marzooqi W, Leeson S (2000) Effect of dietary lipase enzyme on gut morphology, gastric motility, and long-term performance of broiler chicks. Poult Sci 79(7):956–960

Amerah A, Plumstead P, Barnard L, Kumar A (2014) Effect of calcium level and phytase addition on ileal phytate degradation and amino acid digestibility of broilers fed corn-based diets. Poult Sci 93(4):906–915

Arsenault R, Lee J, Latham R, Carter B, Kogut M (2017) Changes in immune and metabolic gut response in broilers fed β-mannanase in β-mannan-containing diets. Poult Sci 96. https://doi.org/10.3382/ps/pex246

Bedford M (2000) Removal of antibiotic growth promoters from poultry diets: implications and strategies to minimise subsequent problems. Worlds Poult Sci J 56(4):347–365. https://doi.org/10.1079/WPS20000024

Bedford MR, Cowieson AJ (2012) Exogenous enzymes and their effects on intestinal microbiology. Anim Feed Sci Technol 173(1):76–85. https://doi.org/10.1016/j.anifeedsci.2011.12.018

Bedford MR, Partridge GG (2010) Enzymes in farm animal nutrition. CABI

Bortoluzzi C, Scapini LB, Ribeiro MV, Pivetta MR, Buzim R, Fernandes JIM (2019) Effects of β-mannanase supplementation on the intestinal microbiota composition of broiler chickens challenged with a coccidiosis vaccine. Livest Sci 228:187–194. https://doi.org/10.1016/j.livsci.2019.09.001

Brundige DR, Maga EA, Klasing KC, Murray JD (2008) Lysozyme transgenic goats' milk influences gastrointestinal morphology in young pigs. J Nutr 138(5):921–926

Brundige DR, Maga EA, Klasing KC, Murray JD (2010) Consumption of pasteurized human lysozyme transgenic goats' milk alters serum metabolite profile in young pigs. Transgenic Res 19(4):563–574

Campbell GL, Classen HL, Goldsmith KA (1983) Effect of fat retention on the rachitogenic effect of Rye fed to broiler chicks. Poult Sci 62(11):2218–2223. https://doi.org/10.3382/ps.0622218

Cani PD, Knauf C (2016) How gut microbes talk to organs: the role of endocrine and nervous routes. Mol Metab 5(9):743–752. https://doi.org/10.1016/j.molmet.2016.05.011

Cani PD, Bibiloni R, Knauf C, Waget A, Neyrinck AM, Delzenne NM, Burcelin R (2008) Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat diet–induced obesity and diabetes in mice. Diabetes 57(6):1470–1481

Choct M, Hughes RJ, Bedford MR (1999) Effects of a xylanase on individual bird variation, starch digestion throughout the intestine, and ileal and caecal volatile fatty acid production in chickens fed wheat. Br Poult Sci 40(3):419–422. https://doi.org/10.1080/00071669987548

Choct M, Kocher A, Waters DL, Pettersson D, Ross G (2004) A comparison of three xylanases on the nutritive value of two wheats for broiler chickens. Br J Nutr 92(1):53–61. https://doi.org/10.1079/bjn20041166

Collins T, Gerday C, Feller G (2005) Xylanases, xylanase families and extremophilic xylanases. FEMS Microbiol Rev 29(1):3–23

Cowieson AJ, Masey O'Neill HV (2013) Effects of exogenous xylanase on performance, nutrient digestibility and caecal thermal profiles of broilers given wheat-based diets. Br Poult Sci 54(3):346–354. https://doi.org/10.1080/00071668.2013.780200

Cowieson AJ, Roos FF (2013) Bioefficacy of a mono-component protease in the diets of pigs and poultry: a meta-analysis of effect on ileal amino acid digestibility. J Appl Anim Nutr 2:e13. https://doi.org/10.1017/jan.2014.5

Cowieson AJ, Bedford MR, Ravindran V (2010) Interactions between xylanase and glucanase in maize-soy-based diets for broilers. Br Poult Sci 51(2):246–257. https://doi.org/10.1080/00071661003789347

Cowieson A, Ruckebusch J, Knap I, Guggenbuhl P, Fru-Nji F (2016) Phytate-free nutrition: a new paradigm in monogastric animal production. Anim Feed Sci Technol 222:180–189

Cowieson A, Lu H, Ajuwon K, Knap I, Adeola O (2017a) Interactive effects of dietary protein source and exogenous protease on growth performance, immune competence and jejunal health of broiler chickens. Anim Prod Sci 57(2):252–261

Cowieson A, Ruckebusch J-P, Sorbara J, Wilson J, Guggenbuhl P, Roos F (2017b) A systematic view on the effect of phytase on ileal amino acid digestibility in broilers. Anim Feed Sci Technol 225:182–194

Craig AD, Khattak F, Hastie P, Bedford MR, Olukosi OA (2020) The similarity of the effect of carbohydrase or prebiotic supplementation in broilers aged 21 days, fed mixed cereal diets and challenged with coccidiosis infection. PLoS One 15(2):e0229281. https://doi.org/10.1371/journal.pone.0229281

Cummings JH, Antoine J-M, Azpiroz F, Bourdet-Sicard R, Brandtzaeg P, Calder PC, Gibson GR, Guarner F, Isolauri E, Pannemans D, Shortt C, Tuijtelaars S, Watzl B (2004) PASSCLAIM1—gut health and immunity. Eur J Nutr 43(2):ii118–ii173. https://doi.org/10.1007/s00394-004-1205-4

Dahiya J, Wilkie D, Van Kessel A, Drew M (2006) Potential strategies for controlling necrotic enteritis in broiler chickens in post-antibiotic era. Anim Feed Sci Technol 129(1–2):60–88

Dal Pont GC, Farnell M, Farnell Y, Kogut MH (2020) Dietary factors as triggers of low-grade chronic intestinal inflammation in poultry. Microorganisms 8(1):139

Dierick NA, Decuypere JA, Molly K, Van Beek E, Vanderbeke E (2002) The combined use of triacylglycerols (TAGs) containing medium chain fatty acids (MCFAs) and exogenous lipolytic enzymes as an alternative to nutritional antibiotics in piglet nutrition: II. In vivo release of MCFAs in gastric cannulated and slaughtered piglets by endogenous and exogenous lipases; effects on the luminal gut flora and growth performance. Livest Prod Sci 76(1):1–16. https://doi.org/10.1016/S0301-6226(01)00331-1

Dierick N, Decuypere J, Molly K, Vanderbeke E (2004) Microbial protease addition to a soybean meal diet for weaned piglets: effects on performance, digestion, gut flora and gut function. EAAP publication, no. 110. Wageningen Pers, Wageningen

Drew M, Syed N, Goldade B, Laarveld B, Van Kessel A (2004) Effects of dietary protein source and level on intestinal populations of Clostridium perfringens in broiler chickens. Poult Sci 83(3):414–420

Ellison RT III (2012) The effects of lactoferrin on gram-negative. Lactoferrin Struct Funct 357:71–72

Fei H, Lin G-D, Zheng C-C, Huang M-M, Qian S-C, Wu Z-J, Sun C, Shi Z-G, Li J-Y, Han B-N (2018) Effects of Bacillus amyloliquefaciens and Yarrowia lipolytica lipase 2 on immunology and growth performance of hybrid sturgeon. Fish Shellfish Immunol 82:250–257. https://doi.org/10.1016/j.fsi.2018.08.031

Gautier A, Walk C, Dilger R (2018) Effects of a high level of phytase on broiler performance, bone ash, phosphorus utilization, and phytate dephosphorylation to inositol. Poult Sci 97(1):211–218

Ghazi S, Rooke J, Galbraith H, Bedford M (2002) The potential for the improvement of the nutritive value of soya-bean meal by different proteases in broiler chicks and broiler cockerels. Br Poult Sci 43(1):70–77

Gonzalez-Uarquin F, Rodehutscord M, Huber K (2020) Myo-inositol: its metabolism and potential implications for poultry nutrition—a review. Poult Sci 99(2):893–905. https://doi.org/10.1016/j.psj.2019.10.014

Goodarzi Boroojeni F, Männer K, Rieger J, Pérez Calvo E, Zentek J (2019) Evaluation of a microbial muramidase supplementation on growth performance, apparent ileal digestibility, and intestinal histology of broiler chickens. Poult Sci 98(5):2080–2086. https://doi.org/10.3382/ps/pey556

Hedemann MS, Jensen BB (2004) Variations in enzyme activity in stomach and pancreatic tissue and digesta in piglets around weaning. Arch Anim Nutr 58(1):47–59. https://doi.org/10.1080/00039420310001656677

Hsiao H-Y, Anderson D, Dale N (2006) Levels of β-mannan in soybean meal. Poult Sci 85(8):1430–1432

Hu Y, Lan D, Zhu Y, Pang H, Mu X, Hu X (2018) Effect of diets with different energy and lipase levels on performance, digestibility and carcass trait in broilers. Asian Australas J Anim Sci 31(8):1275

Hughes R, Magee E, Bingham S (2000) Protein degradation in the large intestine: relevance to colorectal cancer. Curr Issues Intest Microbiol 1(2):51–58

Humphrey B, Klasing K (2004) Modulation of nutrient metabolism and homeostasis by the immune system. Worlds Poult Sci J 60(1):90–100

Humphrey BD, Huang N, Klasing KC (2002) Rice expressing lactoferrin and lysozyme has antibiotic-like properties when fed to chicks. J Nutr 132(6):1214–1218. https://doi.org/10.1093/jn/132.6.1214

Jackman JA, Boyd RD, Elrod CC (2020) Medium-chain fatty acids and monoglycerides as feed additives for pig production: towards gut health improvement and feed pathogen mitigation. J Anim Sci Biotechnol 11(1):44. https://doi.org/10.1186/s40104-020-00446-1

Jang J-C, Kim KH, Jang YD, Kim YY (2020) Effects of dietary β-mannanase supplementation on growth performance, apparent total tract digestibility, intestinal integrity, and immune responses in weaning pigs. Animals 10(4):703

Jiang XR, Awati A, Agazzi A, Vitari F, Ferrari A, Bento H, Crestani M, Domeneghini C, Bontempo V (2015) Effects of a blend of essential oils and an enzyme combination on nutrient digestibility,

ileum histology and expression of inflammatory mediators in weaned piglets. Animal 9(3):417–426. https://doi.org/10.1017/s1751731114002444

Jiang SQ, Lamont SJ, Persia ME (2018) Differential growth performance and intestinal immune gene expression in diverse genetic lines of growing chickens fed a high concentration of supplemental phytase. J Agric Sci 156(2):258–264. https://doi.org/10.1017/S0021859618000096

Józefiak D, Kaczmarek S, Rutkowski A, Józefiak A, Jensen BB, Engberg RM (2005) Fermentation in broiler chicken gastrointestinal tract as affected by high dietary inclusion of barley and by β-glucanase supplementation. J Anim Feed Sci 14(4):695–704. https://doi.org/10.22358/jafs/67156/2005

Juanpere J, Perez-Vendrell A, Angulo E, Brufau J (2005) Assessment of potential interactions between phytase and glycosidase enzyme supplementation on nutrient digestibility in broilers. Poult Sci 84(4):571–580

Kim KA, Gu W, Lee IA, Joh EH, Kim DH (2012) High fat diet-induced gut microbiota exacerbates inflammation and obesity in mice via the TLR4 signaling pathway. PLoS One 7(10):e47713. https://doi.org/10.1371/journal.pone.0047713

Kogut MH (2019) The effect of microbiome modulation on the intestinal health of poultry. Anim Feed Sci Technol 250:32–40

Kogut MH, Genovese KJ, He H, Arsenault RJ (2016) AMPK and mTOR: sensors and regulators of immunometabolic changes during salmonella infection in the chicken. Poult Sci 95(2):345–353. https://doi.org/10.3382/ps/pev349

Kogut MH, Genovese KJ, Swaggerty CL, He H, Broom L (2018) Inflammatory phenotypes in the intestine of poultry: not all inflammation is created equal. Poult Sci 97(7):2339–2346. https://doi.org/10.3382/ps/pey087

Langhout P (2000) New additives for broiler chickens. World Poult 16(3):22–27

Latorre JD, Hernandez-Velasco X, Bielke LR, Vicente JL, Wolfenden R, Menconi A, Hargis BM, Tellez G (2015) Evaluation of a Bacillus direct-fed microbial candidate on digesta viscosity, bacterial translocation, microbiota composition and bone mineralisation in broiler chickens fed on a rye-based diet. Br Poult Sci 56(6):723–732. https://doi.org/10.1080/00071668.2015.1101053

Laudadio V, Dambrosio A, Normanno G, Khan RU, Naz S, Rowghani E, Tufarelli V (2012) Effect of reducing dietary protein level on performance responses and some microbiological aspects of broiler chickens under summer environmental conditions. Avian Biol Res 5(2):88–92. https://doi.org/10.3184/175815512x13350180713553

Laugerette F, Furet J-P, Debard C, Daira P, Loizon E, Géloën A, Soulage CO, Simonet C, Lefils-Lacourtablaise J, Bernoud-Hubac N (2012) Oil composition of high-fat diet affects metabolic inflammation differently in connection with endotoxin receptors in mice. Am J Physiol-Endocrinol Metab 302(3):E374–E386

Lee S, Bedford M (2016) Inositol-an effective growth promotor? Worlds Poult Sci J 72(4):743–760

Li S-A, Jiang W-D, Feng L, Liu Y, Wu P, Jiang J, Kuang S-Y, Tang L, Tang W-N, Zhang Y-A, Tang X, Shi H-Q, Zhou X-Q (2017) Dietary myo-inositol deficiency decreased the growth performances and impaired intestinal physical barrier function partly relating to nrf2, jnk, e2f4 and mlck signaling in young grass carp (Ctenopharyngodon idella). Fish Shellfish Immunol 67:475–492. https://doi.org/10.1016/j.fsi.2017.06.032

Li Q, Gabler NK, Loving CL, Gould SA, Patience JF (2018a) A dietary carbohydrase blend improved intestinal barrier function and growth rate in weaned pigs fed higher fiber diets1. J Anim Sci 96(12):5233–5243. https://doi.org/10.1093/jas/sky383

Li S-A, Jiang W-D, Feng L, Liu Y, Wu P, Jiang J, Kuang S-Y, Tang L, Tang W-N, Zhang Y-A (2018b) Dietary myo-inositol deficiency decreased intestinal immune function related to NF-κB and TOR signaling in the intestine of young grass carp (Ctenopharyngodon idella). Fish Shellfish Immunol 76:333–346

Lichtenberg J, Perez Calvo E, Madsen K, Østergaard Lund T, Kramer Birkved F, van Cauwenberghe S, Mourier M, Wulf-Andersen L, Jansman AJM, Lopez-Ulibarri R (2017)

Safety evaluation of a novel muramidase for feed application. Regul Toxicol Pharmacol 89:57–69. https://doi.org/10.1016/j.yrtph.2017.07.014

Liu S, Selle P, Court S, Cowieson A (2013) Protease supplementation of sorghum-based broiler diets enhances amino acid digestibility coefficients in four small intestinal sites and accelerates their rates of digestion. Anim Feed Sci Technol 183(3–4):175–183

Liu S, Feng L, Jiang W-D, Liu Y, Jiang J, Wu P, Zeng Y-Y, Xu S-D, Kuang S-Y, Tang L, Tang W-N, Zhang Y-A, Zhou X-Q (2016) Impact of exogenous lipase supplementation on growth, intestinal function, mucosal immune and physical barrier, and related signaling molecules mRNA expression of young grass carp (Ctenopharyngodon idella). Fish Shellfish Immunol 55:88–105. https://doi.org/10.1016/j.fsi.2016.05.006

Liu J, Cao S, Liu J, Pu J, Chen L, Zhang H (2018) Effects of dietary energy and lipase levels on nutrient digestibility, digestive physiology and noxious gas emission in weaning pigs. Asian Australas J Anim Sci 31(12):1963

Lu H, Kühn I, Bedford MR, Whitfield H, Brearley C, Adeola O, Ajuwon KM (2019) Effect of phytase on intestinal phytate breakdown, plasma inositol concentrations, and glucose transporter type 4 abundance in muscle membranes of weanling pigs1. J Anim Sci 97(9):3907–3919. https://doi.org/10.1093/jas/skz234

Luo D, Yang F, Yang X, Yao J, Shi B, Zhou Z (2009) Effects of xylanase on performance, blood parameters, intestinal morphology, microflora and digestive enzyme activities of broilers fed wheat-based diets. Asian-Australas J Anim Sci 22(9):1288–1295. https://doi.org/10.5713/ajas.2009.90052

Maga EA, Walker RL, Anderson GB, Murray JD (2006) Consumption of milk from transgenic goats expressing human lysozyme in the mammary gland results in the modulation of intestinal microflora. Transgenic Res 15(4):515–519

Mathlouthi N, Mallet S, Saulnier L, Quemener B, Larbier M (2002) Effects of xylanase and $\beta $-glucanase addition on performance, nutrient digestibility, and physico-chemical conditions in the small intestine contents and caecal microflora of broiler chickens fed a wheat and barley-based diet. Anim Res 51(05):395–406

Morgan NK, Keerqin C, Wallace A, Wu S-B, Choct M (2019a) Effect of arabinoxylo-oligosaccharides and arabinoxylans on net energy and nutrient utilization in broilers. Anim Nutr 5(1):56–62. https://doi.org/10.1016/j.aninu.2018.05.001

Morgan NK, Keerqin C, Wallace A, Wu S-B, Choct M (2019b) Effect of arabinoxylo-oligosaccharides and arabinoxylans on net energy and nutrient utilization in broilers. Anim Nutr 5(1):56–62

Neuman H, Debelius JW, Knight R, Koren O (2015) Microbial endocrinology: the interplay between the microbiota and the endocrine system. FEMS Microbiol Rev 39(4):509–521. https://doi.org/10.1093/femsre/fuu010

Nyachoti CM, Kiarie E, Bhandari SK, Zhang G, Krause DO (2012) Weaned pig responses to Escherichia coli K88 oral challenge when receiving a lysozyme supplement1,2. J Anim Sci 90(1):252–260. https://doi.org/10.2527/jas.2010-3596

Oliver WT, Wells JE (2013) Lysozyme as an alternative to antibiotics improves growth performance and small intestinal morphology in nursery pigs1. J Anim Sci 91(7):3129–3136. https://doi.org/10.2527/jas.2012-5782

Oliver WT, Wells JE (2015) Lysozyme as an alternative to growth promoting antibiotics in swine production. J Anim Sci Biotechnol 6(1):35–35. https://doi.org/10.1186/s40104-015-0034-z

Oliver WT, Wells JE, Maxwell CV (2014) Lysozyme as an alternative to antibiotics improves performance in nursery pigs during an indirect immune challenge1,2. J Anim Sci 92(11):4927–4934. https://doi.org/10.2527/jas.2014-8033

Oster M, Just F, Büsing K, Wolf P, Polley C, Vollmar B, Muráni E, Ponsuksili S, Wimmers K (2016) Toward improved phosphorus efficiency in monogastrics—interplay of serum, minerals, bone, and immune system after divergent dietary phosphorus supply in swine. Am J Phys Regul Integr Comp Phys 310(10):R917–R925

Palacios MC, Haros M, Sanz Y, Rosell CM (2008) Selection of lactic acid bacteria with high phytate degrading activity for application in whole wheat breadmaking. LWT-Food Sci Technol 41(1):82–92

Passos AA, Park I, Ferket P, von Heimendahl E, Kim SW (2015) Effect of dietary supplementation of xylanase on apparent ileal digestibility of nutrients, viscosity of digesta, and intestinal morphology of growing pigs fed corn and soybean meal based diet. Anim Nutr 1(1):19–23. https://doi.org/10.1016/j.aninu.2015.02.006

Peek H, Van Der Klis J, Vermeulen B, Landman W (2009a) Dietary protease can alleviate negative effects of a coccidiosis infection on production performance in broiler chickens. Anim Feed Sci Technol 150(1–2):151–159

Peek HW, van der Klis JD, Vermeulen B, Landman WJM (2009b) Dietary protease can alleviate negative effects of a coccidiosis infection on production performance in broiler chickens. Anim Feed Sci Technol 150(1):151–159. https://doi.org/10.1016/j.anifeedsci.2008.08.006

Png CW, Lindén SK, Gilshenan KS, Zoetendal EG, McSweeney CS, Sly LI, McGuckin MA, Florin TH (2010) Mucolytic bacteria with increased prevalence in IBD mucosa augment in vitro utilization of mucin by other bacteria. Am J Gastroenterol 105(11):2420–2428

Ptak A, Bedford MR, Świątkiewicz S, Żyła K, Józefiak D (2015) Phytase modulates ileal microbiota and enhances growth performance of the broiler chickens. PLoS One 10(3): e0119770

Raza A, Bashir S, Tabassum R (2019) An update on carbohydrases: growth performance and intestinal health of poultry. Heliyon 5(4):e01437. https://doi.org/10.1016/j.heliyon.2019.e01437

Rodriguez-Sanchez R, Tres A, Sala R, Guardiola F, Barroeta A (2019) Evolution of lipid classes and fatty acid digestibility along the gastrointestinal tract of broiler chickens fed different fat sources at different ages. Poult Sci 98(3):1341–1353

Roofchaei A, Rezaeipour V, Vatandour S, Zaefarian F (2019) Influence of dietary carbohydrases, individually or in combination with phytase or an acidifier, on performance, gut morphology and microbial population in broiler chickens fed a wheat-based diet. Anim Nutr 5(1):63–67. https://doi.org/10.1016/j.aninu.2017.12.001

Rooke J, Slessor M, Fraser H, Thomson J (1998) Growth performance and gut function of piglets weaned at four weeks of age and fed protease-treated soya-bean meal. Anim Feed Sci Technol 70(3):175–190

Sais M, Barroeta AC, López-Colom P, Nofrarías M, Majó N, Lopez-Ulibarri R, Pérez Calvo E, Martín-Orúe SM (2019) Evaluation of dietary supplementation of a novel microbial muramidase on gastrointestinal functionality and growth performance in broiler chickens. Poult Sci. https://doi.org/10.3382/ps/pez466

Saleh AA, El-Far AH, Abdel-Latif MA, Emam MA, Ghanem R, Abd El-Hamid HS (2018) Exogenous dietary enzyme formulations improve growth performance of broiler chickens fed a low-energy diet targeting the intestinal nutrient transporter genes. PLoS One 13(5):e0198085

Scapini LB, de Cristo AB, Schmidt JM, Buzim R, Nogueira LK, Palma SC, Fernandes JIM (2019) Effect of β-mannanase supplementation in conventional diets on the performance, immune competence and intestinal quality of broilers challenged with Eimeria sp. J Appl Poult Res 28(4):1048–1057. https://doi.org/10.3382/japr/pfz066

Schliffka W, Zhai H-X, Pérez Calvo E, van Cauwenberghe S, Walsh MC, Lopez-Ulibarri R (2019) Safety and efficacy evaluation of a novel dietary muramidase for swine. Heliyon 5(10):e02600. https://doi.org/10.1016/j.heliyon.2019.e02600

Selle PH, Ravindran V (2007) Microbial phytase in poultry nutrition. Anim Feed Sci Technol 135 (1–2):1–41

Selle PH, Ravindran V, Partridge GG (2009) Beneficial effects of xylanase and/or phytase inclusions on ileal amino acid digestibility, energy utilisation, mineral retention and growth performance in wheat-based broiler diets. Anim Feed Sci Technol 153(3):303–313. https://doi.org/10.1016/j.anifeedsci.2009.06.011

Silvester KR, Cummings JH (1995) Does digestibility of meat protein help explain large bowel cancer risk? Nutr Cancer 24(3):279–288

Singh B, Satyanarayana T (2015) Fungal phytases: characteristics and amelioration of nutritional quality and growth of non-ruminants. J Anim Physiol Anim Nutr 99(4):646–660

Sun Q, Liu D, Guo S, Chen Y, Guo Y (2015) Effects of dietary essential oil and enzyme supplementation on growth performance and gut health of broilers challenged by Clostridium perfringens. Anim Feed Sci Technol 207:234–244

Swidsinski A, Dörffel Y, Loening-Baucke V, Theissig F, Rückert JC, Ismail M, Rau WA, Gaschler D, Weizenegger M, Kühn S (2011) Acute appendicitis is characterised by local invasion with fusobacterium nucleatum/necrophorum. Gut 60(1):34–40

Taylor AE, Bedford MR, Miller HM (2018) The effects of xylanase on grower pig performance, concentrations of volatile fatty acids and peptide YY in portal and peripheral blood. Animal 12(12):2499–2504. https://doi.org/10.1017/S1751731118000277

Tellez G, Latorre JD, Kuttappan VA, Hargis BM, Hernandez-Velasco X (2015) Rye affects bacterial translocation, intestinal viscosity, microbiota composition and bone mineralization in Turkey poults. PLoS One 10(4):e0122390. https://doi.org/10.1371/journal.pone.0122390

Teymouri H, Zarghi H, Golian A (2018) Evaluation of hull-less barley with or without enzyme cocktail in the finisher diets of broiler chickens. J Agric Sci Technol 20(3):469–483

Thammarutwasik P, Hongpattarakere T, Chantachum S, Kijroongrojana K, Itharat A, Reanmongkol W, Tewtrakul S, Ooraikul B (2009) Prebiotics-a review. Songklanakarin J Sci Technol 31(4):401–408

Tilocca B, Witzig M, Rodehutscord M, Seifert J (2016) Variations of phosphorous accessibility causing changes in microbiome functions in the gastrointestinal tract of chickens. PLoS One 11(10):e0164735

Toden S, Bird AR, Topping DL, Conlon MA (2005) Resistant starch attenuates colonic DNA damage induced by higher dietary protein in rats. Nutr Cancer 51(1):45–51

Waititu S, Sanjayan N, Hossain M, Leterme P, Nyachoti C (2018) Improvement of the nutritional value of high-protein sunflower meal for broiler chickens using multi-enzyme mixtures. Poult Sci 97(4):1245–1252

Walk CL, Bedford MR (2020) Application of exogenous enzymes: is digestibility an appropriate response variable? Anim Prod Sci 60(8):993–998. https://doi.org/10.1071/AN19437

Walk C, Bedford M, Olukosi O (2018) Effect of phytase on growth performance, phytate degradation and gene expression of myo-inositol transporters in the small intestine, liver and kidney of 21 day old broilers. Poult Sci 97(4):1155–1162

Wang H, Guo Y, Shih JC (2008) Effects of dietary supplementation of keratinase on growth performance, nitrogen retention and intestinal morphology of broiler chickens fed diets with soybean and cottonseed meals. Anim Feed Sci Technol 140(3–4):376–384

Wang Y, Yan J, Zhang X, Han B (2018) Tolerance properties and growth performance assessment of Yarrowia lipolytic lipase in broilers. J Appl Anim Res 46(1):486–491

Weber HC (2010) Editorial overview: gastrointestinal regulatory peptides. Curr Opin Endocrinol Diabetes Obes 17(1):31–32. https://doi.org/10.1097/MED.0b013e328335366a

Wells JE, Berry ED, Kalchayanand N, Rempel LA, Kim M, Oliver WT (2015) Effect of lysozyme or antibiotics on faecal zoonotic pathogens in nursery pigs. J Appl Microbiol 118(6):1489–1497. https://doi.org/10.1111/jam.12803

Wilkie DC, Van Kessel AG, White LJ, Laarveld B, Drew MD (2005) Dietary amino acids affect intestinal Clostridium perfringens populations in broiler chickens. Can J Anim Sci 85(2):185–193

Windey K, De Preter V, Verbeke K (2012) Relevance of protein fermentation to gut health. Mol Nutr Food Res 56(1):184–196

Woyengo T, Nyachoti C (2013) Anti-nutritional effects of phytic acid in diets for pigs and poultry–current knowledge and directions for future research. Can J Anim Sci 93(1):9–21

Woyengo TA, Slominski BA, Jones RO (2010) Growth performance and nutrient utilization of broiler chickens fed diets supplemented with phytase alone or in combination with citric acid and multicarbohydrase. Poult Sci 89(10):2221–2229. https://doi.org/10.3382/ps.2010-00832

Woyengo T, Rodriguez-Lecompte J, Adeola O, Nyachoti C (2011) Histomorphology and small intestinal sodium-dependent glucose transporter 1 gene expression in piglets fed phytic acid and phytase-supplemented diets. J Anim Sci 89(8):2485–2490

Wu Y, Ravindran V, Hendriks W (2004) Influence of exogenous enzyme supplementation on energy utilisation and nutrient digestibility of cereals for broilers. J Sci Food Agric 84(14):1817–1822

Yildiz T, Ceylan N, Zafer A, Karademir E, ERTEKİN B (2018) Effect of corn distillers dried grains with soluble with or without xylanase supplementation in laying hen diets on performance, egg quality and intestinal viscosity. Kafkas Üniversitesi Veteriner Fakültesi Dergisi 24(2):273–280

Yin D, Yin X, Wang X, Lei Z, Wang M, Guo Y, Aggrey SE, Nie W, Yuan J (2018) Supplementation of amylase combined with glucoamylase or protease changes intestinal microbiota diversity and benefits for broilers fed a diet of newly harvested corn. J Anim Sci Biotechnol 9(1):24. https://doi.org/10.1186/s40104-018-0238-0

Zanu HK, Keerqin C, Kheravii SK, Morgan NK, Wu SB, Bedford MR, Swick RA (2020) Influence of meat and bone meal, phytase, and antibiotics on broiler chickens challenged with subclinical necrotic enteritis: 1. Growth performance, intestinal pH, apparent ileal digestibility, cecal microbiota, and tibial mineralization. Poult Sci 99(3):1540–1550. https://doi.org/10.1016/j.psj.2019.11.021

Zeller E, Schollenberger M, Kühn I, Rodehutscord M (2015) Effect of diets containing enzyme supplements and microwave-treated or untreated wheat on inositol phosphates in the small intestine of broilers. Anim Feed Sci Technol 204:42–51. https://doi.org/10.1016/j.anifeedsci.2015.03.007

Zheng C, Wu J, Jin Z, Zf Y, Yang S, Yq S, Fei H (2020) Exogenous enzymes as functional additives in finfish aquaculture. Aquac Nutr 26(2):213–224

Zhou Y, Jiang Z, Lv D, Wang T (2009) Improved energy-utilizing efficiency by enzyme preparation supplement in broiler diets with different metabolizable energy levels. Poult Sci 88(2):316–322

Zuo J, Ling B, Long L, Li T, Lahaye L, Yang C, Feng D (2015a) Effect of dietary supplementation with protease on growth performance, nutrient digestibility, intestinal morphology, digestive enzymes and gene expression of weaned piglets. Anim Nutr 1(4):276–282. https://doi.org/10.1016/j.aninu.2015.10.003

Zuo J, Ling B, Long L, Li T, Lahaye L, Yang C, Feng D (2015b) Effect of dietary supplementation with protease on growth performance, nutrient digestibility, intestinal morphology, digestive enzymes and gene expression of weaned piglets. Anim Nutr 1(4):276–282. https://doi.org/10.1016/j.aninu.2015.10.003

Butyrate and Intestinal Homeostasis: Effects on the Intestinal Microbiota and Epithelial Hypoxia

4

Cristiano Bortoluzzi, Fernanda Lima de Souza Castro, and Michael Kogut

Abstract

The intestinal microbiota's role on the health of the host is of paramount importance. The bacteria inhabiting the gastrointestinal tract may be considered the first line of defense against the invasion and proliferation of pathogens. The commensal microbiota not only produces a large array of molecules with antimicrobial properties, but also confers colonization resistance and produces short-chain fatty acids (SCFA), specially butyrate. Butyrate is essential to regulate the metabolism of intestinal epithelial cells and their metabolite production, keeping the balance of the microbial community. However, inflammation may decrease the number of butyrate-producing bacteria, change the epithelial metabolism, lead to accumulation of O_2 and L-lactate, and drive further expansion of pathogens such as *Salmonella*. Moreover, the epithelial SCFA metabolism might be a determinant factor for the physiologic hypoxia in the intestinal cells. Indeed, the metabolism of butyrate and other SCFA in the epithelial cells of the distal gut was shown to reduce the local O_2 levels, which activates pathways that allow the cells to respond to hypoxia. Therefore, the objective of this review chapter is to give further insights into the role played by butyrate, and how it can be used to support the intestinal health of broiler chickens.

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Keywords

Butyrate · Chickens · Intestinal hypoxia · Microbiota

4.1 Introduction

The maintenance of the intestinal homeostasis is essential for production of fast-growing animals that yield high-quality protein in a relatively short amount of time. The intestine possesses important functions besides its well-known role in the digestion and absorption of nutrients. The interface between intestinal immune system and microbiota controls many aspects of the intestinal health of animals and must be considered as an additional organ that fulfills important functions with specific nutritional requirements. The bacteria present in the gastrointestinal tract (GIT) is in contact with the host and its immune system, producing a large array of molecules that are able to modulate many biological functions.

The intestinal microbiota plays a critical role in the animal's productivity because of the strict associations between the microorganisms and the host. These beneficial interactions contribute to maintain the health, integrity of the gut, and the homeostasis of the microbial community in the GIT (Pedroso et al. 2012). The first line of defense against pathogens is the commensal microbiota of the intestine. Many commensal bacteria produce organic acids, such as lactic acid, propionic, and butyric, as well as bacteriocins that have effect against Gram-positive and Gram-negative bacteria (Ding et al. 2017; Kers et al. 2018). Furthermore, these molecules are capable of regulating the health of the host and coordinate a balanced immune response that tolerates commensal microorganisms. However, several factors including pathogens and feed ingredients can break this balance and lead to dysbiosis.

Throughout the years, the advances obtained in poultry production were due, in part, to the improvements in the sanitary management of the birds, specially through the use of antimicrobial growth promoters (AGP) as feed supplements. A great number of active compounds, when used in low doses, prevent the colonization and proliferation of enteric pathogens in the GIT, control enteric diseases, and improve feed efficiency and the growth performance of animals. Several countries have now restricted the use of AGP in diets of poultry. This is mostly due to the consumer perception that antibiotic-free produced poultry is superior to conventionally raised poultry in spite of a lack of supporting scientific data. A widely accepted definition of antibiotic-free poultry is "no use of antibiotics (including ionophore anticoccidials) at the farm" (Cervantes 2015), meaning that the use of nutritional strategies is key to prevent intestinal infections and losses in growth performance. Therefore, this article aims to discuss the role of butyrate (endogenously produced or added to the diets) in maintaining the homeostasis of the intestinal epithelium, its relationship with the microbiota balance, and its impact on poultry production.

4.2 What Is a Healthy Microbiota?

Commercial chicks hatch with the GIT nearly devoid of microorganisms mainly due to the absence of contact with the hen during incubation and after hatching. Some strategies can be adopted to speed the initial colonization of the GIT with beneficial bacteria, including spraying the surface of the eggs with a selection of beneficial bacteria at the moment of transfer of the eggs to the hatcher and *in ovo* inoculation of probiotics. For example, Pedroso et al. (2016) inoculated a competitive-exclusion product into eggs at day 18 of incubation and observed that the inoculation increased diversity and affected the composition of the chick intestinal microbiota, even though this effect was transient. Cecal microbiota from good and poor feed efficiency birds was used to spray the surface of eggs prior to the hatching and to study the initial colonization profile of the GIT (Donaldson et al. 2017). Although the performance of the donors was not transferred to the recipient birds, the cecal treatment reduced bird-to-bird variation, which may lead to a better uniformity in terms of performance to the treated flocks.

In the last few years, numerous publications have elaborated on factors affecting the intestinal microbiota, mainly because of the large influence that these microorganisms play on the GIT (Kers et al. 2018). It has been estimated that the intestinal microbial community is composed of more than 800 species of bacteria (Laparra and Sanz 2010) and about nine million bacterial genes (Huang et al. 2018). In broiler chickens, dietary composition, host characteristics (breed, age, sex), and external environment factors affect microbial communities in the GIT (Kers et al. 2018). Litter quality and other management conditions influence the composition of the intestinal microbiota, directly as a source of bacteria, or indirectly by its effect on the physical barrier and defense of the intestine (Apajalahti and Vienola 2016). The luminal microbiota may be regulated by the influx of nutrients from the diet, by the passage rate of the intestinal content, and by the level and activity of antimicrobial substances (Koutsos and Arias 2006). It has been shown that the microbiota present in the feed influences more the microbiota in the ileum and excreta than the cecal microbiota (Haberecht et al. 2020). Therefore, nutrients that are not used by the normal digestive processes may be used by the microbiota or pathogens present in the feed, leading to a state of dysbiosis.

In coordination with the intestinal mucosa, the intestinal microbiota is responsible for the first line of defense in the animals, and it works by regulating cellular permeability, altering the expression of genes in goblet cells for increased mucus production, and stimulating secretion of antimicrobial peptides (Laparra and Sanz 2010). As such, a well-established intestinal microbiota brings benefits to the host due to production of vitamins, immune modulation, and inhibition of pathogens, whereas microbial imbalance may contribute to the development of metabolic and immunologic diseases (Jeurissen et al. 2002) and to the increased competition for nutrients with the host (Yang et al. 2009). Under the influence of diet, the composition of the gut microbiome as well as the commensal-derived nutrients and metabolites (lipids, SCFA, amino acids, vitamins) is altered (Turnbaugh et al. 2008). These diet-induced changes in microbiome composition and derived

metabolites have profound direct and indirect effects on host immunity via alterations in signaling pathways and gene transcription of effector immune cells, development of immune cells, and receptor recognition/sensing of immune cells (Round and Mazmanian 2009; Hooper and Macpherson 2010). It has been demonstrated, in mice, that the microbiota changes the DNA methylation profile of intestinal epithelial cells, indicating that these epigenetic modifications caused by the microbiota are essential for re-establishing homeostasis following an inflammation event (Ansari et al. 2020), most likely by the production of microbial metabolites, such as butyrate.

The understanding and monitoring of the intestinal microbial ecosystem are paramount to develop strategies and interventions to modulate the microbiota and reduce the occurrence of enteric diseases. As a "second genome" of the vertebrate host, the gut microbiome acts as a critical regulator of both the innate and acquired components of mucosal immunity (Belkaid and Hand 2014). The accumulation of intestinal immune cells, physical barriers, and soluble mediators contains and controls the microbiota (Macpherson et al. 2009; Hooper and Macpherson 2010; Belkaid and Hand 2014) by acting as "molecular firewalls" (Macpherson et al. 2009) that prevent microbiota-specific acquired responses against commensal microbes. For instance, Byndloss et al. (2019) have proposed the term "microbiota-nourishing system." The term refers to a separated component of the immune system that balances the microbiota composition to establish colonization resistance, which protects the host against pathogens. Therefore, from this point forward, we will give insights into the role of the commensal microbiota in keeping the epithelial hypoxia and how butyrate regulates this system to maintain the well-functioning of the intestine.

4.3 Intestinal Epithelial Hypoxia

The intestinal tissue has a unique and dynamic oxygenation profile. Under physiological conditions, it is expected a daily fluctuation in oxygen partial pressure (pO_2) mainly dictated by the blood flow and metabolic demands of this tissue. For instance, in a fasting state, lower blood perfusion is found in the gut, representing approximately 5% of the total blood volume, whereas during the fed state, blood perfusion increases to approximately 30% of total blood volume (Taylor and Colgan 2007; Colgan and Taylor 2010). Moreover, after a meal, the need for ATP to support the active sodium and glucose transport increases, as these transport systems are part of the absorptive process (Ramakrishnan and Shah 2016). It was estimated that approximately 79% of ATP to support digestion and absorption comes from oxidative phosphorylation (Del Castillo et al. 1991), leading to an increase in O_2 need as electron acceptor, which is not fully supplied by the intestinal blood circulation (Chou 1983). Additionally, there is a significant pO_2 gradient moving from the virtually anoxic lumen towards the highly vascularized submucosa, with reported O_2 levels in the small intestine of rats being approximately 2% in the lumen, 3% in the tip of villus, and 8% in the intestinal wall (Fisher et al. 2013). Therefore, it is

expected that the intestinal epithelial cells are at a constant physiologic hypoxia state, even at baseline levels (Shepherd 1982; Zheng et al. 2015).

However, when facing disease and inflammation, the intestinal tissue might experience hypoxic stress, in which the cellular O_2 demand is higher than the supply (Glover and Colgan 2011; Colgan et al. 2013). The higher O_2 demand could be associated to the increased number of innate immune cells in the site as well as the invading pathogens and the resident cells (Colgan and Taylor 2010; Campbell et al. 2014). Moreover, the recruitment of activated polymorphonuclear neutrophils has been shown to generate reactive oxygen species (ROS) and increase the O_2 demand by almost 50-fold, significantly contributing to the generation of hypoxic stress during infection and inflammation (Glover and Colgan 2011; Colgan et al. 2013).

Because O_2 supply is essential for the proper functioning of most cells, including enterocytes, these cells have developed a repertoire of transcriptional and posttranscriptional changes to prevent them from entering a hypoxia state (Semenza 1999; Taylor and Colgan 1999, 2007; Cummins and Crean 2017). A known regulator of these transcriptional changes as a response to hypoxia is the hypoxia-inducible factor (HIF) family (Semenza and Wang 1992; Wang et al. 1995). The HIF family, a group of heterodimeric transcription factors, is formed by three O_2-regulated cytoplasmatic α-subunits (HIF-1α, HIF-2 α, and HIF-3α) and a nuclear constitutively expressed HIF-β subunit (Wang et al. 1995; Ramakrishnan and Shah 2016; Manresa and Taylor 2017). The expression of HIF-1α was shown to be regulated by cellular O_2 levels in a model using hepatoma human cell lines (Hep-3B) (Wang et al. 1995). When Hep-3B cells were exposed to 1% of O_2 the HIF-1α expression was upregulated, whereas the exposure level of 20% of O_2 resulted in the downregulation of this gene (Wang et al. 1995). The HIF response mechanism to hypoxia has been extensively described in the literature (Taylor and Colgan 1999; Semenza 2012; Zheng et al. 2015; Shah 2016; Ramakrishnan and Shah 2016), and HIF has been shown to regulate genes involved in inhibition of cell apoptosis (Cummins et al. 2008), erythropoiesis (Semenza and Wang 1992), angiogenesis (Rey and Semenza 2010), intestinal barrier integrity (Furuta et al. 2001; Manresa and Taylor 2017; Sun et al. 2019), and iron homeostasis (Shah et al. 2009; Mastrogiannaki et al. 2009; Taylor et al. 2011), ultimately improving tissue oxygenation. In this context, HIF system activity could be beneficial in intestinal inflammatory diseases, such as inflammatory bowel disease (IBD) in humans (Biddlestone et al. 2015), feed-induced inflammation or bacterial infections in chickens, by modulating cell apoptosis and intestinal barrier integrity.

4.4 Epithelial Hypoxia and Microbiota Balance: Role of Butyrate

The short-chain fatty acids (SCFA) are a group of molecules that contain from one to seven carbons produced within the intestinal lumen by bacterial fermentation of plant materials such as cellulose, fibers, starches, and sugars that animals cannot digest due to the lack of necessary enzymes (Guilloteau et al. 2010). The SCFA with

higher abundance in the GIT are acetate, propionate, and butyrate (Bedford and Gong 2018). During homeostatic state, the microbiota can directly or indirectly utilize non-digestible carbohydrates (NDCs) to produce butyrate (Fu et al. 2019). NDCs are directly fermented by butyrate-producing bacteria or, through a cross-feeding mechanism; NDCs can be used by bifidobacteria to yield large amounts of acetate and lactate, which will be used by butyrate-producing bacteria such as *Roseburia* and *Faecalibacterium prausnitzii* to produce butyrate (Fu et al. 2019). Butyrate is synthesized from two molecules of acetyl-CoA, yielding acetoacetyl-CoA, which is further converted to butyryl-CoA via b-hydroxybutyryl-CoA and crotonyl-CoA (Koh et al. 2016).

Butyrate is essential to maintain the proper interaction between the host and its intestinal microbiota (Byndloss et al. 2019). It has been hypothesized that the butyrate endogenously produced by the microbiota is metabolized by the intestinal epithelial cells through beta-oxidation and regulates T cells and epithelial cells to generate epithelial hypoxia. In return, the hypoxia state helps maintain anaerobiosis in the intestinal lumen, thereby balancing the microbiota to confer colonization resistance (Byndloss et al. 2019). Inflammation may decrease the number of butyrate-producing bacteria, change the epithelial metabolism, lead to accumulation of O_2 and L-lactate, and drive further expansion of pathogens such as *Salmonella* (Gillis et al. 2018). Indeed, Gillis et al. (2019) reported that sensing of host-derived metabolites (O_2 and L-lactate) induces the transcription of L-lactate utilization genes in *S*. Typhimurium, which confers a fitness advantage and ensures successful *S*. Typhimurium outgrowth. Another pathogenesis strategy that may be used by *Citrobacter rodentium*, a member of the attaching and effacing pathogens, relates to the presence of the type III secretion system virulence factor (Luperchio et al. 2000). These virulence factors trigger crypt hyperplasia to induce epithelial oxygenation in mice, which drives further expansion of *C. rodentium* (Lopez et al. 2016). It is becoming more evident that inflammation-induced dysbiosis changes the production of metabolites within the intestinal lumen, the metabolism of intestinal epithelial cells, and, therefore, breaks the microbiota-nourishing immunity by disrupting the anaerobic state of the intestine (Byndloss et al. 2019).

Furthermore, recent studies have shown a dynamic relationship of the microbiota regulating the intestinal oxygenation and health through a cross-talk between microbiota-produced SCFA and HIF (Kelly et al. 2015). It has been suggested that the mucosal-associated organisms might play a role in the generation of the aforementioned O_2 gradient between the intestinal mucosa and lumen by actively consuming host-derived oxygen (Zheng et al. 2015). Moreover, the epithelial SCFA metabolism might be a determinant factor for the physiologic hypoxia in the intestinal cells (Kelly et al. 2015). Indeed, the metabolism of butyrate and other SCFA in the epithelial cells of the distal gut was shown to reduce the local O_2 levels, leading to the stabilization of HIF, which allows the cells to respond to hypoxia (Kelly et al. 2015). Moreover, when antibiotic-treated and germ-free mice were used, the expression of HIF-1α was reduced and the epithelial pO_2 was increased. Interestingly, the restoration of luminal butyrate was able to reestablish the classical physiological hypoxia in the antibiotic-treated subjects (Kelly et al. 2015). More evidence of the

role of SCFA in controlling the oxygenation of intestinal cells was provided by Rivera-Chávez et al. (2016). The authors observed higher O_2 levels in mice colonocytes after the depletion of butyrate-producing Clostridia by a streptomycin treatment. As a consequence of the increased epithelial O_2 level, there was an aerobic expansion of *Salmonella enterica* serovar Typhimurium, which is commonly associated to human gastroenteritis (Majowicz et al. 2010). Therefore, the presence of SCFA-producing microorganisms modulates the epithelial tissue oxygenation status, with potential influence on the microbiota diversity.

4.5 Butyrate Use in Diets of Broiler Chickens

Butyrate can be produced by the intestinal microorganisms through the fermentation of NDCs (Fu et al. 2019), or even by the action of other molecules, such as riboflavin, in stimulation butyrate-producing bacteria (Steinert et al. 2020). Exogenous butyrate can be supplied in the feed as Na, K, Mg, or Ca salts which are odorless and easier to be incorporated into the feed (Guilloteau et al. 2010). Sodium butyrate (SB) is the sodium salt of butyric acid which contains a sodium atom in place of the hydrogen atom in the -OH group (Ahsan et al. 2016), and it has been the subject of many studies in poultry (Liu et al. 2014; Zhou et al. 2017; Song et al. 2017; Bortoluzzi et al. 2017, 2018). However, there is a lack of studies trying to deeply understand the mechanism of action of butyrate, specially studies that describe the immune and metabolic changes promoted by butyrate on the intestine of chickens.

Butyrate can have several effects on the host, including the ability to regulate the production of cytokines, antimicrobial peptides, mucin, tight junction proteins, and the intestinal microbiota (Guilloteau et al. 2010; Song et al. 2017; Bortoluzzi et al. 2017, 2018; Zou et al. 2019). Butyrate leads to epigenetic adaptations and increase the proportion of cholinergic enteric neurons that affects the release of hormones in the enteric nervous system and the endocrine signaling (van de Wouw et al. 2017), which affects and is affected by the microbiota. Butyrate seems to have an anti-inflammatory effect mediated by signaling pathways (Meijer et al. 2010), such as modulating pro-inflammatory cytokines via impairment in NF-kB activation (Guilloteau et al. 2010). Besides its effects on immune modulation, butyrate serves as an energy source for enterocytes and colonocytes, stimulates mucus synthesis, promotes intestinal cell proliferation, differentiation, and maturation, controls intestinal barrier function, decreases apoptosis of normal cells, and has antimicrobial effects against pathogenic bacteria (Guilloteau et al. 2010).

In a previous study, Bortoluzzi et al. (2017) demonstrated that the nutritional reduction of energy and amino acids impaired the performance of broiler chickens in terms of body weight gain and feed conversion ratio, but the supplementation of SB partially counteracted this negative effect. The cecal microbiota of chickens showed a large amount of fiber degraders and SCFA producers, especially in the groups fed a nutritionally reduced diet supplemented with SB. The nutritional reduction changed the predicted functions performed by the microbiota, and the SB supplementation

reduced this variation. Moreover, the frequency of bacterial species presenting the butyryl-CoA:acetate CoA-transferase gene related to butyrate production was increased in the microbiota of chickens fed a nutritionally reduced diet and reduced with SB supplementation (Bortoluzzi et al. 2017), likely due to the butyrate being supplied through the diet. Current research is being performed by our laboratory to better understand the mechanism by which SB supplementation improved growth performance of broilers fed nutritionally reduced diet, starting after 28 days of age (unpublished data). The hypothesis of this action being in part due to changes in the immunometabolism of the enterocytes, but the role of the microbiota cannot be discarded.

Most of the studies have used encapsulation technologies to guarantee that the butyrate would reach the portion of the GIT where it needs to be released, which could otherwise be absorbed in the beginning of the GIT (Liu et al. 2014; Zhou et al. 2017; Zou et al. 2019; Tugnoli et al. 2020). It has been shown, indeed, that the supplementation of a coated source of SB at 300 ppm reduced levels of D-lactate, IL-6, and IL-1β, but increased the IL-10. The SB treatment did not affect the diversity of the intestinal microbiota during the induction of intestinal inflammation but altered its composition (Zou et al. 2019). Taken together, these results suggest that SB has anti-inflammatory effects and modulates the microbial community in broilers. On the other hand, Zhou et al. (2017) observed that coated SB had no significant effect on the cecal microbiota of healthy chickens but balanced the shifts of microbial composition caused by *Eimeria tenella* infection.

The intestinal barrier function is another aspect to be considered when developing products to maintain gut homeostasis. Enteric infections that are routinely found in flocks of broilers may lead to degradation and/or reorganization of the tight junction proteins (Fasano and Nataro 2004), originating what is referred to as "leaky gut." A leaking intestine is thought to contribute to the severity of clinical signs, being a dominant characteristic of pathogenesis of many enteric diseases (Awad et al. 2017). Therefore, dietary or immunotherapy interventions could keep or restore, at least partially, the ability of the intestinal barrier to perform its function. For instance, Wang et al. (2012) showed that butyrate upregulated the expression of claudin 1 and redistributed zonula occludens-1 (ZO-1) and occludin in the cellular membrane. Additionally, Song et al. (2017) showed that SB upregulated the expression of claudin 1 and 4, ZO-1, and occludin in necrotic enteritis induced broiler chickens. Even though the expression of genes that encode for tight junction proteins is an important indicator of the integrity of the intestinal barrier, it is important to associate those findings with other techniques to assess the intestinal permeability and integrity, such as passage of substances from the intestinal lumen to the blood. Furthermore, as reviewed by Parada Venegas et al. (2019), the beneficial effects of SCFA, especially butyrate, on the epithelial integrity may also be related to the production of antimicrobial peptide molecules by the host cells, which also regulates the host–microbiota interface.

4.6 Final Considerations

Here we discussed some aspects of the intestinal metabolism that are regulated by the intestinal microbiota and its metabolites. We have shown, based on the literature available, that the metabolism of intestinal epithelial cells controls and is controlled by the commensal microbiota. However, there is a lack of data demonstrating how the endogenously produced or exogenous butyrate controls the epithelial metabolism in chickens. One hypothesis that can be elaborated is: butyrate modifies the immune metabolism of intestinal epithelial cells which in turn controls the intestinal microbiota and makes the chickens more efficient in using the energy and nutrients available in the diet, and establishes a stronger colonization resistance. The confirmation of this hypothesis would help to understand the mechanism behind the growth promoting effects of butyrate in nutritionally reduced fed broiler.

References

Ahsan U, Cengiz Ö, Raza I et al (2016) Sodium butyrate in chicken nutrition: the dynamics of performance, gut microbiota, gut morphology, and immunity. Worlds Poult Sci J 72:265–276. https://doi.org/10.1017/S0043933916000210

Ansari I, Raddatz G, Gutekunst J et al (2020) The microbiota programs DNA methylation to control intestinal homeostasis and inflammation. Nat Microbiol 5:610–619. https://doi.org/10.1038/s41564-019-0659-3

Apajalahti J, Vienola K (2016) Interaction between chicken intestinal microbiota and protein digestion. Anim Feed Sci Technol 221:323–330. https://doi.org/10.1016/j.anifeedsci.2016.05.004

Awad WA, Hess C, Hess M (2017) Enteric pathogens and their toxin-induced disruption of the intestinal barrier through alteration of tight junctions in chickens. Toxins 9:60. https://doi.org/10.3390/toxins9020060

Bedford A, Gong J (2018) Implications of butyrate and its derivatives for gut health and animal production. Anim Nutr 4:151–159. https://doi.org/10.1016/j.aninu.2017.08.010

Belkaid Y, Hand T (2014) Role of the microbiota in immunity and inflammation. Cell 157:121–141. https://doi.org/10.1016/j.cell.2014.03.011

Biddlestone J, Bandarra D, Rocha S (2015) The role of hypoxia in inflammatory disease (review). Int J Mol Med 35:859–869. https://doi.org/10.3892/ijmm.2015.2079

Bortoluzzi C, Pedroso AA, Mallo JJ et al (2017) Sodium butyrate improved performance while modulating the cecal microbiota and regulating the expression of intestinal immune-related genes of broiler chickens. Poult Sci 96:3981–3993. https://doi.org/10.3382/ps/pex218

Bortoluzzi C, Rothrock MJ, Vieira BS et al (2018) Supplementation of protected sodium butyrate alone or in combination with essential oils modulated the cecal microbiota of broiler chickens challenged with Coccidia and *Clostridium perfringens*. Front Sustain Food Syst 2. https://doi.org/10.3389/fsufs.2018.00072

Byndloss MX, Litvak Y, Bäumler AJ (2019) Microbiota-nourishing immunity and its relevance for ulcerative colitis. Inflamm Bowel Dis 25:811–815. https://doi.org/10.1093/ibd/izz004

Campbell EL, Bruyninckx WJ, Kelly CJ et al (2014) Transmigrating neutrophils shape the mucosal microenvironment through localized oxygen depletion to influence resolution of inflammation. Immunity 40:66–77. https://doi.org/10.1016/j.immuni.2013.11.020

Cervantes HM (2015) Antibiotic-free poultry production: is it sustainable? J Appl Poult Res 24:91–97. https://doi.org/10.3382/japr/pfv006

Chou CC (1983) Splanchnic and overall cardiovascular hemodynamics during eating and digestion. Fed Proc 42:1658–1661

Colgan SP, Curtis VF, Campbell EL (2013) The inflammatory tissue microenvironment in IBD. Inflamm Bowel Dis 19:2238–2244. https://doi.org/10.1097/MIB.0b013e31828dcaaf

Colgan SP, Taylor CT (2010) Hypoxia: an alarm signal during intestinal inflammation. Nat Rev Gastroenterol Hepatol 7:281–287. https://doi.org/10.1038/nrgastro.2010.39

Cummins EP, Crean D (2017) Hypoxia and inflammatory bowel disease. Microbes Infect 19:210–221. https://doi.org/10.1016/j.micinf.2016.09.004

Cummins EP, Seeballuck F, Keely SJ et al (2008) The hydroxylase inhibitor dimethyloxalylglycine is protective in a murine model of colitis. Gastroenterology 134:156–165.e1. https://doi.org/10.1053/j.gastro.2007.10.012

Del Castillo JR, Ricabarra B, Sulbarán-Carrasco MC (1991) Intermediary metabolism and its relationship with ion transport in isolated Guinea pig colonic epithelial cells. Am J Phys 260: C626–C634. https://doi.org/10.1152/ajpcell.1991.260.3.C626

Ding J, Dai R, Yang L et al (2017) Inheritance and establishment of gut microbiota in chickens. Front Microbiol 8. https://doi.org/10.3389/fmicb.2017.01967

Donaldson EE, Stanley D, Hughes RJ, Moore RJ (2017) The time-course of broiler intestinal microbiota development after administration of cecal contents to incubating eggs. PeerJ 5: e3587. https://doi.org/10.7717/peerj.3587

Fasano A, Nataro JP (2004) Intestinal epithelial tight junctions as targets for enteric bacteria-derived toxins. Adv Drug Deliv Rev 56:795–807. https://doi.org/10.1016/j.addr.2003.10.045

Fisher EM, Khan M, Salisbury R, Kuppusamy P (2013) Noninvasive monitoring of small intestinal oxygen in a rat model of chronic mesenteric ischemia. Cell Biochem Biophys 67:451–459. https://doi.org/10.1007/s12013-013-9611-y

Fu X, Liu Z, Zhu C et al (2019) Nondigestible carbohydrates, butyrate, and butyrate-producing bacteria. Crit Rev Food Sci Nutr 59:S130–S152. https://doi.org/10.1080/10408398.2018.1542587

Furuta GT, Turner JR, Taylor CT et al (2001) Hypoxia-inducible factor 1–dependent induction of intestinal trefoil factor protects barrier function during hypoxia. J Exp Med 193:1027–1034

Gillis CC, Hughes ER, Spiga L et al (2018) Dysbiosis-associated change in host metabolism generates lactate to support Salmonella growth. Cell Host Microbe 23:54–64.e6. https://doi.org/10.1016/j.chom.2017.11.006

Gillis CC, Winter MG, Chanin RB et al (2019) Host-derived metabolites modulate transcription of Salmonella genes involved in l-lactate utilization during gut colonization. Infect Immun:87. https://doi.org/10.1128/IAI.00773-18

Glover LE, Colgan SP (2011) Hypoxia and metabolic factors that influence inflammatory bowel disease pathogenesis. Gastroenterology 140:1748–1755. https://doi.org/10.1053/j.gastro.2011.01.056

Guilloteau P, Martin L, Eeckhaut V et al (2010) From the gut to the peripheral tissues: the multiple effects of butyrate. Nutr Res Rev 23:366–384. https://doi.org/10.1017/S0954422410000247

Haberecht S, Bajagai YS, Moore RJ et al (2020) Poultry feeds carry diverse microbial communities that influence chicken intestinal microbiota colonisation and maturation. AMB Express 10:143. https://doi.org/10.1186/s13568-020-01077-5

Hooper LV, Macpherson AJ (2010) Immune adaptations that maintain homeostasis with the intestinal microbiota. Nat Rev Immunol 10:159–169. https://doi.org/10.1038/nri2710

Huang G, Tang X, Bi F et al (2018) *Eimeria tenella* infection perturbs the chicken gut microbiota from the onset of oocyst shedding. Vet Parasitol 258:30–37. https://doi.org/10.1016/j.vetpar.2018.06.005

Jeurissen SHM, Lewis F, Klis VD, et al (2002) Parameters and techniques to determine intestinal health of poultry as constituted by immunity, integrity and functionality

Kelly CJ, Zheng L, Campbell EL et al (2015) Crosstalk between microbiota-derived short-chain fatty acids and intestinal epithelial HIF augments tissue barrier function. Cell Host Microbe 17: 662–671. https://doi.org/10.1016/j.chom.2015.03.005

Kers JG, Velkers FC, Fischer EAJ et al (2018) Host and environmental factors affecting the intestinal microbiota in chickens. Front Microbiol 9. https://doi.org/10.3389/fmicb.2018.00235

Koh A, De Vadder F, Kovatcheva-Datchary P, Bäckhed F (2016) From dietary fiber to host physiology: short-chain fatty acids as key bacterial metabolites. Cell 165:1332–1345. https://doi.org/10.1016/j.cell.2016.05.041

Koutsos EA, Arias VJ (2006) Intestinal ecology: interactions among the gastrointestinal tract, nutrition, and the microflora. J Appl Poult Res 15:161–173. https://doi.org/10.1093/japr/15.1.161

Laparra JM, Sanz Y (2010) Interactions of gut microbiota with functional food components and nutraceuticals. Pharmacol Res 61:219–225. https://doi.org/10.1016/j.phrs.2009.11.001

Liu W, Yang Y, Zhang J et al (2014) Effects of dietary microencapsulated sodium butyrate on growth, intestinal mucosal morphology, immune response and adhesive bacteria in juvenile common carp (*Cyprinus carpio*) pre-fed with or without oxidised oil. Br J Nutr 112:15–29. https://doi.org/10.1017/S0007114514000610

Lopez CA, Miller BM, Rivera-Chávez F et al (2016) Virulence factors enhance *Citrobacter rodentium* expansion through aerobic respiration. Science 353:1249–1253. https://doi.org/10.1126/science.aag3042

Luperchio SA, Newman JV, Dangler CA, et al (2000) *Citrobacter rodentium*, the causative agent of transmissible murine colonic hyperplasia, exhibits clonality: synonymy of *C. rodentium* and mouse-pathogenic *Escherichia coli*, J Clin Microbiol 38:4343–4350

Macpherson AJ, Slack E, Geuking MB, McCoy KD (2009) The mucosal firewalls against commensal intestinal microbes. Semin Immunopathol 31:145–149. https://doi.org/10.1007/s00281-009-0174-3

Majowicz SE, Musto J, Scallan E et al (2010) The global burden of nontyphoidal *Salmonella gastroenteritis*. Clin Infect Dis Off Publ Infect Dis Soc Am 50:882–889. https://doi.org/10.1086/650733

Manresa MC, Taylor CT (2017) Hypoxia inducible factor (HIF) hydroxylases as regulators of intestinal epithelial barrier function. Cell Mol Gastroenterol Hepatol 3:303–315. https://doi.org/10.1016/j.jcmgh.2017.02.004

Mastrogiannaki M, Matak P, Keith B et al (2009) HIF-2α, but not HIF-1α, promotes iron absorption in mice. J Clin Invest 119:1159–1166. https://doi.org/10.1172/JCI38499

Meijer K, de Vos P, Priebe MG (2010) Butyrate and other short-chain fatty acids as modulators of immunity: what relevance for health? Curr Opin Clin Nutr Metab Care 13:715. https://doi.org/10.1097/MCO.0b013e32833eebe5

Parada Venegas D, De la Fuente MK, Landskron G et al (2019) Short chain fatty acids (SCFAs)-mediated gut epithelial and immune regulation and its relevance for inflammatory bowel diseases. Front Immunol 10. https://doi.org/10.3389/fimmu.2019.00277

Pedroso AA, Batal AB, Lee MD (2016) Effect of in ovo administration of an adult-derived microbiota on establishment of the intestinal microbiome in chickens. Am J Vet Res 77:514–526. https://doi.org/10.2460/ajvr.77.5.514

Pedroso AA, Maurer J, Cheng Y, Lee MD (2012) Remodeling the intestinal ecosystem toward better performance and intestinal health. J Appl Poult Res 21:432–443. https://doi.org/10.3382/japr.2011-00401

Ramakrishnan SK, Shah YM (2016) Role of intestinal HIF-2α in health and disease. Annu Rev Physiol 78:301–325. https://doi.org/10.1146/annurev-physiol-021115-105202

Rey S, Semenza GL (2010) Hypoxia-inducible factor-1-dependent mechanisms of vascularization and vascular remodelling. Cardiovasc Res 86:236–242. https://doi.org/10.1093/cvr/cvq045

Rivera-Chávez F, Zhang LF, Faber F et al (2016) Depletion of butyrate-producing Clostridia from the gut microbiota drives an aerobic luminal expansion of Salmonella. Cell Host Microbe 19:443–454. https://doi.org/10.1016/j.chom.2016.03.004

Round JL, Mazmanian SK (2009) The gut microbiota shapes intestinal immune responses during health and disease. Nat Rev Immunol 9:313–323. https://doi.org/10.1038/nri2515

Semenza GL (1999) Perspectives on oxygen sensing. Cell 98:281–284. https://doi.org/10.1016/S0092-8674(00)81957-1

Semenza GL (2012) Hypoxia-inducible factors in physiology and medicine. Cell 148:399–408. https://doi.org/10.1016/j.cell.2012.01.021

Semenza GL, Wang GL (1992) A nuclear factor induced by hypoxia via de novo protein synthesis binds to the human erythropoietin gene enhancer at a site required for transcriptional activation. Mol Cell Biol 12:5447–5454. https://doi.org/10.1128/MCB.12.12.5447

Shah YM (2016) The role of hypoxia in intestinal inflammation. Mol Cell Pediatr 3. https://doi.org/10.1186/s40348-016-0030-1

Shah YM, Matsubara T, Ito S et al (2009) Intestinal hypoxia-inducible transcription factors are essential for iron absorption following iron deficiency. Cell Metab 9:152–164. https://doi.org/10.1016/j.cmet.2008.12.012

Shepherd AP (1982) Metabolic control of intestinal oxygenation and blood flow. Fed Proc 41: 2084–2089

Song B, Li H, Wu Y et al (2017) Effect of microencapsulated sodium butyrate dietary supplementation on growth performance and intestinal barrier function of broiler chickens infected with necrotic enteritis. Anim Feed Sci Technol 232:6–15. https://doi.org/10.1016/j.anifeedsci.2017.07.009

Steinert RE, Lee Y-K, Sybesma W (2020) Vitamins for the gut microbiome. Trends Mol Med 26: 137–140. https://doi.org/10.1016/j.molmed.2019.11.005

Sun L, Li T, Tang H, et al (2019) Intestinal epithelial cells-derived hypoxia-inducible factor-1α is essential for the homeostasis of intestinal intraepithelial lymphocytes. Front Immunol 10. https://doi.org/10.3389/fimmu.2019.00806

Taylor CT, Colgan SP (2007) Hypoxia and gastrointestinal disease. J Mol Med 85:1295–1300. https://doi.org/10.1007/s00109-007-0277-z

Taylor CT, Colgan SP (1999) Therapeutic targets for hypoxia-elicited pathways. Pharm Res 16: 1498–1505. https://doi.org/10.1023/A:1011936016833

Taylor M, Qu A, Anderson ER et al (2011) Hypoxia-inducible factor-2α mediates the adaptive increase of intestinal ferroportin during iron deficiency in mice. Gastroenterology 140:2044–2055. https://doi.org/10.1053/j.gastro.2011.03.007

Tugnoli B, Piva A, Sarli G, Grilli E (2020) Tributyrin differentially regulates inflammatory markers and modulates goblet cells number along the intestinal tract segments of weaning pigs. Livest Sci 234:103996. https://doi.org/10.1016/j.livsci.2020.103996

Turnbaugh PJ, Bäckhed F, Fulton L, Gordon JI (2008) Diet-induced obesity is linked to marked but reversible alterations in the mouse distal gut microbiome. Cell Host Microbe 3:213–223. https://doi.org/10.1016/j.chom.2008.02.015

van de Wouw M, Schellekens H, Dinan TG, Cryan JF (2017) Microbiota-gut-brain axis: modulator of host metabolism and appetite. J Nutr 147:727–745. https://doi.org/10.3945/jn.116.240481

Wang GL, Jiang BH, Rue EA, Semenza GL (1995) Hypoxia-inducible factor 1 is a basic-helix-loop-helix-PAS heterodimer regulated by cellular O2 tension. Proc Natl Acad Sci 92:5510–5514. https://doi.org/10.1073/pnas.92.12.5510

Wang H-B, Wang P-Y, Wang X et al (2012) Butyrate enhances intestinal epithelial barrier function via up-regulation of tight junction protein Claudin-1 transcription. Dig Dis Sci 57:3126–3135. https://doi.org/10.1007/s10620-012-2259-4

Yang Y, Iji PA, Choct M (2009) Dietary modulation of gut microflora in broiler chickens: a review of the role of six kinds of alternatives to in-feed antibiotics. Worlds Poult Sci J 65:97–114. https://doi.org/10.1017/S0043933909000008

Zheng L, Kelly CJ, Colgan SP (2015) Physiologic hypoxia and oxygen homeostasis in the healthy intestine. A review in the theme: cellular responses to hypoxia. Am J Physiol – Cell Physiol 309: C350–C360. https://doi.org/10.1152/ajpcell.00191.2015

Zhou Z, Nie K, Huang Q et al (2017) Changes of cecal microflora in chickens following Eimeria tenella challenge and regulating effect of coated sodium butyrate. Exp Parasitol 177:73–81. https://doi.org/10.1016/j.exppara.2017.04.007

Zou X, Ji J, Qu H et al (2019) Effects of sodium butyrate on intestinal health and gut microbiota composition during intestinal inflammation progression in broilers. Poult Sci 98:4449–4456. https://doi.org/10.3382/ps/pez279

Gut Microbiome and Poultry Health

5

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Abstract

Intestinal microbiota is involved in a variety of metabolic and immunological functions and has been implicated in both gastrointestinal and extra-intestinal disorders. Advances in high-throughput sequencing and mass spectrometry-based technologies have expanded our understanding of the intestinal microbiota composition, function, and their interplay with the host. The 16S rRNA gene sequencing and meta-omics techniques have revealed the association between dysbiosis of the intestinal microbiota and poultry diseases. Lactic acid bacteria and short-chain fatty acid-producing bacteria are generally reduced, while opportunistic pathogens are flourished in the intestine of chickens with coccidiosis or necrotic enteritis. Strategies to modulate the intestinal microbiome through probiotics, prebiotics, postbiotics, phytochemicals, or microbiota transplantation are showing promises in enhancing disease resistance and production efficiency in poultry. A better understanding of the structure and function of the intestinal microbiome and its interactions with the host have potential to identify microbial and host signatures associated with diseases and facilitate the development of novel antibiotic alternatives to improve the health and productivity of poultry.

Keywords

Microbiome · Microbiota · Dysbiosis · Intestinal health · Coccidiosis · Necrotic enteritis · Poultry

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5.1 Introduction

The gastrointestinal tract (GI) of humans and animals is inhabited by trillions of diverse microorganisms including bacteria, archaea, protists, fungi, and viruses, which are collectively known as the microbiota (Clavijo and Flórez 2018; Aggeletopoulou et al. 2019). The intestinal microbiota plays a critical role in maintaining host health by aiding in nutrient digestion, metabolism, and absorption, providing competitive exclusion of pathogens, and promoting immune development (Clavijo and Flórez 2018; Aggeletopoulou et al. 2019). Advancement in the microbiome analysis techniques such as 16S rRNA gene sequencing, metagenomics, metatranscriptomics, and metametabolomics allows for a detailed understanding of the structure and function of the microbial community (Zhang et al. 2019; Knight et al. 2018; Johnson et al. 2016). Applications of these techniques has revealed that intestinal microbiota dysbiosis is linked to a variety of poultry intestinal and extra-intestinal diseases such as necrotic enteritis (NE) (Kiu et al. 2019; Lacey et al. 2018; Latorre et al. 2018), coccidiosis (Lu et al. 2020; Chen et al. 2020; Macdonald et al. 2017), influenza (Yitbarek et al. 2018), Marek's disease (Perumbakkam et al. 2014), and infectious bronchitis virus (Xu et al. 2020).

In the case of NE, chickens show reduced richness of the intestinal microbiota after infection (Stanley et al. 2014a; Yang et al. 2019; Kim et al. 2015) and beta diversity is generally changed in NE chickens as well (Lacey et al. 2018; Latorre et al. 2018; Stanley et al. 2014a; Yang et al. 2019; Hernandez-Patlan et al. 2019). The intestinal microbiota of NE chickens is characterized by reduced abundance of lactic acid bacteria such as *Lactobacillus* and *Weissella* (Lacey et al. 2018; Yang et al. 2019; Hernandez-Patlan et al. 2019). Moreover, short-chain fatty acid (SCFA)-producing bacteria such as *Blautia, Coprococcus,* and *Eubacterium hallii* are diminished in NE chickens (Kiu et al. 2019; Lacey et al. 2018; Li et al. 2017; Stanley et al. 2012). In coccidiosis, the cecal microbiota is largely stable in response to *Eimeria maxima* or *E. tenella* in broilers (Lu et al. 2020; Chen et al. 2020; Macdonald et al. 2017), while opportunistic pathogens such as *C. perfringens* and *Enterobacteriaceae* are increased with a concomitant reduction of lactic acid and SCFA-producing bacteria in the ceca (Lu et al. 2020; Chen et al. 2020; Macdonald et al. 2017; Kimura et al. 1976). Additional investigations are warranted to characterize the microbiota signatures associated with various diseases and understand the microbiota–host interactions.

Modulation of the intestinal microbiota has proved to be beneficial in alleviating many of poultry diseases. For example, administration of probiotics such as *Lactobacillus johnsonii* (Qing et al. 2017), *Lactobacillus acidophilus* (Li et al. 2017), and *Bacillus* (Hernandez-Patlan et al. 2019) has been found to increase lactic acid bacteria in the intestine and alleviate NE in broilers. Prebiotic oligosaccharides favor the growth of lactic acid bacteria and SCFA producers, but suppress pathogen colonization in the chicken intestine (Davani-Davari et al. 2019; Teng and Kim 2018). With antimicrobial and many other activities, essential oils are beneficial in maintaining the microbiota balance (Zeng et al. 2015; Hashemi and Davoodi 2011). Fecal microbiota transplantation has become a standard treatment to *C. difficile*

infections in humans (van Nood et al. 2013). Cloacal administration of ileal or cecal contents from NE-resistant chickens protects naïve chickens against NE (Keerqin et al. 2017). More research is needed to investigate the potential of microbiome-based, antibiotic-free approaches to improving poultry health and productivity.

5.2 Analysis of the Microbiome

5.2.1 Basic Concepts in the Microbiome Analysis

The microbiome is defined as "a characteristic microbial community occupying a reasonably well-defined habitat which has distinct physiochemical properties" (Whipps et al. 1988) and consists of the microbial community, microbial structural elements and metabolites, and the environmental conditions (Berg et al. 2020). The microbiota refers only to the microbial community of the microbiome, which is represented by a collection of all living microorganisms including bacteria, fungi, viruses, archaea, and protists (Berg et al. 2020). Alpha and beta diversity are commonly used metrics in the measurement of the diversity of a microbial community. Alpha diversity refers to species diversity within a community and is measured by richness and evenness (Whittaker 1972). The richness estimates the number of species in a community and can be measured by the number of observed species, Chao1, and abundance-based coverage estimators (ACE), etc. (Knight et al. 2018; Hughes et al. 2001). On the other hand, evenness characterizes the equitability of species within a community (Whittaker 1972). To measure both evenness and richness of a microbial community, Shannon index and Simpson index are frequently used (Knight et al. 2018).

Beta diversity evaluates the differences between microbial communities by computing pairwise distance metrics (Knight et al. 2018; Whittaker 1972). Jaccard and unweighted UniFrac are qualitative metrics of beta diversity measuring the presence or absence of microbial species, while quantitative indices such as Bray–Curtis and weighted UniFrac account for species abundance as well as their presence or absence (Knight et al. 2018). In contrast to Jaccard or Bray–Curtis, UniFrac takes the phylogenetic relationships of the species into consideration when measuring the distance among microbial communities (Lozupone and Knight 2005).

5.2.2 16s rRNA Gene Sequencing

Molecular methods, such as PCR amplification of small subunit ribosomal RNA (SSU rRNA) genes, have allowed culture-independent profiling of bacterial communities (Su et al. 2012). The 16S rRNA gene sequencing, also known as amplicon sequencing, is the most commonly used technology for bacterial profiling by taking advantage of the ubiquity and high sequence conservation of the 16S rRNA gene in bacteria (Janda and Abbott 2007). Though mostly conserved, the 16S rRNA gene has nine "hypervariable" regions (V1–V9) that are interspersed among

extremely conserved regions and can be targeted with universal primers located in the conserved regions for bacterial identification (James 2010). The V3 (approximately 180–200 bp) and V4 (approximately 250 bp) regions are frequently targeted in microbiome studies. Kozich et al. (2013) found that amplification of V4 had the lowest error rate (0.01%), followed by V3–V4 (0.10–0.21%) and V4–V5 (0.36–0.64%) (Kozich et al. 2013). Species richness estimates generated by sequencing V4, V5–V6, and V6–V7 fragments are comparable to the richness estimate of the full-length 16S rRNA gene sequencing, while sequencing the V3 region underestimates species richness (Youssef et al. 2009). V4, V5, and V6 regions appear to be the most reliable in representing the full-length gene sequence in the phylogenic analysis (Yang et al. 2016). V2 and V3 regions are better suited to identify bacteria at the genus level than relatively less variable V4, V5, and V7 regions (Chakravorty et al. 2007).

After deep sequencing, raw reads of the 16S sequencing data are subjected to pairing, trimming, alignment, clustering, classification, and statistical analysis. The two most popular pipelines for processing and analyzing bacterial 16S sequencing reads are mothur (Schloss et al. 2009) and QIIME 2 (Bolyen et al. 2019). Both are similar in their capabilities, but differ in their default alignment, clustering, and classification algorithms. The differences in output between two appear to be linked to the reference database used, rather than the software itself (López-García et al. 2018). Additionally, UPARSE and DADA2 can also be used for analyzing 16S sequencing data (Niu et al. 2018). Clustering has traditionally been performed based on sequence identity by binning sequencing reads that differ by less than a fixed, arbitrary dissimilarity threshold into "operational taxonomic units" (OTUs), which are operationally equivalent to species. Generally, sequences of no less than 97% identity are combined and assigned to the same OTU, 95% identity to the same genus, and 80% identity to the same phylum (Schloss and Handelsman 2005). Recently, amplicon sequence variants (ASVs) were introduced and recommended to replace the OTU clustering method (Knight et al. 2018; Callahan et al. 2017). Instead of binning the sequences with 97% identity or above as a single OTU, every unique sequence is treated separately as an ASV. As such, sequencing variations are distinguished by a single nucleotide change, which helps increase the resolution of taxonomic classification and allows a direct comparison of the outcomes among different studies. Algorithms such as DADA2 (Callahan et al. 2016) and Deblur (Amir et al. 2017) also produce ASVs.

Taxonomic assignment of 16S sequencing reads is then achieved by comparing OTUs or ASVs with known sequences in reference databases. GreenGenes, SILVA, and RDP are the most popular 16S reference databases used (Knight et al. 2018). Taxonomy information on sequence reads can also be confirmed through BLAST search against the National Center for Biotechnology Information (NCBI) nucleotide databases. The R software is a powerful and widely used tool for downstream statistical analysis and visualization (R Core Team. R 2019). The 16S rRNA gene sequencing is the most cost-effective approach to study the composition of a microbial community. In addition to analysis of alpha and beta diversity and taxonomic profile, 16S rRNA sequencing data can be used to predict functional

potential of a microbial community using a method like PICRUSt (Langille et al. 2013).

5.3 Metagenomics, Metatranscriptomics, Metaproteomics, and Metametabolomics

To further reveal the functions of a microbial community, meta-omics techniques such as metagenomics, metatranscriptomics, metaproteomics, and metametabolomics can be employed (Nyholm et al. 2020). These techniques quantify different microbial genomes, RNA transcripts, proteins, and metabolites in a microbial community, providing the information on the functional and metabolic activities of the microbiota and its interactions with the environment. Metagenomics employs whole genome shotgun (WGS) sequencing and computational tools to sequence, assemble, classify, and annotate microbial DNA, to reveal the genome diversity and functions of a microbial community (Handelsman 2004; Oulas et al. 2015). Instead of sequencing a fragment of the 16S rRNA gene, metagenomics shears and sequences the entire collection of the microbial genomic DNA, allowing the identification of microbes at the strain level and providing more detailed genetic information (Knight et al. 2018). Tools such as MetaPhlAn2, Kraken, and MG-RAST classify WGS sequences to the species level, while Sigma, PanPhlan, StrainPhlAn, ConStrains, and LSA can identify taxa with a strain-level resolution (Niu et al. 2018). Along with the identification of gene families, encoded proteins and relevant metabolic pathways can be quantified in the metagenomics analysis. Kyoto Encyclopedia of Genes and Genomes (KEGG), SEED, eggNOG, and COG are widely used databases for functional annotation of genomes (Oulas et al. 2015). For example, Huang et al. (2018) revealed the changes in the microbial composition and key metabolic pathways in response to a plant-derived growth promoter in chickens (Huang et al. 2018). Xiong et al. (2018) defined antibiotic-induced shifts in the microbiota composition and antibiotic resistance genes in the feces of broilers using metagenomics (Xiong et al. 2018).

Metatranscriptomics is a method of sequencing all gene transcripts in a microbial community yielding information on the microbial mRNA expression profile and active functionality (Knight et al. 2018). RNA sequencing (RNA-Seq) has become the method of choice for comprehensive analysis of the microbial transcriptome (Wickramasinghe et al. 2014). The workflow for RNA-Seq generally includes RNA extraction, rRNA removal, library preparation, RNA-seq, read filtering, and aligning reads to a reference sequence and de novo assembly, annotation, and statistical analysis. Pipelines like MG-RAST, HUMAnN2, MetaTrans, and SAMSA can be applied for metatranscriptomic analysis (Niu et al. 2018). Khalique et al. (2019) identified differentially expressed mRNAs and KEGG pathways related to liver inflammation induced by subclinical NE in chickens (Khalique et al. 2019).

Metaproteomics measures the protein expression profile of a microbial community, thus providing further insights into microbial functions (Zhang et al. 2019). Major steps for the metaproteome analyses include protein extraction, protein

separation, mass spectrometry, taxonomical and functional identification, and bioinformatics analysis (Heyer et al. 2017). Functional annotation of proteins can be achieved using gene ontologies (GO) and UniProtKB keywords. Moreover, metabolic pathways of identified proteins can be mapped using repositories such as MetaCyc and KEGG (Heyer et al. 2017). The microbial proteome present in the fecal microbiota of chickens has been identified using metaproteomics (Tang et al. 2014).

Metametabolomics aims to profile the entire repertoire of metabolites present in a microbial community and is commonly used to identify biomarkers and elucidate the involvement of certain metabolic pathways (Johnson et al. 2016). Mass spectrometry and nuclear magnetic resonance are two major approaches for metabolite measurement (Fuhrer and Zamboni 2015). Metabolites can be identified using metabolite databases such as METLIN, HMDB, MassBank, and GMD (Johnson et al. 2016). Metabolic pathway databases such as KEGG and MetaCyc provide comprehensive information on biological functions of metabolites. The application of metametabolomics has revealed molecules and pathways associated with white striping in broilers (Boerboom et al. 2018).

5.4 Intestinal Microbiome of Poultry

5.4.1 Succession and Maturation

In comparison with other livestock species, poultry is minimally influenced by the maternal microbiota due to the sanitation practices of modern commercial hatcheries. Eggs are washed or fumigated prior to placement in a sanitized hatching environment, eliminating contact with pathogens but also parental microbiota. Instead, newly-hatched chicks are more likely to be exposed to non-avian microbial sources such as egg incubators, human handlers, transport containers, bedding material, feed, and water (Stanley et al. 2013a, 2014b). It is conceivable that the GI tract of day-old chicks is lightly inhabited by microbes, but varies greatly among individuals. Total bacterial populations of day-old chicks are approximately 10^8 and 10^{10} CFU/g digesta in the ileum and the cecum, respectively (Apajalahti et al. 2004), and plateau at 10^8–10^9 CFU/g in the ileum and 10^{11}–10^{12} CFU/g in the cecum and the cloaca (Yadav and Jha 2019).

As chickens develop and mature, the intestinal microbiota composition constantly shifts along the GI tract until approximately 3–4 weeks of age, after which the bacterial composition remains relatively stable in each intestinal segment for the duration of the animal's productive life (Ballou et al. 2016; Ranjitkar et al. 2016). Overall, Firmicutes is predominant (70%) along the entire GI tract, followed by Bacteroidetes (12.3%) and Proteobacteria (9.3%) (Feye et al. 2020; Waite and Taylor 2014). Lu et al. (2003) found that the family *Lactobacillaceae* dominates the ileum, followed by *Clostridiaceae*, *Streptococcaceae*, and *Enterococcaceae*; the cecum, on the other hand, is dominated by *Clostridiaceae*, followed by Actinobacteria, *Lactobacillaceae*, and *Bacteroidaceae* (Lu et al. 2003). The

nutrient-dense, hypoxic conditions of the jejunum and the ileum favor the growth of facultative anaerobes, including *Lactobacillus*, *Enterococcus*, and *Streptococcus*, while the anoxic environment of the distal cecum favors polysaccharide fermentation and SCFA production by the obligate anaerobes of the order Clostridiales (including *Lachnospiraceae* and *Ruminococcaceae*) (Apajalahti and Vienola 2016). SCFAs, majorly including acetate, propionate, and butyrate, contribute to host health with multifaceted benefits, such as providing energy to intestinal epithelial cells and improving epithelial integrity and immune defense (Koh et al. 2016; Sun et al. 2017).

5.4.2 Role in Digestion

Intestinal microbiome provides a myriad of nutritional benefits to the host. It helps break down potentially toxic compounds in the diet and also synthesizes the B vitamins, vitamin K, amino acids, SCFAs, lactic acid, etc. (Yadav and Jha 2019; Aggeletopoulou et al. 2019; Rinttilä and Apajalahti 2013). For example, only certain intestinal microbes, but not animals or plants, encode the key enzymes needed to form vitamin B12. Starter diets for broiler chicks consist of carbohydrate-rich mash or crumble, which favor the growth of lactic acid bacteria. Lactic acid produced in turn lowers the pH of the duodenum and jejunum, discouraging the growth of Proteobacteria (Rinttilä and Apajalahti 2013). Lactobacilli are found throughout the GI tract, as their β-glucanase and bile salt hydrolase activities are important for non-starch polysaccharide and lipid metabolism, respectively (Torok et al. 2008). Clostridiales, however, is the dominant order of the cecum and colon. Sergeant et al. (2014) analyzed the cecal metagenome and found genes involved in poly- and oligosaccharide degradation and carbohydrate fermentation to produce SCFAs (Sergeant et al. 2014). Members of Clostridiales are particularly effective at degrading starch and cellulose found in plant material, while *Bacteroides*, *Prevotella*, *Parabacteroides*, and *Alistipes* (members of Bacteroidetes) are also associated with carbohydrate and SCFA production (Stanley et al. 2013b).

5.4.3 Role in Immunological Development and Host Defense

In chickens, the adaptive immune system is not mature until the end of the first week of life, forcing newly-hatched chicks to rely heavily on their innate defense (Bar-Shira and Friedman 2006). Intestinal microbiome is essential for the development of primary and secondary immune organs and particularly B-cell activation and proliferation, which in turn promotes the secretion of mucosal immunoglobulin A (IgA) and activation of T-cells (Lex and Azizi 2017). Additionally, the intestinal microbes have been linked to higher goblet cell density and increased mucin-2 gene expression, leading to secretion of protective mucus (Broom and Kogut 2018). Intestinal microbiota is also well-known to reduce colonization of pathogens via competitive exclusion (Nisbet 2002) and appears to enhance the efficacy of vaccines

through low-grade stimulation of the intestinal immune system (Nothaft et al. 2017; Redweik et al. 2020).

5.5 Perturbation of the Intestinal Microbiome by Enteric Pathogens

5.5.1 *Eimeria*

The protozoan *Eimeria*, belonging to the phylum Apicomplexa, is responsible for coccidiosis, a major parasitic disease in poultry. Impaired growth, reduced feed efficiency, and even mortality are associated with coccidiosis, which is estimated to cost over $3 billion annually to the global poultry industry (Dalloul and Lillehoj 2006). *E. tenella*, *E. necatrix*, *E. acervulina*, *E. maxima*, *E. brunetti*, *E. mitis*, and *E. praecox* are major *Eimeria* species that cause avian coccidiosis, with site specificity and varying pathogenicities (Chapman 2014). Studies have shown that alpha and beta diversities of the cecal microbiota are relatively stable, but its composition shows alterations in response to infection with *E. maxima* or *E. tenella* in broilers (Lu et al. 2020; Chen et al. 2020; Macdonald et al. 2017). *Eimeria* infections also promote the proliferation of *C. perfringens* in the chicken intestine (Kimura et al. 1976; Arakawa and Ohe 1975). Infections with *Eimeria* spp. favor *C. perfringens* growth by damaging mucosa and impairing digestion to provide nutrients for *C. perfringens*, making coccidial infection a predisposing factor for the NE development (Hauck 2017). Culturing methods and 16S rRNA gene sequencing have also revealed that infections with *Eimeria* spp. lead to an overgrowth of *Enterobacteriaceae* in the chicken intestine (Lu et al. 2020; Macdonald et al. 2017; Kimura et al. 1976; Arakawa and Ohe 1975). Moreover, *E. tenella* infection increases the abundance of opportunistic pathogens such as *Enterococcus* and *Streptococcus* (Chen et al. 2020).

On the other hand, *Eimeria* spp. also reduces the abundances of certain intestinal bacteria such as lactic acid bacteria and SCFA-producing bacteria. Lactobacilli are obviously diminished after *E. tenella* infection in the ceca of broilers (Chen et al. 2020; Macdonald et al. 2017; Kimura et al. 1976). *E. tenella* inoculation also decreases cecal probiotic *Bifidobacteria* (O'Callaghan and van Sinderen 2016). Additionally, SCFA producers such as *Romboutsia*, *Shuttleworthia, Faecalibacterium*, *Bacteroides,* and *Bacillales* are also declined in the ceca of chickens challenged with *E. tenella* (Lu et al. 2020; Chen et al. 2020; Macdonald et al. 2017). These studies suggest that *Eimeria* disturbs the intestinal microbial community by suppressing beneficial or commensal bacteria, while facilitating the growth of opportunistic pathogens in chickens.

5.5.2 *Clostridium perfringens*

C. perfringens, a spore-forming Gram-positive bacterium within the genus *Clostridium*, is the etiological agent of avian NE. NE is the most common and economically devastating enteric disease in poultry, leading to approximately $6 billion loss annually to the industry worldwide (Wade and Keyburn 2015). NetB toxin produced by *C. perfringens* is a critical virulence factor in the pathogenesis of NE (Keyburn et al. 2008). This disease occurs commonly in 2- to 6-week-old broilers and the pathology is mainly restricted in the small intestine (Williams 2005). Reduced feed efficiency and growth suppression are associated with subclinical NE, while 10–40% mortality, signs of inappetence, dehydration, and diarrhea are manifested in clinical NE (Williams 2005; Shojadoost et al. 2012). NE is a multifactorial disease, in which feeding indigestible non-starch polysaccharides, high protein diets, coccidial infections, and immunosuppression may favor the disease development (Shojadoost et al. 2012). These factors, particularly coccidia, are thus often used in combination with *C. perfringens* to induce experimental NE (Shojadoost et al. 2012).

Disturbances of the intestinal microbiome have been described in NE (Lacey et al. 2018; Stanley et al. 2014a; Kim et al. 2015; Feng et al. 2010) and the extent of the disturbance is strongly correlated with the severity of the infection (Yang et al. 2021). As NE is progressed, *C. perfringens* colonization is increased. While the *C. perfringens* population is normally $<10^2$–10^4 CFU/g contents in the small intestine of healthy chickens, the bacterial load may rise to 10^5–10^8 CFU/g in NE birds (Kondo 1988). Associated with an increase in relative abundance of *C. perfringens* in NE, richness and Shannon index in the jejunal or ileal microbiota is evidently reduced (Yang et al. 2019). Additionally, beta diversity of the small intestinal microbiota is also altered by NE (Lacey et al. 2018; Yang et al. 2019; Hernandez-Patlan et al. 2019). However, the cecal or fecal microbiota is not as much affected by NE as the small intestinal microbiota (Latorre et al. 2018; Hernandez-Patlan et al. 2019), presumably due to a greater diversity of the cecal or fecal microbiota and the fact that only the small intestine are mainly affected by NE.

Compositionally, the intestinal microbiota is altered by NE. *Lactobacillus* such as *L. aviaries* is declined in the intestine of chickens with NE (Yang et al. 2019; Hernandez-Patlan et al. 2019; Feng et al. 2010). *Weissella*, another genus of lactic acid bacteria, is diminished in NE chickens as well (Kiu et al. 2019; Lacey et al. 2018; Yang et al. 2019; Stanley et al. 2012). Moreover, depletion of SCFA-producing bacteria such as *Eubacterium, Blautia,* and *Coprococcus* is obvious in NE-afflicted chickens (Kiu et al. 2019; Lacey et al. 2018; Li et al. 2017; Stanley et al. 2012). Concomitantly, opportunistic pathogens such as *Escherichia/Shigella* or *Enterobacteriaceae* are increased in the chicken intestine in response to NE (Yang et al. 2019; Qing et al. 2017; Du et al. 2015). Taken together, NE-induced dysbiosis is obvious and somewhat similar to the dysbiosis observed in coccidiosis. A strategy to reverse the dysbiosis may prove beneficial in the control and prevention of both coccidiosis and NE.

5.6 Manipulation of the Intestinal Microbiome to Enhance Animal Health and Productivity

5.6.1 Current Practices

Manipulation of the intestinal microbiome with probiotics, prebiotics, or through fecal microbiota transplantation has emerged as a novel strategy to improve human and animal health (Clavijo and Flórez 2018; Aggeletopoulou et al. 2019). Probiotics are live microorganisms that have a beneficial effect to host health by improving the microbiota balance, inhibiting pathogen colonization, improving nutrient digestion and absorption, and stimulation of immune development (Clavijo and Flórez 2018; Gibson and Roberfroid 1995; FAO/WHO 2002). *Lactobacillus*, *Bacillus*, *Enterococcus,* and *Bifidobacterium* as well as yeasts have been used as probiotics in poultry (Hume 2011). Administration of probiotics such as *L. johnsonii* (Qing et al. 2017), *L. acidophilus* (Li et al. 2017), and *Bacillus* (Hernandez-Patlan et al. 2019) has been shown to improve the lactic acid bacteria abundance in the intestine and alleviate NE in broilers.

Prebiotics are non-digestible food ingredients that selectively favors the growth of beneficial bacteria in the GI tract and thus improve the health of the host (Gibson and Roberfroid 1995). Prebiotics are mostly oligosaccharides that can be fermented by intestinal bacteria to produce lactic acid and SCFAs (Davani-Davari et al. 2019; Teng and Kim 2018). Prebiotics like mannan oligosaccharides, β-glucans, and fructans promote intestinal microbiota balance and modulate immune responses partly through increasing *Lactobacillus, Bifidobacteria*, *Bacteroides*, and *Faecalibacterium prausnitzii*, while inhibiting colonization of *E. coli, Salmonella, C. perfringens,* or *Campylobacter* in broilers (Teng and Kim 2018). Furthermore, phytochemicals such as essential oils (e.g., oregano and thymol) have also shown positive effects on improving intestinal microbiota and promoting performance in poultry (Zeng et al. 2015; Hashemi and Davoodi 2011).

5.7 Future Directions

Manipulation of the intestinal microbiome has emerged as a promising approach to improve animal health and productivity. Transplantation of the entire or selected species of the intestinal microbiota is actively being investigated (Aggeletopoulou et al. 2019). With the success of fecal microbiota transplantation in the treatment of *C. difficile* infections (van Nood et al. 2013), this technique is being explored as a promising therapeutic approach for other enteric diseases such as intestinal bowel disease (Aggeletopoulou et al. 2019). Although microbiota transplantation has been evaluated in poultry aiming to enhance NE resistance (Keerqin et al. 2017) and feed efficiency (Metzler-Zebeli et al. 2019; Siegerstetter et al. 2018), additional research is warranted. Moreover, current poultry microbiome studies are mainly focused on the intestinal microbial compositional measurements, not much about the microbiota functions and its interactions with the host. Little has investigated the impact of the

microbiota on the pathogenesis and therapy of extra-intestinal diseases. The application and integration of meta-omics techniques will provide further insights to the functional and metabolic activities of the microbiota as well as the interplay between the microbiota and the host in the context of intestinal and extra-intestinal diseases. Undoubtedly, the outcomes of these investigations will provide more avenues to the development of novel antibiotic alternative strategies to enhance disease resistance and production efficiency in poultry.

5.8 Conclusion

Intestinal microbiota plays a vital role in animal health. Meta-omics techniques are essential in the analysis of the structure and function of the microbiome. Dysbiosis of the intestinal microbiota is associated with a variety of intestinal and extra-intestinal diseases in poultry. Manipulation of the intestinal microbiota through probiotics, prebiotics, postbiotics, phytochemicals, or microbiota transplantation holds potential to promote growth and improve disease resistance. Further research is warranted to understand the functional alterations of the microbiota under different disease conditions and how the microbiota interacts with the host, which may facilitate the development of novel effective antibiotic-free strategies to enhance health and productivity in poultry.

References

Aggeletopoulou I, Konstantakis C, Assimakopoulos SF, Triantos C (2019) The role of the gut microbiota in the treatment of inflammatory bowel diseases. Microb Pathog 137:103774

Amir A, McDonald D, Navas-Molina JA, Kopylova E, Morton JT, Zech XZ et al (2017) Deblur rapidly resolves singlenucleotide community sequence patterns. mSystems 2(2):e00191-16

Apajalahti J, Vienola K (2016) Interaction between chicken intestinal microbiota and protein digestion. Anim Feed Sci Technol 221:323–330

Apajalahti J, Kettunen A, Graham H (2004) Characteristics of the gastrointestinal microbial communities, with special reference to the chicken. Worlds Poult Sci J 60(2):223–232

Arakawa A, Ohe O (1975) Reduction of Clostridium perfringens by feed additive antibiotics in the ceca of chickens infected with Eimeria tenella. Poult Sci 54(4):1000–1007

Ballou AL, Ali RA, Mendoza MA, Ellis JC, Hassan HM, Croom WJ et al (2016) Development of the chick microbiome: how early exposure influences future microbial diversity. Front Vet Sci 3: 2

Bar-Shira E, Friedman A (2006) Development and adaptations of innate immunity in the gastrointestinal tract of the newly hatched chick. Dev Comp Immunol 30(10):930–941

Berg G, Rybakova D, Fischer D, Cernava T, Vergès MC, Charles T et al (2020) Microbiome definition re-visited: old concepts and new challenges. Microbiome 8(1):103

Boerboom G, van Kempen T, Navarro-Villa A, Pérez-Bonilla A (2018) Unraveling the cause of white striping in broilers using metabolomics. Poult Sci 97(11):3977–3986

Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA et al (2019) Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat Biotechnol 37(8):852–857

Broom LJ, Kogut MH (2018) Inflammation: friend or foe for animal production? Poult Sci 97 (2):510–514

Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJ, Holmes SP (2016) DADA2: high-resolution sample inference from Illumina amplicon data. Nat Methods 13(7):581–583
Callahan BJ, McMurdie PJ, Holmes SP (2017) Exact sequence variants should replace operational taxonomic units in marker-gene data analysis. ISME J 11(12):2639–2643
Chakravorty S, Helb D, Burday M, Connell N, Alland D (2007) A detailed analysis of 16S ribosomal RNA gene segments for the diagnosis of pathogenic bacteria. J Microbiol Methods 69(2):330–339
Chapman HD (2014) Milestones in avian coccidiosis research: a review. Poult Sci 93(3):501–511
Chen H-L, Zhao X-Y, Zhao G-X, Huang H-B, Li H-R, Shi C-W et al (2020) Dissection of the cecal microbial community in chickens after *Eimeria tenella* infection. Parasit Vectors 13(1):56
Clavijo V, Flórez MJV (2018) The gastrointestinal microbiome and its association with the control of pathogens in broiler chicken production: a review. Poult Sci 97(3):1006–1021
Dalloul RA, Lillehoj HS (2006) Poultry coccidiosis: recent advancements in control measures and vaccine development. Expert Rev Vaccines 5(1):143–163
Davani-Davari D, Negahdaripour M, Karimzadeh I, Seifan M, Mohkam M, Masoumi SJ et al (2019) Prebiotics: definition, types, sources, mechanisms, and clinical applications. Foods 8 (3):92
Du E, Gan L, Li Z, Wang W, Liu D, Guo Y (2015) In vitro antibacterial activity of thymol and carvacrol and their effects on broiler chickens challenged with *Clostridium perfringens*. J Anim Sci Biotechnol 6(1):58
FAO/WHO (2002) Guidelines for the evaluation of probiotics in food. Food and Agriculture Organization of the United Nations/World Health Organization, London
Feng Y, Gong J, Yu H, Jin Y, Zhu J, Han Y (2010) Identification of changes in the composition of ileal bacterial microbiota of broiler chickens infected with *Clostridium perfringens*. Vet Microbiol 140(1):116–121
Feye KM, Baxter MFA, Tellez-Isaias G, Kogut MH, Ricke SC (2020) Influential factors on the composition of the conventionally raised broiler gastrointestinal microbiomes. Poult Sci 99 (2):653–659
Fuhrer T, Zamboni N (2015) High-throughput discovery metabolomics. Curr Opin Biotechnol 31: 73–78
Gibson GR, Roberfroid MB (1995) Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. J Nutr 125(6):1401–1412
Handelsman J (2004) Metagenomics: application of genomics to uncultured microorganisms. Microbiol Mol Biol Rev 68(4):669–685
Hashemi SR, Davoodi H (2011) Herbal plants and their derivatives as growth and health promoters in animal nutrition. Vet Res Commun 35(3):169–180
Hauck R (2017) Interactions between parasites and the bacterial microbiota of chickens. Avian Dis 61(4):428–436
Hernandez-Patlan D, Solis-Cruz B, Pontin KP, Hernandez X, Merino-Guzman R, Adhikari B et al (2019) Impact of a Bacillus direct-fed microbial on growth performance, intestinal barrier integrity, necrotic enteritis lesions and ileal microbiota in broiler chickens using a laboratory challenge model. Front Vet Sci 6:108
Heyer R, Schallert K, Zoun R, Becher B, Saake G, Benndorf D (2017) Challenges and perspectives of metaproteomic data analysis. J Biotechnol 261:24–36
Huang P, Zhang Y, Xiao K, Jiang F, Wang H, Tang D et al (2018) The chicken gut metagenome and the modulatory effects of plant-derived benzylisoquinoline alkaloids. Microbiome 6(1):211
Hughes JB, Hellmann JJ, Ricketts TH, Bohannan BJ (2001) Counting the uncountable: statistical approaches to estimating microbial diversity. Appl Environ Microbiol 67(10):4399–4406
Hume ME (2011) Historic perspective: prebiotics, probiotics, and other alternatives to antibiotics. Poult Sci 90(11):2663–2669
James G (2010) Universal bacterial identification by PCR and DNA sequencing of 16S rRNA gene. In: Schuller M, Sloots TP, James GS, Halliday CL, Carter IWJ (eds) PCR for clinical microbiology: an Australian and international perspective. Springer, Dordrecht, pp 209–214

Janda JM, Abbott SL (2007) 16S rRNA gene sequencing for bacterial identification in the diagnostic laboratory: pluses, perils, and pitfalls. J Clin Microbiol 45(9):2761–2764

Johnson CH, Ivanisevic J, Siuzdak G (2016) Metabolomics: beyond biomarkers and towards mechanisms. Nat Rev Mol Cell Biol 17(7):451–459

Keerqin C, Morgan N, Wu S, Svihus B, Choct M (2017) Reintroduction of microflora from necrotic enteritis-resistant chickens reduces gross lesions and improves performance of necrotic enteritis-challenged broilers. J Appl Poult Res 26(3):449–457

Keyburn AL, Boyce JD, Vaz P, Bannam TL, Ford ME, Parker D et al (2008) NetB, a new toxin that is associated with avian necrotic enteritis caused by *Clostridium perfringens*. PLoS Pathog 4(2): e26

Khalique A, Zeng D, Wang H, Qing X, Zhou Y, Xin J et al (2019) Transcriptome analysis revealed ameliorative effect of probiotic lactobacillus johnsonii BS15 against subclinical necrotic enteritis induced hepatic inflammation in broilers. Microb Pathog 132:201–207

Kim JE, Lillehoj HS, Hong YH, Kim GB, Lee SH, Lillehoj EP et al (2015) Dietary Capsicum and Curcuma longa oleoresins increase intestinal microbiome and necrotic enteritis in three commercial broiler breeds. Res Vet Sci 102:150–158

Kimura N, Mimura F, Nishida S, Kobayashi A, Mitsuoka T (1976) Studies on the relationship between intestinal flora and cecal coccidiosis in chicken. Poult Sci 55(4):1375–1383

Kiu R, Brown J, Bedwell H, Leclaire C, Caim S, Pickard D et al (2019) Genomic analysis on broiler-associated *Clostridium perfringens* strains and exploratory caecal microbiome investigation reveals key factors linked to poultry necrotic enteritis. Anim Microbiome 1:12

Knight R, Vrbanac A, Taylor BC, Aksenov A, Callewaert C, Debelius J et al (2018) Best practices for analysing microbiomes. Nat Rev Microbiol 16(7):410–422

Koh A, De Vadder F, Kovatcheva-Datchary P, Bäckhed F (2016) From dietary fiber to host physiology: short-chain fatty acids as key bacterial metabolites. Cell 165(6):1332–1345

Kondo F (1988) In vitro lecithinase activity and sensitivity to 22 antimicrobial agents of *Clostridium perfringens* isolated from necrotic enteritis of broiler chickens. Res Vet Sci 45(3):337–340

Kozich JJ, Westcott SL, Baxter NT, Highlander SK, Schloss PD (2013) Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform. Appl Environ Microbiol 79(17):5112–5120

Lacey JA, Stanley D, Keyburn AL, Ford M, Chen H, Johanesen P et al (2018) Clostridium perfringens-mediated necrotic enteritis is not influenced by the pre-existing microbiota but is promoted by large changes in the post-challenge microbiota. Vet Microbiol 227:119–126

Langille MG, Zaneveld J, Caporaso JG, McDonald D, Knights D, Reyes JA et al (2013) Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. Nat Biotechnol 31(9):814–821

Latorre JD, Adhikari B, Park SH, Teague KD, Graham LE, Mahaffey BD et al (2018) Evaluation of the epithelial barrier function and ileal microbiome in an established necrotic enteritis challenge model in broiler chickens. Front Vet Sci 5:199

Lex JR, Azizi A (2017) Microbiota, a forgotten relic of vaccination. Expert Rev Vaccines 16 (12):1171–1173

Li Z, Wang W, Liu D, Guo Y (2017) Effects of *Lactobacillus acidophilus* on gut microbiota composition in broilers challenged with *Clostridium perfringens*. PLoS One 12(11):e0188634

López-García A, Pineda-Quiroga C, Atxaerandio R, Pérez A, Hernández I, García-Rodríguez A et al (2018) Comparison of mothur and QIIME for the analysis of rumen microbiota composition based on 16S rRNA amplicon sequences. Front Microbiol 9:3010

Lozupone C, Knight R (2005) UniFrac: a new phylogenetic method for comparing microbial communities. Appl Environ Microbiol 71(12):8228–8235

Lu J, Idris U, Harmon B, Hofacre C, Maurer JJ, Lee MD (2003) Diversity and succession of the intestinal bacterial community of the maturing broiler chicken. Appl Environ Microbiol 69 (11):6816–6824

Lu M, Li RW, Zhao H, Yan X, Lillehoj HS, Sun Z et al (2020) Effects of *Eimeria maxima* and *Clostridium perfringens* infections on cecal microbial composition and the possible correlation with body weight gain in broiler chickens. Res Vet Sci 132:142–149

Macdonald SE, Nolan MJ, Harman K, Boulton K, Hume DA, Tomley FM et al (2017) Effects of *Eimeria tenella* infection on chicken caecal microbiome diversity, exploring variation associated with severity of pathology. PLoS One 12(9):e0184890

Metzler-Zebeli BU, Siegerstetter S-C, Magowan E, Lawlor PG, O'Connell NE, Zebeli Q (2019) Fecal microbiota transplant from highly feed efficient donors affects ccecal physiology and microbiota in low- and high-feed efficient chickens. Front Microbiol 10:1576

Nisbet D (2002) Defined competitive exclusion cultures in the prevention of enteropathogen colonisation in poultry and swine. Antonie Van Leeuwenhoek 81(1–4):481–486

Niu SY, Yang J, McDermaid A, Zhao J, Kang Y, Ma Q (2018) Bioinformatics tools for quantitative and functional metagenome and metatranscriptome data analysis in microbes. Brief Bioinform 19(6):1415–1429

Nothaft H, Perez-Muñoz ME, Gouveia GJ, Duar RM, Wanford JJ, Lango-Scholey L et al (2017) Coadministration of the campylobacter jejuni N-glycan-based vaccine with probiotics improves vaccine performance in broiler chickens. Appl Environ Microbiol 83(23):e01523–e01517

Nyholm L, Koziol A, Marcos S, Botnen AB, Aizpurua O, Gopalakrishnan S et al (2020) Holo-Omics: integrated host-microbiota multi-omics for basic and applied biological research. iScience 23(8):101414

O'Callaghan A, van Sinderen D (2016) Bifidobacteria and their role as members of the human gut microbiota. Front Microbiol 7:925

Oulas A, Pavloudi C, Polymenakou P, Pavlopoulos GA, Papanikolaou N, Kotoulas G et al (2015) Metagenomics: tools and insights for analyzing next-generation sequencing data derived from biodiversity studies. Bioinform Biol Insights 9:75–88

Perumbakkam S, Hunt HD, Cheng HH (2014) Marek's disease virus influences the core gut microbiome of the chicken during the early and late phases of viral replication. FEMS Microbiol Ecol 90(1):300–312

Qing X, Zeng D, Wang H, Ni X, Liu L, Lai J et al (2017) Preventing subclinical necrotic enteritis through *Lactobacillus johnsonii* BS15 by ameliorating lipid metabolism and intestinal microflora in broiler chickens. AMB Express 7(1):139

R Core Team (2019) A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna

Ranjitkar S, Lawley B, Tannock G, Engberg RM (2016) Bacterial succession in the broiler gastrointestinal tract. Appl Environ Microbiol 82(8):2399–2410

Redweik GAJ, Stromberg ZR, Van Goor A, Mellata M (2020) Protection against avian pathogenic *Escherichia coli* and salmonella Kentucky exhibited in chickens given both probiotics and live Salmonella vaccine. Poult Sci 99(2):752–762

Rinttilä T, Apajalahti J (2013) Intestinal microbiota and metabolites—implications for broiler chicken health and performance. J Appl Poult Res 22(3):647–658

Schloss PD, Handelsman J (2005) Introducing DOTUR, a computer program for defining operational taxonomic units and estimating species richness. Appl Environ Microbiol 71 (3):1501–1506

Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB et al (2009) Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl Environ Microbiol 75(23):7537–7541

Sergeant MJ, Constantinidou C, Cogan TA, Bedford MR, Penn CW, Pallen MJ (2014) Extensive microbial and functional diversity within the chicken cecal microbiome. PLoS One 9(3):e91941

Shojadoost B, Vince AR, Prescott JF (2012) The successful experimental induction of necrotic enteritis in chickens by *Clostridium perfringens*: a critical review. Vet Res 43:74

Siegerstetter SC, Petri RM, Magowan E, Lawlor PG, Zebeli Q, O'Connell NE et al (2018) Fecal microbiota transplant from highly feed-efficient donors shows little effect on age-related

changes in feed-efficiency-associated fecal microbiota from chickens. Appl Environ Microbiol 84(2):e02330–e02317
Stanley D, Keyburn AL, Denman SE, Moore RJ (2012) Changes in the caecal microflora of chickens following *Clostridium perfringens* challenge to induce necrotic enteritis. Vet Microbiol 159(1):155–162
Stanley D, Geier MS, Hughes RJ, Denman SE, Moore RJ (2013a) Highly variable microbiota development in the chicken gastrointestinal tract. PLoS One 8(12):e84290
Stanley D, Geier MS, Denman SE, Haring VR, Crowley TM, Hughes RJ et al (2013b) Identification of chicken intestinal microbiota correlated with the efficiency of energy extraction from feed. Vet Microbiol 164(1–2):85–92
Stanley D, Wu SB, Rodgers N, Swick RA, Moore RJ (2014a) Differential responses of cecal microbiota to fishmeal, Eimeria and *Clostridium perfringens* in a necrotic enteritis challenge model in chickens. PLoS One 9(8):e104739
Stanley D, Hughes RJ, Moore RJ (2014b) Microbiota of the chicken gastrointestinal tract: influence on health, productivity and disease. Appl Microbiol Biotechnol 98(10):4301–4310
Su C, Lei L, Duan Y, Zhang KQ, Yang J (2012) Culture-independent methods for studying environmental microorganisms: methods, application, and perspective. Appl Microbiol Biotechnol 93(3):993–1003
Sun M, Wu W, Liu Z, Cong Y (2017) Microbiota metabolite short chain fatty acids, GPCR, and inflammatory bowel diseases. J Gastroenterol 52(1):1–8
Tang Y, Underwood A, Gielbert A, Woodward MJ, Petrovska L (2014) Metaproteomics analysis reveals the adaptation process for the chicken gut microbiota. Appl Environ Microbiol 80 (2):478–485
Teng P-Y, Kim WK (2018) Review: roles of prebiotics in intestinal ecosystem of broilers. Front Vet Sci 5:245
Torok VA, Ophel-Keller K, Loo M, Hughes RJ (2008) Application of methods for identifying broiler chicken gut bacterial species linked with increased energy metabolism. Appl Environ Microbiol 74(3):783–791
van Nood E, Vrieze A, Nieuwdorp M, Fuentes S, Zoetendal EG, de Vos WM et al (2013) Duodenal infusion of donor feces for recurrent *Clostridium difficile*. N Engl J Med 368(5):407–415
Wade B, Keyburn A (2015) The true cost of necrotic enteritis. World Poult 31(7):16–17
Waite DW, Taylor MW (2014) Characterizing the avian gut microbiota: membership, driving influences, and potential function. Front Microbiol 5:223
Whipps JM, Lewis K, Cooke RC (1988) Mycoparasitism and plant disease control. In: Burge MN (ed) Fungi in biological control systems. Manchester University Press, pp 161–187
Whittaker RH (1972) Evolution and measurement of species diversity. Taxon 21(2–3):213–251
Wickramasinghe S, Cánovas A, Rincón G, Medrano JF (2014) RNA-sequencing: a tool to explore new frontiers in animal genetics. Livest Sci 166:206–216
Williams RB (2005) Intercurrent coccidiosis and necrotic enteritis of chickens: rational, integrated disease management by maintenance of gut integrity. Avian Pathol 34(3):159–180
Xiong W, Wang Y, Sun Y, Ma L, Zeng Q, Jiang X et al (2018) Antibiotic-mediated changes in the fecal microbiome of broiler chickens define the incidence of antibiotic resistance genes. Microbiome 6(1):34
Xu P, Shi Y, Liu P, Yang Y, Zhou C, Li G et al (2020) 16S rRNA gene sequencing reveals an altered composition of the gut microbiota in chickens infected with a nephropathogenic infectious bronchitis virus. Sci Rep 10(1):3556
Yadav S, Jha R (2019) Strategies to modulate the intestinal microbiota and their effects on nutrient utilization, performance, and health of poultry. J Anim Sci Biotechnol 10:2
Yang B, Wang Y, Qian PY (2016) Sensitivity and correlation of hypervariable regions in 16S rRNA genes in phylogenetic analysis. BMC Bioinform 17:135
Yang WY, Lee Y, Lu H, Chou CH, Wang C (2019) Analysis of gut microbiota and the effect of lauric acid against necrotic enteritis in *Clostridium perfringens* and Eimeria side-by-side challenge model. PLoS One 14(5):e0205784

Yang Q, Liu J, Wang X, Robinson K, Whitmore MA, Stewart SN, Zhao J, Zhang G (2021) Identification of an intestinal microbiota signature associated with the severity of necrotic enteritis. Front Microbiol 12:703693

Yitbarek A, Weese JS, Alkie TN, Parkinson J, Sharif S (2018) Influenza A virus subtype H9N2 infection disrupts the composition of intestinal microbiota of chickens. FEMS Microbiol Ecol 94(1):fix165

Youssef N, Sheik CS, Krumholz LR, Najar FZ, Roe BA, Elshahed MS (2009) Comparison of species richness estimates obtained using nearly complete fragments and simulated pyrosequencing-generated fragments in 16S rRNA gene-based environmental surveys. Appl Environ Microbiol 75(16):5227–5236

Zeng Z, Zhang S, Wang H, Piao X (2015) Essential oil and aromatic plants as feed additives in non-ruminant nutrition: a review. J Anim Sci Biotechnol 6(1):7

Zhang X, Li L, Butcher J, Stintzi A, Figeys D (2019) Advancing functional and translational microbiome research using meta-omics approaches. Microbiome 7(1):154

The Gut Mycobiome and Animal Health

6

Katie Lynn Summers and Ann M. Arfken

Abstract

The gut microbiome plays a critical role in animal health through its ability to alter nutrition, immune development, inflammation, prevent potential pathogens, and participate in fungal–bacterial–host interactions. The mycobiome (fungal microbiome) in the gut is a critical component in animal health despite being numerically inferior to the bacteriome (bacterial microbiome). Currently, studies on the gut mycobiome in agricultural animals have been lacking due to limitations in technology, but the recent use of high throughput sequencing techniques has furthered the field. While challenges remain in DNA isolation, primer design, PCR parameters, and database accuracies, fungi have been found to have complex interactions in the gut milieu. Further, fungi do not demonstrate the α-diversity or succession seen in the gut bacteriome, suggesting a distinct colonization pattern. Studies have also shown the ability of the mycobiome to be manipulated by environmental factors, such as diet, more readily than the bacteriome, making it an excellent candidate for dietary interventions to promote animal growth and health. In this chapter we will examine the limitations to the field, assess the current knowledge of the gut mycobiome in agricultural animals, investigate known fungal–bacterial interactions, and review what is known regarding fungal immunity promoting gut homeostasis.

Keywords

Mycobiome · Mycobiota · Fungi · Mycoses · ITS · 18S

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6.1 Introduction

Fungi are a ubiquitous part of our world and the mycobiome (fungal microbiome) has co-evolved over time into a complex relationship with the microbiome and the gastrointestinal (GI) tracts of animals. In agricultural settings, these diverse eukaryotic microorganisms are found in the air that animals breathe in and on the feed provided to animals, and in the soil and dust of farms (Martin et al. 1996; Frac et al. 2018; Krnjaja et al. 2009). Starting at birth or hatch, animals are exposed to environmental microbes, including those present on the maternal skin and in feces. These fungi can become autochthonous or allochthonous members of the GI tract of agricultural animals. These fungi are considered part of the "rare biosphere" (less than 1% of the microorganisms in an ecosystem) as they never reach the numerical density of bacteria that simultaneously colonize the gut (Dubos et al. 1965), but they contribute significantly to host development, immune responses, and microbial interactions (Iliev et al. 2012; Rizzetto et al. 2014; Underhill and Iliev 2014).

While fungi play a critical role in host homeostasis, under the right conditions, including gut dysbiosis, fungi can switch to opportunistic pathogens that are associated with multiple diseases including allergic airway disease, atopic dermatitis, obesity, and inflammatory bowel disease (Huseyin et al. 2017a; Limon et al. 2017; Iliev and Leonardi 2017; Heisel et al. 2017). In agricultural animals, these yeasts and molds can frequently cause mycoses (fungal diseases) that are a financial burden to the industry, but the mechanism behind the fungal pathogen switch is not well understood. While species like *Cryptococcus neoformans* can persist in the lungs of humans for years at low levels, they can go on to "bloom" and cause illness (Buchanan and Murphy 1998). *Candida albicans* is another indigenous fungus in humans that can become an opportunistic pathogen when a human becomes immunocompromised or takes broad-spectrum antibiotics. However, not all commensal fungi are potential pathogens; some are beneficial, such as *Saccharomyces cerevisiae* var. *boulardii*. *S. boulardii* has been found to be protective against *Clostridium difficile* in mice through increased IgA production leading to reduced efficacy of *C. difficile* toxin A (Qamar et al. 2001) and to conserve tight junctions of enterocytes in the small intestine (Czerucka et al. 2000; Sougioultzis et al. 2006). *S. boulardii* also increases short chain fatty acids (SCFAs), such as butyrate, that may induce a protective gut environment and may provide a food source to other microbes (Schneider et al. 2005). Fungal species in agricultural animals have been associated with reduced fertility and predisposition to enhanced secondary infections, but the underlying role of fungi in health remains to be fully understood (Weissenbacher-Lang et al. 2016; Kanora and Maes 2009).

Though it is tempting to lump the bacteriome and mycobiome together in overall microbiota studies, the low relative abundance of the mycobiome can result in sequencing technologies missing or biasing critical species. Further, the composition and diversity of the mycobiome is significantly different than the bacteriome; fungi in a healthy host tend to have lower α-diversity overall but maintain a higher α-diversity in the upper GI tract (Nash et al. 2017; Ward et al. 2018), whereas the highest bacterial diversity in healthy hosts is found in the lower GI tract (Hillman

et al. 2017) and low bacterial α-diversity is associated with host disease and dysbiosis (Turnbaugh et al. 2009; Carriere et al. 2014; Kang et al. 2013). High variability in the early life mycobiome has also been shown (Ward et al. 2018), which may indicate a potentially stronger link between mycobiome development and agricultural exposure than that of the bacteriome (Arfken et al. 2020; Summers et al. 2019). Several studies have demonstrated the longitudinal travel of fungi through the GI tract as fungi found in feces are also found in saliva and/or food (Auchtung et al. 2018; Oh et al. 2014; Hallen-Adams et al. 2015; Ghannoum et al. 2010). The effects of these transient fungi on animals during digestive transit remain to be understood, but the mycobiome has been found to be more malleable than the bacteriome, as it is more easily altered by simple interventions such as diet (Arfken et al. 2019, 2020; Mar Rodriguez et al. 2015). The ability to manipulate the mycobiome, and thus alter animal growth and health, is an important topic to assess in future animal performance studies.

For the purposes of this chapter, we will be focusing on the GI tract as the ecological setting for the mycobiome and its interactions and assess what is known about the role of the immune system in developing mycobiome homeostasis. We will evaluate the current technologies for studying the mycobiome and what is known about the mycobiome in cows, pigs, and poultry.

6.2 Mycobiome–Bacteriome Interactions

The mycobiome and bacteriome interact with each other in a variety of ways within the host. Competition for physical space and resources within the gut and the resulting physical and/or chemical interactions create modulated ecological settings and alter host health. In addition, some autochthonous microbes appear to have co-evolved with the host to allow for enhanced survival in certain gut niches. One example is the ability of certain fungi to survive and persist in the harsh, low pH environment found in the stomach. While certain bacteria like *Lactobacillus* can survive the acidic organ, *Candida* is also acid-tolerant and can colonize without apparent inflammation in mice (Savage and Dubos 1967). Many studies have investigated the ability of these microbes to interact with each other and multiple groups have found antagonism between *Lactobacillus* and *Candida*, preventing overgrowth of this opportunistic fungal pathogen (Mason et al. 2012a, b; Tso et al. 2018). But, the presence of *Candida* during antibiotic treatment can result in reduced lactobacilli numbers and an overall altered bacteriome and mucosal immune response that does not always return to the pre-antibiotic state long term (Erb Downward et al. 2013; Mason et al. 2012a, b). *Candida* overgrowth is associated with *Enterococcus faecalis* overgrowth and while this interaction is not fully understood, *E. faecalis* is often associated with disease states or dysbiosis with significant implications for animal health (Mason et al. 2012a, b; Garsin and Lorenz 2013). *E. faecalis* and *Pseudomonas aeruginosa* have both been shown to inhibit hyphal morphogenesis in *C. albicans*, preventing invasion and overall virulence potential, which may promote commensalism of this fungus in the gut (Hogan et al. 2004).

But, *C. albicans* produces farnesol that has been found to alter quorum sensing molecules produced by *P. aeruginosa*, altering its virulence and growth (Cugini et al. 2007; Peleg et al. 2010). SCFAs produced by anaerobic bacterial fermentation can also inhibit hyphae and protect intestinal epithelial cells through promoting epithelial integrity (Noverr and Huffnagle 2004; Schauber et al. 2003; Otte et al. 2009; Bulgasem et al. 2016; Nguyen et al. 2011).

In other organs, the bacteriome is effective at preventing fungal overgrowth as seen in germfree mouse models, where *Candida* colonizes at higher levels compared to non-germfree mice (Naglik et al. 2008). *Candida* overgrowth can lead to inflammation or disease states (Savage and Dubos 1967; Helstrom and Balish 1979; Savage 1969) and so, in this way, lactobacilli and other bacteria have been found to be critical in the control and prevention of fungal overgrowth and disease. Bacterial-fungal antagonism has also been found in the oral cavity between *Streptococcus mutans and C. albicans*. Quorum sensing molecules secreted by *S. mutans* were found to be antifungal through the inhibition of fungal biofilms, filamentation reduction, and decreased candidiasis in a murine model (dos Santos et al. 2020). Another study found that the presence of *C. albicans* can enhance *S. mutans* growth in biofilms, suggesting complex interactions occurring between the two microbes beyond simple antagonism (Kim et al. 2017; Jenkinson et al. 1990). Other antagonisms found have included gut bacteria preventing fungal growth through the production of antifungal molecules such as p-cresol by *C. difficile*. P-cresol reduces *C. albicans* virulence through the inhibition of hyphal transformation and biofilm formation (van Leeuwen et al. 2016).

Fungal species have been found to alter the microbiome through the production of metabolites that promote or inhibit bacterial and/or fungal growth. *Saccharomyces cerevisiae* is a commonly studied fungus that interacts with the bacteriome in multiple ways. *S. cerevisiae* produces ethanol during its growth that can trigger the growth of *Acinetobacter* (Smith et al. 2004). *S. boulardii* forms capric acid that alters biofilm formation, adhesion ability, and hyphal transformation in *C. albicans* (Shareck and Belhumeur 2011). *Penicillium*, *Fusidium*, and *Aspergillus* species are capable of forming metabolites such as griseofulvin, fusidic acid, mevastatin, and lovastatin that have the ability to alter microbes in the vicinity (Banani et al. 2016; Curbete and Salgado 2016; Manzoni and Rollini 2002). Further, the presence of elevated *Wallemia* spp. in the gut leads to altered pulmonary immune responses and intensified airway inflammation, demonstrating the mucosal immune response connection across the body that is seen in other gut fungi such as *A. fumigatus* and *C. albicans* (Noverr et al. 2001, 2005; Erb Downward et al. 2013; Skalski et al. 2018; McAleer et al. 2016).

In agriculture, antagonistic relationships have been found between bacterial and fungal organisms. A recent study screening 130 fungal and bacterial isolates on plants found seven different microorganisms able to inhibit *Aspergillus* spp. in vitro (Kasfi et al. 2018). Two *Bacillus* spp. and five fungal isolates were found to have anti-*Aspergillus* activity, including *Candida membranifaciens* (two strains) and *Meyerozyma guilliermondii* (three strains). But it is not always the bacteria inhibiting the fungi. *Aspergillus fumigatus* has been found to inhibit *P. aeruginosa* and is

implicated in altering the microbiome through its production of gliotoxin, a potent mycotoxin (a toxic, fungal secondary metabolite) (Reece et al. 2018). It is expected that as our understanding of the mycobiome grows in agriculture, extensive interactions between bacteria and fungi in the gut environment will be found that have important implications in animal health.

Fungi are important players in host health despite being numerically inferior to the bacteriome. Studies of the mycobiome and its interactions in the agricultural-animal gut ecosystem are severely lacking but antifungal drug regimens have been shown to be rapidly metabolized, resulting in little drug reaching the lower GI tract, and thus, partial efficacy. Further, long-term use can result in unintended diseases (Wheeler et al. 2016; Li et al. 2018). Very few studies have assessed the effect of antifungals on the microbiome, but one murine study demonstrated that antifungals decreased fungal diversity but also unintendedly increased bacterial diversity (Qiu et al. 2015). These effects and the role of diet on fungal gut outcomes need to be addressed in agricultural animals as fungal contaminants in feed sources are well documented.

6.3 Antifungal Immunology and Host Interactions

Fungal infections are well known for their contradictory overactive or underactive inflammatory responses. What immune response(s) allows for gut mycobiome homeostasis? The critical paradigm of how fungal members persist within the gut without rejection remains to be understood. In the agricultural setting, sequencing technologies have significantly aided in the characterization of the mycobiota in recent years. Fungi are diverse, ubiquitous microbes that are estimated to comprise 0.01-0.1% of the gut microbiome, but have the ability to significantly alter the host immune response (Li et al. 2019; Richard and Sokol 2019). Certain species are known to be commensals in healthy individuals, but the tissue homeostasis involved in this symbiosis remains to be determined.

When a fungus is ingested by an animal, the immune system is not ignorant of its presence and recognition occurs in multiple ways. One such mechanism is through pathogen associated molecular patterns (PAMPs) recognized by pattern recognition receptors (PRRs) expressed on host cells. The most common major cell wall components in pathogenic fungi are β-glucans, chitin, and mannans. These cell wall components are PAMPs that can be recognized by the PRRs on host cells. Specifically, Toll-like receptors (TLRs), C-type lectin receptors (CLRs), and galectin family proteins are used to detect fungi. Phagocytes will start an immune system response and subsequent fungal clearing through activation of PRRs. Innate immune cells such as monocytes and macrophages aid in the clearing of fungi, but gut cells such as intestinal epithelial and endothelial cells can also assist through PRRs on their surface (Liu et al. 2010). This immune response is controlled to prevent a too robust immune response that can cause host cell damage leading to fungal infection or invasion. Further, the presence of fungi in early life has been shown to be critical in the maturation of secondary lymphoid organs, through the induction of

lymphocyte homing to gut associated lymphoid tissues and peripheral lymph nodes via dendritic cells trafficking (Zhang et al. 2016).

Caspase recruitment domain-containing protein 9 (CARD9) is an antifungal molecule downstream of antifungal receptors such as C-lectin receptors (Sokol et al. 2013). IL-17 is implicated in animal and human studies of fungal immunity as an important receptor in the mucosal response to fungi, but its role in gut mycobiome homeostasis is unknown (Conti and Gaffen 2015; Netea et al. 2015; Plato et al. 2015). IL-22 has been shown to regulate fungi, such as *C. albicans*, in the gut (De Luca et al. 2010). Both IL-17 and IL-22 induce antimicrobial peptides production by epithelial cells, and these AMPs play a role in clearing mucosal infections of *Candida* spp. and *Aspergillus* spp. (Puel et al. 2010; Gessner et al. 2012; De Luca et al. 2013; Conti et al. 2016). IL-17 and IL-22 produced by Th17 cells are essential in preventing *Candida* overgrowth and invasion (van de Veerdonk et al. 2011). T regulatory (Treg) cells are also important when determining clearance or tolerance of fungi. As recently reviewed, when Treg cells are active, there will be reduced host cell damage, but this reduction in damage can lead to fungal persistence (Romani 2011; Romani and Puccetti 2006).

One common subversion technique utilized by fungi is altering the expression of certain cell wall components to evade immune detection. For example, the β-(1,3)-glucan on *C. albicans* is masked on hyphae so that hyphal growth can avoid immune recognition (Hernandez-Chavez et al. 2017). *Cryptococcus neoformans* creates a capsule that encompasses the entire cell wall to avoid PRR detection (Vecchiarelli 2007; Aimanianda et al. 2009). *A. fumigatus* coats its conidia with hydrophobins and melanin to avoid immune recognition and can evade immune response through hyphal transformation, which results in low recognition by TLR4. When this morphological switching occurs, the TLR2 response mediates the IL-10 pathway that shifts toward the generation of Treg cells and a Th2-type response that can result in fungal persistence (Netea et al. 2003). Signaling between fungi and the host immune response has been recently reviewed in detail in humans (Underhill and Iliev 2014; Iliev and Leonardi 2017), and noted that dectin-1 is a crucial PRR in shaping fungal immunity (Iliev and Leonardi 2017; Moyes and Naglik 2011). Dectin-1 recognizes β-(1,3)-glucan motifs on yeast cell walls and has been extensively studied in murine and human models of colitis, with dectin-1 deficiencies resulting in increased colonization of *Candida* species (Plantinga et al. 2009; van der Velden et al. 2013; Iliev and Leonardi 2017). However, fungal cells have found ways to evade dectin-1 detection through hyphal coating with mannan that hides the β-glucans on yeast (Gantner et al. 2005).

Members of the bacteriome alter the host immune response to fungi in multiple mechanisms. *Bacteroides fragilis* produces polysaccharide A that induces Tregs through TLR2 stimulation and inhibits the IL-17 response, therefore altering important components of the antifungal immune response (Round et al. 2011). Gut bacteria can release HIF-1α that stimulates host intestinal epithelial cells to create antimicrobial peptides that prevent *Candida* growth (Fan et al. 2015). Other bacteria, including lactobacilli, produce metabolites that activate IL-22 production by T helper cells to prevent *Candida* colonization (Zelante et al. 2013; Lamas et al.

2016; Kiss et al. 2011). Oever and Netea suggest that numerous bacterial species, such as *Lactobacillus*, *Clostridium*, segmented filamentous bacteria and *Bacteroides* are likely to alter the fungal immune response at the mucosal interface (Oever and Netea 2014). *Bacteroides thetaiotaomicron* and *Blautia* spp. metabolites induce the secretion of antifungal peptides by the colonic epithelium (Fan et al. 2015). All of these studies indicate that the bacteriome, mycobiome, and host exist in a complicated milieu of interactions; under certain situations, commensal fungi can become opportunistic pathogens. Despite extensive progress in bacteriome studies, fungal investigations have lagged due to multiple issues including difficulty in DNA isolation, primer design, choice of marker gene, fungal database complications, and limitations of analyses. Next, we assess the current state of technology and limitations associated with experimental design.

6.4 Methods for Investigating Fungi and Their Limitations

Prior to high throughput sequencing technologies, culture-dependent methods were utilized to investigate fungi in the environment. But the diversity in the fungal kingdom prevents in vitro studies from fully assessing fungi present in any sample due to limitations of culture conditions. Next generation sequencing has rapidly improved our understanding of the mycobiome in health and disease in human and murine samples, but this information remains lacking in agricultural animals. Despite the transient nature of some gut fungi, it is important to recognize that these species are likely to have a sustained effect on animal health. There is a need to distinguish transient members from true colonizers and identify their role in the GI microbial milieu. Through a combination of culture-dependent and culture-independent methods, our current understanding suggests several fungal genera are normal commensals in the gut of humans, but under the right circumstances (e.g., antibiotic use, immune suppression) some of these fungi act as opportunistic pathogens (Scanlan and Marchesi 2008; Hallen-Adams et al. 2015; Hallen-Adams and Suhr 2017; Limon et al. 2017; Huffnagle and Noverr 2013; Liggenstoffer et al. 2010; Kittelmann et al. 2012). Current studies suggest that this phenomenon is present in agricultural animals as well, but much work remains to be done on elucidating the mechanism behind these disease states.

Nilsson et al. recently reviewed the technologies and primers utilized in investigating the mycobiome including 454 pyrosequencing, Ion Torrent PGM and Gene Studio, Illumina MiSeq, HiSeq, and NovaSeq, PacBio RSII and Sequel, and Oxford Nanopore MinION, GridION, and PromethION and demonstrated the importance of reproducibility and availability of accurate, public fungal databases (Nilsson et al. 2019). While full details of each technology are beyond the scope of this chapter, it is worth noting the major biases and caveats associated with these technologies (Table 6.1). When investigating the mycobiome, investigators must carefully assess which techniques to use for DNA isolation, primer choice, polymerase choice, sequencing platform, and database used for analyses. Major biases have been seen when comparing freezing and storage protocols as well as biases arising

Table 6.1 Considerations for mycobiome analyses

Table 1. Considerations for Mycobiome Analyses	
Sample Type and DNA Extraction	
	Environmental contamination during sampling
	Freezing and storage conditions
	Commercial kit or sample specific
	Mechanical or enzymatic cell lysis
PCR parameters	
	Oligo target choice: 18S, ITS-1, ITS-2
	Oligo design
	Combinatorial PCR with multiple primers
	Polymerase choice
	Low abundance bias
	Amplicon length bias
	Poor species-level resolution
Data Analysis	
	Platform choice: Illumina, IonTorrent, PacBio
	Quality control and read processing
	OTU (clustering) or ASV assignment of reads
	Database selection
	Taxonomic assignments
	Bioinformatic and statistical software packages

from different DNA isolation techniques due to the presence and composition of fungal cell walls (reviewed in (Enaud et al. 2018)). A universal fungal DNA or RNA extraction method will not be viable across all mycobiome samples due to fungal diversity. There is no current consensus on whether mechanical disruption or enzymatic cell lysis is best for isolation, hence each new investigation of fungi requires careful assessment of DNA extraction methods. Only recently have commercial kits begun to be optimized for fungal extraction, and these must be more fully tested for reproducibility across laboratories and environmental samples. Once isolated, marker bias suggests that the ribosomal RNA operon copy numbers can vary greatly between fungi making overrepresentation a significant concern when assessing fungal abundance in a sample. Currently, the internal transcribed spacer (ITS) region is preferred for primer targeting, but studies have shown that ITS does not always provide sufficient resolution of all fungal species on its own, but ITS primers have been shown to discriminate between most major fungal pathogens (Schubert et al. 2007; Irinyi et al. 2015).

Fig. 6.1 ITS-1 and ITS-2 primer targets tested. Primers targeting ITS-1 (ITS1-27F, ITS1-217R) and ITS-2 (ITS3, ITS4) were assessed for amplicon accuracy

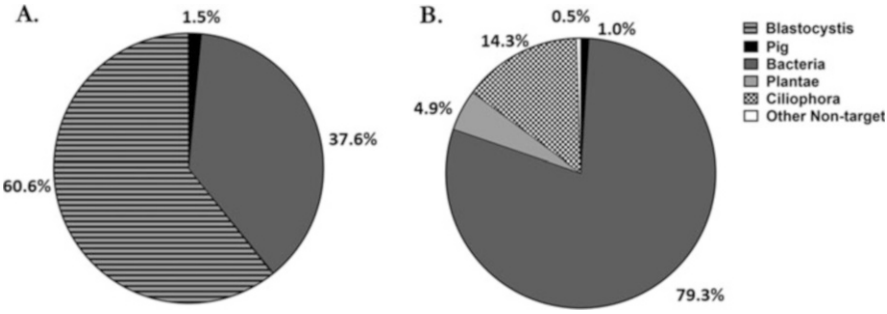

Fig. 6.2 ITS primers amplify non-fungal targets. Primers targeting ITS-1 (**a**) and ITS-2 (**b**) were assessed for percent composition of non-fungal reads amplified from pig feces. Non-fungal targets were classified to the closest match using BLAST and the NCBI NT database; non-targets were designed as having $\geq$ 80% coverage and identity to known reference sequences

ITS-targeting primers have been noted in the literature to amplify other non-fungal species such as the protozoa, *Blastocystis*, a known gut inhabitant (Martin and Rygiewicz 2005; Bellemain et al. 2010; AbuOdeh et al. 2016; Stensvold and Clark 2016). To demonstrate the differences seen between ITS primers in agricultural-animal samples, our laboratory assessed commonly used ITS-1 based primers (Usyk et al. 2017) and ITS-2 based primers (White et al. 1990), on the Illumina MiSeq platform, amplifying fungal DNA isolated from piglet feces (Fig. 6.1). Despite identical DNA isolation and PCR parameters, substantial differences were seen when comparing non-fungal amplicons between the two primer sets (Fig. 6.2). After identifying and removing fungal amplicons based on the UNITE database classification, non-targets were classified using BLAST with the NCBI nucleotide database. Non-targets were identified as amplicons having $\geq$80% coverage and identity with database reference sequences. For relative abundance of total sequences from piglet feces, non-target amplification was significantly higher in ITS-1 primers (55.3%) compared to ITS-2 primers (15.7%). ITS-1 primers (ITS1-27F, ITS1-217R) predominantly amplified non-target protozoa *Blastocystis* (60.6%) and bacteria (37.6%) (Fig. 6.2a). A small amount of host (pig) DNA was amplified as well (1.5%). Substantial differences were seen in the ITS-2 targeting primers (ITS3, ITS4) where the most dominant non-fungal amplicons were bacteria (79.3%) followed by protozoa *Ciliophora* (14.3%) and *Plantae* (4.9%), with a small amount of pig DNA also seen (1.0%) (Fig. 6.2b). Our data demonstrates the

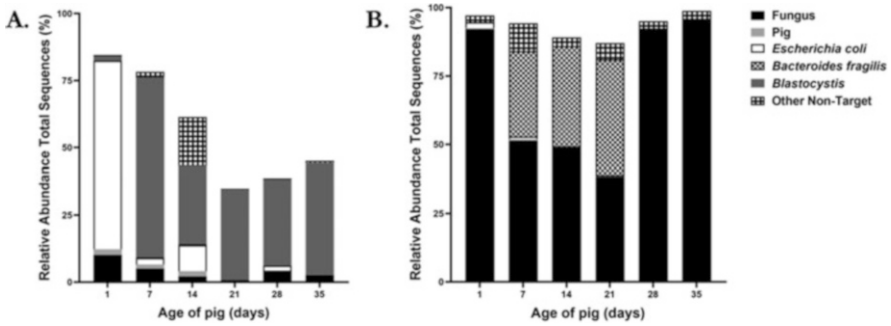

Fig. 6.3 Temporal changes to amplicons from ITS primers. Relative abundance of total sequences amplified from piglet feces from day 1 of age through day 35 (2 weeks post-weaning) utilizing ITS-1 (**a**) and ITS-2 (**b**) primers. Fungal targets were classified using the UNITE database. Non-fungal targets were classified to the closest match using BLAST and the NCBI NT database; non-targets were defined as having ≥80% coverage and identity to known reference sequences. Sequences with ≤80% coverage or identity were considered non-hits and are not included in the graph

substantial differences seen between amplicons utilizing different primer sets and highlights the non-fungal diversity and composition amplification seen.

Our laboratory also assessed the temporal differences seen in piglet fecal samples over time utilizing different primers to determine if age could alter non-fungal amplification effects. Temporal effects were strong when analyzing amplification in piglet feces (Fig. 6.3). ITS-1 primers (ITS1-27F and ITS1-217R) amplified a large amount of *Escherichia coli* from feces from 1-day-old piglets but by day 7, *Blastocystis* became the most frequent non-fungal amplicon (Fig. 6.3a). Interestingly, with this primer set, fungal amplicons were never the most abundant amplicons (Fig. 6.3a). When assessing ITS-2 primers (ITS3 and ITS4), temporal changes were also seen but less dramatically with fungi being the predominant amplicon at most time points (Fig. 6.3b). The ITS-1 primers analyzed in this data were unable to adequately amplify fungus in the piglet feces, but this is not a surprising phenomenon in the mycobiome field. While one primer set may accurately amplify one type of sample, it may be insufficient in another sample type. In our piglet feces, some of the increased non-target bacterial amplification corresponded to known temporal bacterial development trends in the pig gut, as well as physical changes in the piglet feces itself over time; together these factors can contribute to challenges in fungal DNA extraction and targeted ITS amplification even from samples from the same host. While ITS-1 and ITS-2 primers are utilized for some samples, 18S rDNA-based primers are favored for different samples. Some studies have begun to suggest using a dual approach, sequencing both 18S and ITS, to amplify the most fungal species possible (Arfi et al. 2012; Reich et al. 2017; Banos et al. 2018). One recent study utilized a combination of ITS-2 and 18S primers to enhance fungal detection of human fecal samples and found that all detectable fungi in current databases were identified by the ITS-2 primers they utilized, but this may

not be true outside of their chosen sample type (Auchtung et al. 2018). A further complication in primer choice is the uneven length of the ITS gene region found in fungal species; this may affect the abundance of certain species when utilizing targeted amplicon sequencing.

PCR bias has been shown in fungal DNA amplification as different polymerases preferentially amplify shorter fragments and introns can cause polymerase issues (Reich and Labes 2017). Therefore, fungal DNA needs to be diluted, amplification cycles kept as low as possible, and high-fidelity polymerases with low GC bias need to be used (Castle et al. 2018; Gohl et al. 2016; D'Amore et al. 2016; Nilsson et al. 2019). Different primer pairs can significantly alter the fungal phyla spectrum and extreme care must be taken in choosing the correct primer pair(s). Testing of multiple primer sets is appropriate when assessing which primers to choose at the onset of a study, as fungi have high diversity amplification potential.

Incomplete fungal bioinformatic databases are a substantial issue as our knowledge of fungal species and taxonomic accuracy is lagging significantly behind that of bacteria. A lack of quality-controlled databases and evolving fungal definitions and taxonomy, complicated by sexual and asexual fungal forms, makes analyses more challenging (Halwachs et al. 2017). The ITS marker gene is often used in studying fungi, but the resolution of fungal identification is sometimes limited to phylum level due to the lack of reference sequences or reference sequence quality (Schoch et al. 2012; Nilsson et al. 2019; Glockner et al. 2017; Yarza et al. 2017). Bioinformatic analyses including preprocessing, OTU/ASV decisions, classification, and database selection can all alter results. Fungal studies are needed to update fungal databases to assist in taxonomic assignment and reduce unclassified fungal results. Additionally, different clustering algorithms have been shown to inaccurately reflect species levels across the fungal kingdom; a suggested approach to reduce clustering errors is to utilize dynamic similarity thresholds or sequence variants (Froslev et al. 2017; Callahan et al. 2017). Unequal sequencing depth is often approached by rarefying to the lowest common number of sequences, but this often results in data loss (McMurdie and Holmes 2014) and it is important to note that due to their low abundance, fungal species are often missed in some large-scale sequencing experiments including metagenome studies.

Evidence supports the important role that fungi have in homeostasis in the GI tract of animals. Fungi can alter immune responses, inflammatory responses, metabolism, and the bacteriome, all of which will significantly impact the health and growth of agricultural animals. Care must be taken in investigating these diverse microorganisms in their respective settings. Factors to consider include storage and freezing, DNA isolation techniques, diversity of environmental sample, primer design, PCR design, database accuracy, and choice of analyses (Huseyin et al. 2017b). In the next section we will address what is known about the mycobiome in cows, pigs, and poultry.

6.5 Cows

The anaerobic fungi in the rumen of cows have been studied for many decades through culture-dependent methods. The rumen environment is a complex ecosystem with microorganisms, food fragments, and water all in close proximity to host GI cells. Diet and water intake can directly alter host health and growth, but it can also change gut microbial health and alter fungal interactions which may lead to altered milk production and animal performance. Studies have shown that despite their low numbers (10^6 CFU/mL of rumen liquid), fungi play an important role in the degradation of plant fiber (Dagar et al. 2011, 2015; Gruninger et al. 2014; Kumar et al. 2015b; Almeida et al. 2012). Rumen fungi degrade cellulose material through the production of multiple enzymes or through invasive rhizoidal growth. For example, cows fed forage diets show elevated *Caecomyces* levels, a fungus that creates bulbous rhizoids which can penetrate and expand inside cellulosic matrix, breaking up plant tissue (Joblin and Naylor 2010; Fliegerova et al. 2010). Anaerobic fungi in ruminants get their energy from fermenting carbohydrates and are adept at utilizing multiple plant monosaccharides, oligosaccharides, and polysaccharides (Gordon and Phillips 1998; Phillips and Gordon 1988). In addition to anaerobic fungi, mycelial fungi have been found to be important in the rumen. One study utilizing classical culture techniques found high levels of mycelial fungi in animals fed sorghum silage, which was attributed to potential feed contamination previously seen in sorghum and maize silages (Mngadi et al. 2008; O'Brien et al. 2007; Richard et al. 2009). Mycelial fungi are well suited to degrade plant polysaccharide cell walls due to their production of diverse enzymes. And in cows fed sugarcane foliage, yeasts were the predominant isolated fungi possibly due to their ability to degrade simple carbohydrates (Kurtzman et al. 2011). One interesting finding of that study is the higher number of yeasts found in the rumen of calves versus cows, regardless of diet, suggesting that there is an age-factor involved in the colonization of the rumen. At birth, the rumen of the calf is germfree but quickly becomes colonized by the microbes in its environment. It has been demonstrated that the saliva, feces, and immediate vegetation provide the first microbes to colonize the developing rumen of the newborn, and the development of the microbiome continues to mature as the transition from milk to solid feed occurs (Oikonomou et al. 2013; Alipour et al. 2018; Takino et al. 2017). This is a period of significant microbial changes and results in elevated fungal colonization in younger animals until the microbiome stabilizes after weaning.

More recently, studies have utilized culture-independent methods to assess the diversity of the cow gut mycobiome. One common fungal group found in the bovine rumen that assists with food degradation was *Neocallimastigomycota* (Liggenstoffer et al. 2010). Other common fungal species found from rumen fluid were *Pichia kudriavzevii* (*Candida krusei*) and *Aspergillus* (Almeida et al. 2012). However, there are many fungal clades that have no classification due to a lack of cultured samples and/or entries into databases, underscoring the need for further fungal studies (Liggenstoffer et al. 2010; Kittelmann et al. 2012; Griffith et al. 2009). Fouts et al. assessed the mycobiome in 12 cows consuming a forage-based diet and found low

fungal diversity using a combinatorial approach with Sanger sequencing and 454 pyrosequencing (Fouts et al. 2012). Despite approximately 1000 sequences/animal, only 21–40 operational taxonomic units (OTUs) were identified as fungal with the most abundant rumen fungi being identified as *Nectria*, *Penicilliopsis*, *Cystofilobasidium*, and *Delphinella*. These authors noted disparities between public repositories and previously deposited sequences that complicated their studies. This lack of sequences or inaccurate sequences continue to be a trend despite consistent findings of their importance in animal health. Kittelmann et al. investigated the rumen of multiple ruminants in an ITS-1 based approach and found 4 novel fungal clades previously undetected due to potential inaccuracies in databases. They went on to suggest that >29% of ITS-1 based sequences are incorrect at the genus level in GenBank, posing significant issues for the mycobiome field. While these authors and others have proposed revised phylogeny and taxonomy, the problem persists (Kittelmann et al. 2012).

Henderson et al. investigated the effects of DNA extraction and sampling techniques on altering the microbial populations in cow and sheep rumens (Henderson et al. 2013). Unsurprisingly, DNA extraction methods altered the resulting bacterial and archaeal communities significantly, with different isolation methods on the same sample resulting in microbial numbers that differed by more than 100-fold (Henderson et al. 2013). This phenomenon has been seen repeatedly before in other studies, due to the cell wall structure of fungi making effective DNA extraction methods difficult without damaging the underlying DNA (as discussed above). Henderson's study was ultimately hampered by correspondingly low numbers of fungal sequencing reads, therefore effects of DNA isolation on fungal community composition were difficult to assess. Further optimization and standardization of fungal DNA extraction from the cow rumen is needed.

Investigations assessing cow manure by culture-independent or combinatorial approaches have demonstrated high fungal diversity, with the presence of *Neocallimastix, Piromyces, Caecomyces,* and *Cyllamyces* (Fliegerova et al. 2010; Griffith et al. 2009). These fungi have long been known to colonize the GI tracts of cows and can persist in the environment in a wide range of temperatures and moisture (McGranaghan et al. 1999). Some fungi were viable after isolation from feces collected after 2 months of exposure to winter frost and rainfall (McGranaghan et al. 1999). When assessing feces, the time between defecation and sampling can alter the species recovered. Griffith et al. found that freezing and thawing fecal samples could lower "most probable numbers" of fungi up to 40%, severely altering results and indicating the inability of these fungi to tolerate environmental and laboratory conditions.

While the microbiota has been studied as a potential source for optimizing milk production and overall performance in dairy cows, a comprehensive culture-independent assessment of the mycobiome of the GI tract in cows remains to be completed at the time of this writing. As in other animal models, significant variations have been found in the mycobiome between different organ sites such as the abomasum, small intestine, the foregut, and the hindgut, reviewed in Khafipour et al. (2016). While cows are fed more high-grain diets to promote

increased milk yield, adverse effects on the microbiota of the GI tract have been found. To combat these changes, supplements including yeasts and yeast culture products have been tested, particularly supplements isolated from *S. cerevisiae*. The results of yeast-based supplements have shown some success in stabilizing the gut environment under certain feed conditions (Al Ibrahim et al. 2012; Chiquette et al. 2015; Li et al. 2016).

6.5.1 Dietary Interventions and the Cow Mycobiome

The role of diet is intrinsically linked to which fungi will thrive, whether on the ability of the fungus to directly utilize carbon sources in the environment/diet or through altered microbial interactions in the gut due to dietary changes. Kumar et al. demonstrated that dietary changes are the main cause of microbial shifts in the rumen, and importantly, modifying methane emissions (Kumar et al. 2013, 2015b; Pitta et al. 2014; Zhou et al. 2009). Overall, the role of diet is the main driving force in altering the fungal communities in the rumen of cows, with high fiber diets enriching anaerobic fungal communities (Kumar et al. 2015b; Kittelmann et al. 2012, 2013; Boots et al. 2013; Lima et al. 2015). One of the most studied interventions in cows is the supplementation of diet with yeast supplements (Bach et al. 2007; Bayat et al. 2015; Malekkhahi et al. 2016; Guedes et al. 2008; Chung et al. 2011; AlZahal et al. 2014; Kalmus et al. 2009; Bitencourt et al. 2011; Tristant and Moran 2015; DeVries and Chevaux 2014).

Yeast supplementation results in numerous effects that we will discuss here. Some studies have pointed to increased fiber degradation in the cow rumen through multiple mechanisms as a result of yeast supplementation. Plant cell wall polymers are abundant in the rumen and are insoluble and unable to be broken down by host enzymes, but in vitro studies have demonstrated the ability of some yeast strains to promote the growth and/or activity of fibrolytic bacteria and fungi. For example, *S. cerevisiae* stimulates *Fibrobacter succinogenes*, *Ruminococcus albus*, *Ruminococcus flavefaciens*, *Butyrivibrio fibrisolvens*, and *Neocallimastix frontalis* (Chaucheyras et al. 1995; Girard and Dawson 1995; Jouany 2006). Desnoyers et al. performed a quantitative meta-analysis over 157 experiments in ruminants in 110 papers, to study the effects of live yeast supplementation on performance in ruminant species and saw a positive effect on rumen pH, organic matter digestibility, milk production and milk fat content (Desnoyers et al. 2009). Not all studies have shown effective results with yeast supplementation as mixed results have occurred due to inconsistencies in yeast strains used, feed alterations, animal stress levels, and fungal viability. Despite these mixed results, active dry yeasts are generally accepted as beneficial in bovine nutrition (Chaucheyras-Durand et al. 2008).

Multiple animal studies have demonstrated alteration of bacterial-fungal interactions due to supplementation with live yeasts. Williams et al. described the pH stabilization effect of feeding active dry yeasts in rumen cannulated dairy cows (Williams et al. 1991). Further, interactions between the bacteriome and mycobiome in the rumen of cows were seen; *S. cerevisiae* was able to outcompete *Streptococcus*

bovis for utilization of sugars, leading to limited lactate production (Chaucheyras et al. 1996). This effect was lost when the yeast cells were heat inactivated. Numerous in vitro studies have demonstrated that multiple live yeasts can stimulate the growth and activity of lactate-utilizing bacteria by providing a source of peptides, amino acids, vitamins, and organic acids that these bacteria require (Nisbet and Martin 1991; Chaucheyras et al. 1996; Rossi et al. 1995; Rossi et al. 2004; Newbold et al. 1998). Both *Aspergillus oryzae* and *S. cerevisiae* have been utilized as dietary supplements and have been found to improve the productivity of ruminants including improved weight gain and total fiber digestibility (Martin and Nisbet 1992; Wallace 1994; Tricario et al. 2008; Di Francia et al. 2008).

6.5.2 Fungal Interactions in the Cow Gut

Fungi, including *Neocallimastix* (Krause et al. 2003), are considered necessary for digestive health, but the mechanisms remain to be understood. Fungi have been implicated in limiting lactate accumulation in the rumen and therefore altering the colonization and viability of lactate-fermenting bacteria (Chaucheyras-Durand et al. 2008). Studies have been done to assess the role of bacterial–fungal competition in the GI tract and have shown both inhibitory and stimulatory interactions. For example, *Megasphaera elsdenii* and *Selenomonas ruminantium* were able to stimulate fungal cellulolysis in co-culture, but other strains can cause inhibition of the same process (reviewed in Gordon and Phillips (1998)). Competitive exclusion has been documented in agricultural settings, resulting in reduced pathogen numbers, reduced pathogen binding, or degradation of toxins produced by pathogenic bacteria. *Saccharomyces* has been seen to reduce growth and survival of *E. coli* O157:H7 and *Listeria monocytogenes* in cow feed (Newbold and Olvera-Ramirez 2006). In calves, *Clostridia* and *Salmonella* numbers have been hindered by the supplementation of active dry yeasts in feed, and *Saccharomyces* reduces diarrhea in calves and promotes body weight gain (Galvao et al. 2005). In particular, *S. boulardii* is effective in degrading the toxin produced by *C. difficile* and reducing clostridiosis (Castagliuolo et al. 1999). One study investigated the ability of *A. oryzae* to alter fungi in the rumen of dairy cows and demonstrated that *A. oryzae* extract increased the zoospores of *N. frontalis*, a cellulolytic fungus, indicating interventions can effectively alter important fungal members in the cow rumen (Schmidt et al. 2004). However, fungi can alter other fungi, including yeasts inhibiting mycelial growth of other fungi (Coelho et al. 2007). Thus, we are just beginning to understand the complicated communications occurring between microbes in the gut and their role in animal health and growth.

6.5.3 Fungal Infections in Cows

Fungi are important opportunistic pathogens under the correct circumstances in cows, including broad-spectrum antibiotic use, reflux of acidic abomasal contents,

metabolic dysfunction, stress, presence of a primary disease, stasis of proventricular content, and postpartum period alterations (Casadevall 2007; Jensen et al. 1994). Ruminitis can be caused by multiple *Aspergillus* spp. and *Mucor* spp. (Jensen et al. 1994; Chihaya et al. 1992). *Candida glabrata* has been associated with diarrhea in newborn calves (Elad et al. 1998; Radostitis et al. 2006). *Candida* species have been found in clinical and subclinical mastitis in ruminants (Spanamberg et al. 2008). *Lichtheimia corymbifera* has been implicated in fungal infections that cause mastitis and bovine abortion (Piancastelli et al. 2009). While many fungi, such as *Trichophyton* (keratin degrading fungus), have been found to cause dermatomycoses in cows, it is worth noting that increased *Trichophyton* levels are seen in cows fed on pastures, suggesting a complex role between the gut and overall animal health (Almeida et al. 2012).

6.6 Pigs

Numerous groups have recently studied the manipulation of the microbiome to improve pig production (Nowland et al. 2019; Niederwerder 2018; Niederwerder et al. 2018; Ober et al. 2017; Aluthge et al. 2019; Arowolo et al. 2020; Alain et al. 2014; Arfken et al. 2019; Campbell et al. 2013; Chen et al. 2017; Frese et al. 2015; Gresse et al. 2017; Guevarra et al. 2018, 2019; Han et al. 2017; Han et al. 2018; Holman et al. 2017; Isaacson and Kim 2012; Kelly et al. 2017; Kim et al. 2011; Looft et al. 2014; Mach et al. 2015; Pajarillo et al. 2014; Quan et al. 2018; Summers et al. 2019; Urubschurov et al. 2008, 2011; Xiao et al. 2018; Yang et al. 2018; Zhao et al. 2015). While many studies investigated the role of age, dietary interventions, and genetic backgrounds on the bacteriome, some studies investigated the temporal development of the bacteriome over the piglet lifetime (Kim et al. 2011; Looft et al. 2012). As in other models, the mycobiome studies lagged behind the bacteriome, but the studies done thus far predominantly focused on the weaning transition, a critical stage in piglet development that can result in enhanced susceptibility to disease and reduced growth performance (Campbell et al. 2013; Guevarra et al. 2018, 2019).

The post-weaning dominance by the fungus *Kazachstania slooffiae* is one important theme commonly seen in pigs (Summers et al. 2019; Urubschurov et al. 2008, 2015, 2017, 2018; Arfken et al. 2019, 2020; Ramayo-Caldas et al. 2020). Urubschurov et al. investigated the yeast composition in pigs' feces using PCR-Denaturing Gradient Gel Electrophoresis (DGGE) with the 26S rRNA gene and DNA sequencing and found *K. slooffiae* to be the dominant yeast in post-weaning piglets. In fact, *K. slooffiae* was the only yeast found using these techniques while culture-dependent studies found *K. slooffiae*, *Galactomyces geotrichum*, *Candida catenulate*, and *C. glabrata* (Urubschurov et al. 2011). All studies of pigs, post-weaning found the presence of *Kazachstania* across geography, sex, and diet, suggesting that this fungus is a normal commensal in the healthy gut of pigs.

The stomach and upper small intestine are the first location exposed to ingested microbes; many fungi can persist or survive short-term, exposing the host to the fungal surface receptors and secreted proteins. Arfken et al. found that despite

identical piglet nursery diets, investigators saw individual variation in the stomach, duodenum, and jejunum in terms of mycobiome composition, noting the potential importance of factors such as host immunity, fungal–fungal interactions, fungal–bacterial interactions and even the amount and timing of a piglet's last meal (Arfken et al. 2019; Villmones et al. 2018). Diet plays a critical role in altering the composition of the fungal community (Arfken et al. 2019; Summers et al. 2019; White et al. 2019; Hoffmann et al. 2013). Studies investigating the effect of high fat diets on the mycobiome composition in pigs found that the *Kazachstania* remains the dominant fungus in pigs after 5 months of dietary changes. However, high fat diets altered mycobiome compositions significantly through reduced levels of *Aspergillus*, *Penicillium*, *Oidiodendron*, and *Wallemia* compared to control diet (Arowolo et al. 2020). Studies showed an association between the presence of lipid oxidation products and elevated levels of *Schwanniomyces* compared to high fat diet alone. The long-term effects of these altered fungal populations remain to be elucidated but indicate that a western diet could be an important factor in altering pig health as well as have implications for humans and chronic disease risk (Arowolo et al. 2020).

In humans, obesity studies show an association between altered fungal communities and obesity, this could be of interest in agricultural models where weight gain is of importance. For example, while fungal richness did not change, the diversity of *Zygomycota* is decreased in obese subjects. *Mucor* and *Nakaseomyces* were the most abundant genera in obese human patients and when patients had diet-induced weight loss, there was altered *Mucor* abundance (Mar Rodriguez et al. 2015). Further, high-density lipoprotein correlated negatively with *Saccharomycetes* and correlated positively with *Eurotiomycetes*. In mice, high fat diets change the mycobiome of the gut significantly through a reduction in *Saccharomyces*, consistent with that seen in humans (Heisel et al. 2017; Borgo et al. 2017). Further, the feeding of the fungal cell wall component, β-glucan, or the live fungus, *S. boulardii*, prevented obesity phenotypes associated with high fat diets (Everard et al. 2014; Neyrinck et al. 2012). These studies implicate fungi in altering obesity and weight gain in pigs and have the potential to influence industry standards.

Unsurprisingly, porcine organ microbiomes show major differences in population diversity and composition (Arfken et al. 2019; Crespo-Piazuelo et al. 2018; Liu et al. 2019; Looft et al. 2014; Isaacson and Kim 2012). For example, the fungal isolates seen in the low pH environment of the stomach are not the same composition or abundance as those found in the colon. While the α-diversity of the bacteriome increases along the GI tract from stomach to colon, the α-diversity of the mycobiome does not follow the same trend (Arfken et al. 2019; Crespo-Piazuelo et al. 2018). The bacteriome has shorter retention times for adherence to host mucus or epithelium, lower pH, and elevated bile acid concentrations, preventing most microorganisms from colonizing (Mackie et al. 1999; Walter and Ley 2011; Donaldson et al. 2016). *Aspergillus* and *Candida* species are known to promote their survival over other microorganisms by actively lowering their surrounding pH (Vylkova 2017). This increased ability to survive low pH may be a reason behind the enhanced diversity seen in the piglet stomach mycobiome post-weaning.

Strati et al. suggested the role of age and gender on gut mycobiome composition, in which younger aged humans have higher fungal richness compared to adults, when utilizing culture-independent methods; this aligns with similar trends seen in pre-weaning vs. post-weaning piglets (Arfken et al. 2019; Strati et al. 2016). Furthermore, differences in gender, such as higher mycobiome diversity in women versus men, may be seen in pigs. Christoforidou et al. found sexual dimorphism in the immune development and response to nutritional changes in neonatal pigs, including more IgA production in mesenteric lymph nodes and increased intestinal barrier function in females under inulin and starch supplementation (Christoforidou et al. 2019). The authors hypothesized that control female piglets may significantly alter their responses to dietary interventions compared to males due to the appearance of increased local immune regulation potential at the gut mucosal surface. The caveat to this study was that the piglets were 21 days old at the onset of the study and investigators followed them for a short period of time. Longer studies will be needed to assess the role of age and gender in local immune regulation.

Investigators historically use pigs as a model for human digestive tract studies due to the physiological similarities, but these similarities are not necessarily seen in the bacteriome and mycobiome upon comparison. One lesson learned from preliminary studies in pigs is that the microbes seen in the human gut environment are not identical to those in the porcine gut environment. While phylum level communities appear similar, the piglet has compromised gut function during the weaning transition and substantial changes in the composition of the microbiome result due to this early life stage (Alper et al. 2018). The fungal changes throughout pig life remain relatively unstudied and therefore comparisons to humans remain difficult. A clear example of this is the role of *C. albicans* in the human gut as a significant commensal and opportunistic pathogen (Sam et al. 2017; Hallen-Adams and Suhr 2017). Much is known about the role of *C. albicans* in altering human health, interacting with bacterial species, and altering immune responses, but the role of *Kazachstania* or other fungi, as potential healthy commensals, in altering piglet growth and immunity remains to be elucidated.

Ramayo-Caldasi et al. investigated genetic variants associated with fungal α-diversity to determine the heritability of eukaryotic communities. The candidate genes found to be associated with fungal heritability all related to immunity, metabolism, and gut homeostasis (IL23R, IL12RB2, PIK3C3, PIK3CD, HNF4A, TNFRSF9) (Ramayo-Caldas et al. 2020). This is an important step toward understanding the mechanism of fungal colonization and alterations of the pig gut environment and immune responses. Fungi can alter gut community structure through genetic exchange, interactions with bacterial species, biofilm formation, secondary metabolite secretion, and antibiotic creation, so their ability to alter the host may be extensive (Frey-Klett et al. 2011; Suhr and Hallen-Adams 2015; Hallen-Adams and Suhr 2017).

Many of the fungal diseases seen in pigs are not in the gut environment, but are the result of dermatophytes, fungi that cause diseases of the skin, hair or nails, such as ringworm. While occasional mucocutaneous candidiasis is seen in pigs, it is not considered a substantial burden in the species (Zlotowski et al. 2006). Due to our

focus on the gut in agricultural animals, we will not go into deeper analysis of mycoses in swine.

6.7 Poultry

Investigations of the gastrointestinal tract mycobiome in poultry remain sparse. To date, most poultry studies have involved the investigation of the fungal populations on the combs or wattles; very few have assessed the indigenous fungi of the GI tract. Historically, investigators examined the mycobiome in poultry through culture-based studies on a limited number of organ sites (Hume et al. 2012; Shokri et al. 2011; Subramanya et al. 2017; Sokol et al. 2018; Cafarchia et al. 2019; Byrd et al. 2017). These studies, taken together, suggest *Candida* spp. or *Aspergillus* spp. as the dominant fungal species in the cecum of chicken and turkeys (Hume et al. 2012; Shokri et al. 2011; Subramanya et al. 2017; Sokol et al. 2018; Cafarchia et al. 2019). Other genera seen through classical culturing techniques were *Trichosporon*, *Geotrichum*, *Rhodotorula,* and *Saccharomyces* (Sokol et al. 2018; Subramanya et al. 2017; Shokri et al. 2011). However, other culture-based studies, such as those by Byrd et al., found much more diversity. When a study cultured over 3000 samples in broiler and layer chickens, it found 88 fungal species in the cecum alone (Byrd et al. 2017). These discrepancies between culture-dependent studies demonstrate the diversity of fungal species and our inability to accurately assess the mycobiome without advanced technologies. Our current understanding suggests the initial colonization of the chick gut occurs by the indigenous microbiota after hatching, along with associated low levels of inflammation and increased IL-8 levels that are hypothesized to play a role in the colonization and stabilization of the microbiota (Bar-Shira and Friedman 2006). These immune responses are necessary in the first week of life in terms of regulating potential inflammation and the development of immune homeostasis (Crhanova et al. 2011). This environment of controlled inflammation eventually results in the establishment of tolerance to the normal commensal microbiota at the mucosal interface (Bar-Shira et al. 2003; Lowenthal et al. 1994; Van Immerseel et al. 2002). While more studies are needed to understand the full mechanism of immune alteration by the microbiota, the composition of the microbiome appears to determine further development of lymphocytes in the lamina propria (Methner et al. 1997; Crhanova et al. 2011). Despite this progress with the overall microbiome, little is known regarding the immune response to the mycobiome in the gut and its role in chick development.

Discrepancies between studies using culture-independent methods have continued as well. *Aspergillus* and *Trichosporon* continue to be found as the top genera in chickens (Shokri et al. 2011; Subramanya et al. 2017; Sokol et al. 2018) but one pyrosequencing-based study found only the presence of genus *Cladosporium* (*Cladosporium spp*. and *C. sphaerospermum*) in the cecum (Hume et al. 2012). Other studies have demonstrated age-based differences in fungal composition; in one study, chicks at 28 days of age were predominantly colonized by *T. asahii* and *S. brevicaulis* (Robinson et al. 2020), while another study found that

Microascus (*Scopulariopsis) brevicaulis* was the most dominant intestinal fungal genus at day 28 (Robinson et al. 2020). As discussed previously in this chapter, discrepancies in DNA isolation, primer choice, sequencing platform, and the database used for analyses can significantly alter the findings of studies and more work must be done to provide consistent, comparable research.

The diet and environment of poultry as a source of fungi cannot be overlooked, as these fungi play a large role in colonizing, or at least surviving, the GI tract environment. These fungi have the ability to survive due to diverse substrate utilization. For example, *S. brevicaulis* can degrade multiple plant substrates (Kumar et al. 2015a) and *T. asahii* can utilize multiple carbon and nitrogen sources (Colombo et al. 2011). Investigations of the mycobiome of agricultural animals must include sampling of the bedding and feed as these contribute to the bacteriome, likely alter the mycobiome as well (Kers et al. 2018; Dong and Gupta 2019). Common chicken-associated fungi such as *Aspergillus*, *Trichosporon*, and *S. brevicaulis* are associated with environmental sources such as feed, wood shavings, soil, and floor contamination (Abbott et al. 1998; Colombo et al. 2011; Sugui et al. 2014; Hubka et al. 2013). *Gibberella* is a commonly found fungus in chickens and the current understanding presumes that it originates from corn in the feed (Munkvold 2003; Robinson et al. 2020).

A characterization of the biogeography of the mycobiota along the GI tract of 28-day-old broiler chicks utilized ITS-2 primers on an Illumina platform and found 4 fungal phyla and 125 genera along the GI tract (Robinson et al. 2020). An interesting finding of this study suggests that the upper GI tract contains more fungal diversity than the lower GI tract, and that these populations transition over time from a *S. brevicaulis*-dominant to a *T. asahii*-dominant population from day 14 to day 28 (Robinson et al. 2020). Another study found decreased fungal populations in the cecum, showing consistency in the reduced diversity of the lower GI tract, which may be unsurprising due to competition from resident cecal bacteria utilizing resources (Robinson et al. 2020; Shokri et al. 2011). This trend is similar to mycobiome trends found in other models, including pigs and cows, which reveal elevated fungal diversity and abundance in the upper GI tract. The ability of the common antibiotic, bacitracin methylene disalicylate, to alter the fungal population in the GI tract of chicks was also assessed by Robinson et al. (Robinson et al. 2020). Interestingly, subtherapeutic levels of this antibiotic resulted in the most drastic effects on the fungal population composition. Further studies are needed to assess the temporal and spatial changes to the mycobiome in poultry, especially as in-feed antibiotics are still used in some settings.

Ward et al. utilized ITS-2 primers to assess the effect of a turkey-specific oral prebiotic compared to low-dose antibiotics on overall turkey performance. *Sarocladium kiliense* was found to be the most common fungus regardless of treatment; no study assessed treatments significantly altered the overall mycobiome (Ward et al. 2019). Despite a lack of significant change in α-diversity, treatment altered the abundance of certain fungal species. Prebiotics, in addition to two different probiotics (one commercial and one turkey-targeted), resulted in an increase in *Candida parapsilosis* and *C. albicans* and a reduction in *Sclerotinia*

sclerotiorum, *Debaryomyces prosopidis*, and *Cladosporium halotolerans* (Ward et al. 2019). Across treatment groups, as investigators administered antibiotics, probiotics, and/or prebiotics, the data showed parallel shifts in the bacteriome, mycobiome, and host gene expression, providing evidence for interkingdom signaling and coordinated changes (Ward et al. 2019). While the authors could not distinguish the cause and effect relationships or interactions occurring, it is perhaps not surprising that when investigators reduced one or more microbial populations, or hindered them by antibiotic use, other microbes (bacterial and fungal) took advantage of the space and resources now available in the gut environment.

As in other species, the field describes many of the mycobiome members as opportunistic pathogens and links them to diseases in the young or immunocompromised (Colombo et al. 2011; Sugui et al. 2014; Iwen et al. 2012; Sandoval-Denis et al. 2013). Further studies must be done to understand the switch from commensal to pathogen and its implications in food and personnel safety. Future studies of the mycobiome in poultry must also take the time to assess which primers and DNA isolation techniques are best for each organ site, environmental site, or feed sample to accurately identify the fungal species present. Different studies show large variations between those that utilize birds of different ages or genetics, and environmental factors such as feed, temperatures, and sanitizing techniques. Once investigators understand the baselines, they can better examine the effect of the mycobiome in altering animal health.

6.7.1 Common Poultry Mycoses of the Mucosa

Mycoses are a significant source of financial loss due to infections and/or the effects of mycotoxins in poultry. Mycotoxins, secondary fungal metabolites found in feed and grains, are outside the scope of this chapter due to the abundance of literature on the topic, but have a long history of reduced animal health in industry and are still the leading cause of immunosuppression in birds (reviewed in (Dhama et al. 2013)). Molds and yeasts can cause diverse illnesses in chicken, including lung, skin, and GI infections under the right circumstances. The poultry environment provides beneficial factors that enhance fungal growth and spore dissemination, including warm temperatures, elevated humidity, poor ventilation, and long-term storage of feed (Kamei and Watanabe 2005; Tell 2005).

Aspergillosis *Aspergillus* is a common mold that can thrive in diverse environments, and under the right conditions can infect young or immunosuppressed birds. The most common form of *Aspergillus*-induced illness is brooder pneumonia, which is an acute infection of the lungs in chicks. Other names for this are avian aspergillosis, mycotic pneumonia, pneumonomycosis, and bronchomycosis. The two major species that cause aspergillosis in poultry are *A. fumigatus* and *A. flavus*. *Aspergillus spp*. are common soil saprophytes and thrive on decaying vegetative matter and feed grains, especially in warm, humid environments often found in poultry farms where infections typically stem from inhalation of spores

leading to a primary infection in the lungs (Arne et al. 2011; Beernaert et al. 2010). While *Microsporum gallinae* and *Trichophyton simii* are well-known fungal mycoses, *Aspergillus flavus* is more costly to industry and is associated with poor sanitation and husbandry conditions (Bond 2010; Mbata 2008). However, multiple *Aspergillus* species have been isolated, including *A. nidulans*, *A. carbonicus, A. fumigatus*, and *A. tereus*, and may lead to financial loss (Taghavi et al. 2014; Miljkovic et al. 2011; Mbata 2008; Kaul and Sumbali 2000).

Young age, antibiotic use, and poor sanitation are the biggest predisposing factors to aspergillosis; young chicks and birds can have morbidity and mortality rates as high as 70–90% (Arne et al. 2011; Beernaert et al. 2010). *Aspergillus* is an effective opportunistic pathogen with the ability to create proteases and secondary metabolites that contribute to its virulence (Tekaia and Latge 2005). Further, effective immune clearance relies on mucosal epithelial cells in the respiratory tract to clear the mycosis, but conidia released by *Aspergillus* can break down the preventative physical barriers of the epithelium (Reese et al. 2006). The fungus also utilizes a mycotoxin called gliotoxin that is an effective immunosuppressant allowing immune evasion. When *Aspergillus* is not cleared effectively, chronic aspergillosis can result leading to immunosuppression in birds (Vanderheyden 1993; Pena et al. 2010). While investigators found turkeys to have higher susceptibility to *Aspergillus* infections, the mechanism behind this susceptibility is not fully understood (Dhama et al. 2013). Systemic aspergillosis can cause bone, skin, eye, and brain infections but these are relatively rare; and prevention is the best means of controlling these diseases in poultry, as treatments are not always effective.

Candidiasis Another common fungal infection in chickens is candidiasis, or thrush, which is often caused by the genus *Candida*. *Candida* is ubiquitous in the environment and is found in the upper GI tract of healthy birds, but upon long-term antibiotic use, *Candida* can overgrow and cause health issues. In this disease, a white, thickened patch forms inside the crop, the mouth, or on the skin of the vent area of a chicken. Signs of *Candida* can be subtle, and chickens simply look disheveled or listless. Typically, candidiasis is not contagious from bird to bird, but can be spread through dirty feeders and/or waterers (Odds 1994a, b). While systemic candidiasis in poultry is rare, *Candida spp*. have multiple ways of evading or dampening the immune response in the host, including the ability of *Candida* to directly bind to complement proteins and secrete aspartyl proteases that cleave complement proteins to prevent the assembly of the membrane attack complex (MAC) (Meri et al. 2004; Gropp et al. 2009). *Candida spp*. also generate adhesins that aid in cell surface attachment, phospholipases to aid hyphal invasion, and many other enzymes, e.g. neuraminidase, proteases. *Candida* can evade immune responses through morphological switching between yeast and filamentous (hyphal) growth. These morphologies are recognized differently by the immune system, aiding fungi in avoiding immune detection.

6.8 Conclusions

Currently there is no consensus on defining a healthy gut mycobiome due to numerous factors including low abundance and high diversity in fungi, temporal instability, high variation between hosts, and a lack of agreement on appropriate techniques, primers and databases to study fungi. Despite these issues, several fungi have repeatedly been found in agricultural studies, including phyla, *Ascomycota*, *Zygomycota* and *Basidiomycota*. Further, the same species are repeatedly reported as colonizers, such as *Candida*, *Cryptococcus*, *Kazachstania*, *Malassezia*, *Aspergillus*, *Saccharomyces*, *Galactomyces*, *Trichosporon*, and *Cladosporium* (Chin et al. 2020; Hallen-Adams et al. 2015; Nash et al. 2017). Fungi can alter immune responses, inflammatory responses, metabolism, and the bacteriome, which can significantly impact the health and growth of agricultural animals. Despite the transient nature of some gut fungi, it is important to recognize that these species are likely to have a sustained effect on animal health. There is a need to distinguish transient members from true colonizers and identify their role in the GI microbial milieu. Human studies have demonstrated that fungal members found in the feces are also found in saliva and/or food and the longitudinal travel of these fungi need to be further assessed (Auchtung et al. 2018; Oh et al. 2014; Hallen-Adams et al. 2015; Ghannoum et al. 2010). Interestingly, many fungi identified through molecular techniques cannot be cultured or are not optimally suited to growth at 37 °C and are unable to adapt to the GI environment. The fungi that are able to colonize and/or grow compete for nutrients must also survive challenges by other microbes and the host immune response.

Fungi are ubiquitous in the environment and therefore impossible to eradicate. Under the right circumstances these fungi can become pathogens, infecting different body sites and resulting in diverse symptoms. Recently, drug-resistant fungi have become a threat to worldwide health and agriculture. *Candida auris* recently emerged as a multi-drug resistant fungal pathogen that has large agricultural implications. Transmission of this fungal pathogen is presumed to be from an environmental source based on phylogenetic analyses (Casadevall et al. 2019; Satoh et al. 2009). Agricultural settings, in particular, have been described as a potential site of interspecies transmission, promoting the emergence of global fungal pathogens transferred zoonotically (Casadevall 2017). Future studies are needed to determine the composition and diversity of the gut mycobiome in different agricultural animals and learn the mechanisms behind the role of fungi in health, growth, and opportunistic infections.

Glossary

Mycobiome	The fungal community in and on an organism, or the fungal members of the microbiome of the gut.
Bacteriome	The bacterial members of the microbiome.
Autochthonous	Native microbial members of the microbiome.

Allochthonous	Transient microbes that are not resident members of the microbiome.
Dysbiosis	Microbial imbalance or changes in microbiome homeostasis that can contribute to disease.
Mycoses	Diseases caused by fungi.
Yeasts	Fungi consisting of single oval cells that reproduce by budding and can convert sugar into alcohol and carbon dioxide.
Molds	Fungi that grows in the form of multicellular filaments called hyphae.
Mycelium	The vegetative part of a fungus that is composed of a network of hyphae.
Hypha/hyphae	A long, branching filamentous structure of a fungus and serves as the main mode of vegetative growth.
ITS	Internal Transcribed Spacer. A region of the fungal ribosomal RNA operon that is utilized as primer targets to distinguish fungal species from each other based on variable sequences within the region.
OTU	Operational Taxonomic Units. OTU is the operational definition used in studies of the microbiome to classify groups of closely related sequences.
ASV	Amplicon Sequence Variant. DNA sequences recovered from high-throughput sequencing technologies and resolve sequences to a single nucleotide. ASVs are an alternative to OTUs.

References

Abbott SP, Sigler L, Currah RS (1998) *Microascus brevicaulis* sp. nov., the teleomorph of *Scopulariopsis brevicaulis*, supports placement of Scopulariopsis with the Microascaceae. Mycologia 90(2):297–302

AbuOdeh R, Ezzedine S, Samie A, Stensvold CR, ElBakri A (2016) Prevalence and subtype distribution of Blastocystis in healthy individuals in Sharjah, United Arab Emirates. Infect Genet Evol 37:158–162. https://doi.org/10.1016/j.meegid.2015.11.021

Aimanianda V, Bayry J, Bozza S, Kniemeyer O, Perruccio K, Elluru SR, Clavaud C, Paris S, Brakhage AA, Kaveri SV, Romani L, Latge JP (2009) Surface hydrophobin prevents immune recognition of airborne fungal spores. Nature 460(7259):1117–1121. https://doi.org/10.1038/nature08264

Al Ibrahim RM, Gath VP, Campion DP, McCarney C, Duffy P, Mulligan FJ (2012) The effect of abrupt or gradual introduction to pasture after calving and supplementation with *Saccharomyces cerevisiae* (Strain 1026) on ruminal pH and fermentation in early lactation dairycows. Anim Feed Sci Technol 178(1-2):40–47

Alain BPE, Chae JP, Balolong MP, Bum Kim H, Kang DK (2014) Assessment of fecal bacterial diversity among healthy piglets during the weaning transition. J Gen Appl Microbiol 60 (4):140–146

Alipour MJ, Jalanka J, Pessa-Morikawa T, Kokkonen T, Satokari R, Hynonen U, Iivanainen A, Niku M (2018) The composition of the perinatal intestinal microbiota in cattle. Sci Rep 8 (1):10437. https://doi.org/10.1038/s41598-018-28733-y

Almeida PNM, Duarte ER, Abrao FO, Freitas CE, Geraseev LC, Rosa CA (2012) Aerobic fungi in the rumen fluid from dairy cattle fed different sources of forage. Rev Bras Zoot 41:2336–2342

Alper J, Anestidou L, Ogilvie J (2018) Animal models for microbiome research: advancing basic and translational science. In: National Academies of Sciences, Engineering, and Medicine, The National Academies Press, Washington, DC. https://doi.org/10.17226/24858

Aluthge ND, Van Sambeek DM, Carney-Hinkle EE, Li YS, Fernando SC, Burkey TE (2019) BOARD INVITED REVIEW: The pig microbiota and the potential for harnessing the power of the microbiome to improve growth and health1. J Anim Sci 97(9):3741–3757. https://doi.org/10.1093/jas/skz208

AlZahal O, Dionissopoulos L, Laarman AH, Walker N, McBride BW (2014) Active dry *Saccharomyces cerevisiae* can alleviate the effect of subacute ruminal acidosis in lactating dairy cows. J Dairy Sci 97(12):7751–7763. https://doi.org/10.3168/jds.2014-8212

Arfi Y, Buee M, Marchand C, Levasseur A, Record E (2012) Multiple markers pyrosequencing reveals highly diverse and host-specific fungal communities on the mangrove trees *Avicennia marina* and *Rhizophora stylosa*. FEMS Microbiol Ecol 79(2):433–444. https://doi.org/10.1111/j.1574-6941.2011.01236.x

Arfken AM, Frey JF, Ramsay TG, Summers KL (2019) Yeasts of burden: exploring the mycobiome-bacteriome of the piglet GI tract. Front Microbiol 10:2286. https://doi.org/10.3389/fmicb.2019.02286

Arfken AM, Frey JF, Summers KL (2020) Temporal dynamics of the gut bacteriome and mycobiome in the weanling pig. Microorganisms 8(6). https://doi.org/10.3390/microorganisms8060868

Arne P, Thierry S, Wang D, Deville M, Le Loc'h G, Desoutter A, Femenia F, Nieguitsila A, Huang W, Chermette R, Guillot J (2011) *Aspergillus fumigatus* in poultry. Int J Microbiol 2011:746356. https://doi.org/10.1155/2011/746356

Arowolo F, Pierre JF, Blaser M, Schanmuganayagam D (2020) Longitudinal effects of dietary oxidized lipids on the gut microbiome and mycobiome in pigs. FASEB J 34(S1)

Auchtung TA, Fofanova TY, Stewart CJ, Nash AK, Wong MC, Gesell JR, Auchtung JM, Ajami NJ, Petrosino JF (2018) Investigating colonization of the healthy adult gastrointestinal tract by fungi. mSphere 3(2). https://doi.org/10.1128/mSphere.00092-18

Bach A, Iglesias C, Devant M (2007) Daily rumen pH pattern of loose-housed dairy cattle as affected by feeding pattern and live yeast supplementation. Anim Feed Sci Technol 136(1-2)

Banani H, Marcet-Houben M, Ballester AR, Abbruscato P, Gonzalez-Candelas L, Gabaldon T, Spadaro D (2016) Genome sequencing and secondary metabolism of the postharvest pathogen *Penicillium griseofulvum*. BMC Genomics 17:19. https://doi.org/10.1186/s12864-015-2347-x

Banos S, Lentendu G, Kopf A, Wubet T, Glockner FO, Reich M (2018) A comprehensive fungi-specific 18S rRNA gene sequence primer toolkit suited for diverse research issues and sequencing platforms. BMC Microbiol 18(1):190. https://doi.org/10.1186/s12866-018-1331-4

Bar-Shira E, Friedman A (2006) Development and adaptations of innate immunity in the gastrointestinal tract of the newly hatched chick. Dev Comp Immunol 30(10):930–941. https://doi.org/10.1016/j.dci.2005.12.002

Bar-Shira E, Sklan D, Friedman A (2003) Establishment of immune competence in the avian GALT during the immediate post-hatch period. Dev Comp Immunol 27(2):147–157. https://doi.org/10.1016/s0145-305x(02)00076-9

Bayat AR, Kairenius P, Stefanski T, Leskinen H, Comtet-Marre S, Forano E, Chaucheyras-Durand F, Shingfield KJ (2015) Effect of camelina oil or live yeasts (*Saccharomyces cerevisiae*) on ruminal methane production, rumen fermentation, and milk fatty acid composition in lactating cows fed grass silage diets. J Dairy Sci 98(5):3166–3181. https://doi.org/10.3168/jds.2014-7976

Beernaert LA, Pasmans F, Van Waeyenberghe L, Haesebrouck F, Martel A (2010) Aspergillus infections in birds: a review. Avian Pathol 39(5):325–331. https://doi.org/10.1080/03079457.2010.506210

Bellemain E, Carlsen T, Brochmann C, Coissac E, Taberlet P, Kauserud H (2010) ITS as an environmental DNA barcode for fungi: an in silico approach reveals potential PCR biases. BMC Microbiol 10:189. https://doi.org/10.1186/1471-2180-10-189

Bitencourt LL, Silva JRM, Oliveira BML, Junior GSD, Lopes F, Junior SS, Zacaroni OF, Pereira MN (2011) Diet digestibility and performance of dairy cows supplemented with live yeast. Sci Agric 68:301–307

Bond R (2010) Superficial veterinary mycoses. Clin Dermatol 28(2):226–236. https://doi.org/10.1016/j.clindermatol.2009.12.012

Boots B, Lillis L, Clipson N, Petrie K, Kenny DA, Boland TM, Doyle E (2013) Responses of anaerobic rumen fungal diversity (phylum Neocallimastigomycota) to changes in bovine diet. J Appl Microbiol 114(3):626–635. https://doi.org/10.1111/jam.12067

Borgo F, Verduci E, Riva A, Lassandro C, Riva E, Morace G, Borghi E (2017) Relative abundance in bacterial and fungal gut microbes in obese children: a case control study. Child Obes 13 (1):78–84. https://doi.org/10.1089/chi.2015.0194

Buchanan KL, Murphy JW (1998) What makes Cryptococcus neoformans a pathogen? Emerg Infect Dis 4(1):71–83. https://doi.org/10.3201/eid0401.980109

Bulgasem BY, Lani MN, Hassan Z, Wan Yusoff WM, Fnaish SG (2016) Antifungal activity of lactic acid bacteria strains isolated from natural honey against pathogenic Candida species. Mycobiology 44(4):302–309. https://doi.org/10.5941/MYCO.2016.44.4.302

Byrd JA, Caldwell DY, Nisbet DJ (2017) The identification of fungi collected from the ceca of commercial poultry. Poult Sci 96(7):2360–2365. https://doi.org/10.3382/ps/pew486

Cafarchia C, Iatta R, Danesi P, Camarda A, Capelli G, Otranto D (2019) Yeasts isolated from cloacal swabs, feces, and eggs of laying hens. Med Mycol 57(3):340–345. https://doi.org/10.1093/mmy/myy026

Callahan BJ, McMurdie PJ, Holmes SP (2017) Exact sequence variants should replace operational taxonomic units in marker-gene data analysis. ISME J 11(12):2639–2643. https://doi.org/10.1038/ismej.2017.119

Campbell JM, Crenshaw JD, Polo J (2013) The biological stress of early weaned piglets. J Anim Sci Biotechnol 4(1):19. https://doi.org/10.1186/2049-1891-4-19

Carriere J, Darfeuille-Michaud A, Nguyen HT (2014) Infectious etiopathogenesis of Crohn's disease. World J Gastroenterol 20(34):12102–12117. https://doi.org/10.3748/wjg.v20.i34.12102

Casadevall A (2007) Determinants of virulence in the pathogenic fungi. Fungal Biol Rev 21 (4):130–132. https://doi.org/10.1016/j.fbr.2007.02.007

Casadevall A (2017) Don't forget the fungi when considering global catastrophic biorisks. Health Secur 15(4):341–342. https://doi.org/10.1089/hs.2017.0048

Casadevall A, Kontoyiannis DP, Robert V (2019) On the emergence of *Candida auris*: climate change, azoles, swamps, and birds. mBio 10(4). https://doi.org/10.1128/mBio.01397-19

Castagliuolo I, Riegler MF, Valenick L, LaMont JT, Pothoulakis C (1999) *Saccharomyces boulardii* protease inhibits the effects of *Clostridium difficile* toxins A and B in human colonic mucosa. Infect Immun 67(1):302–307

Castle SC, Song Z, Gohl DM, Gutknecht JLM, Rosen CJ, Sadowsky MJ, Samac DA, Kinkel LL (2018) DNA template dilution impacts amplicon sequencing-based estimates of soil fungal diversity. Phytobiomes 2(2)

Chaucheyras F, Fonty G, Bertin G, Gouet P (1995) Effects of live *Saccharomyces cerevisiae* cells on zoospore germination, growth, and cellulolytic activity of the rumen anaerobic fungus, *Neocallimastix frontalis* MCH3. Curr Microbiol 31(4):201–205. https://doi.org/10.1007/BF00298373

Chaucheyras F, Fonty G, Bertin G, Salmon JM, Gouet P (1996) Effects of a strain of *Saccharomyces cerevisiae* (Levucell SC1), a microbial additive for ruminants, on lactate metabolism in vitro. Can J Microbiol 42:927–933

Chaucheyras-Durand F, Walker ND, Bach A (2008) Effects of active dry yeasts on the rumen microbial ecosystem: past, present and future. Anim Feed Sci Technol 145:5–26

Chen L, Xu Y, Chen X, Fang C, Zhao L, Chen F (2017) The maturing development of gut microbiota in commercial piglets during the weaning transition. Front Microbiol 8:1688. https://doi.org/10.3389/fmicb.2017.01688

Chihaya Y, Matsukawa K, Ohshima K, Matsui Y, Ogasa K, Furusawa Y, Okada H (1992) A pathological study of bovine alimentary mycosis. J Comp Pathol 107(2):195–206. https://doi.org/10.1016/0021-9975(92)90036-t

Chin VK, Yong VC, Chong PP, Amin Nordin S, Basir R, Abdullah M (2020) Mycobiome in the gut: a multiperspective review. Mediators Inflamm 2020:9560684. https://doi.org/10.1155/2020/9560684

Chiquette J, Lagrost J, Girard CL, Talbot G, Li S, Plaizier JC, Hindrichsen IK (2015) Efficacy of the direct-fed microbial *Enterococcus faecium* alone or in combination with *Saccharomyces cerevisiae* or *Lactococcus lactis* during induced subacute ruminal acidosis. J Dairy Sci 98 (1):190–203. https://doi.org/10.3168/jds.2014-8219

Christoforidou Z, Mora Ortiz M, Poveda C, Abbas M, Walton G, Bailey M, Lewis MC (2019) Sexual dimorphism in immune development and in response to nutritional intervention in neonatal piglets. Front Immunol 10:2705. https://doi.org/10.3389/fimmu.2019.02705

Chung YH, Walker ND, McGinn SM, Beauchemin KA (2011) Differing effects of 2 active dried yeast (*Saccharomyces cerevisiae*) strains on ruminal acidosis and methane production in nonlactating dairy cows. J Dairy Sci 94(5):2431–2439. https://doi.org/10.3168/jds.2010-3277

Coelho AR, Celli MG, Ono EYS, Wosiacki G, Hoffmann FL, Pagnocca FC, Hirooka EY (2007) Braz Aarch Biol Technol 50(4). https://doi.org/10.1590/S1516-89132007000400019

Colombo AL, Padovan AC, Chaves GM (2011) Current knowledge of Trichosporon spp. and Trichosporonosis. Clin Microbiol Rev 24(4):682–700. https://doi.org/10.1128/CMR.00003-11

Conti HR, Gaffen SL (2015) IL-17-mediated immunity to the opportunistic fungal pathogen *Candida albicans*. J Immunol 195(3):780–788. https://doi.org/10.4049/jimmunol.1500909

Conti HR, Bruno VM, Childs EE, Daugherty S, Hunter JP, Mengesha BG, Saevig DL, Hendricks MR, Coleman BM, Brane L, Solis N, Cruz JA, Verma AH, Garg AV, Hise AG, Richardson JP, Naglik JR, Filler SG, Kolls JK, Sinha S, Gaffen SL (2016) IL-17 receptor signaling in oral epithelial cells is critical for protection against oropharyngeal candidiasis. Cell Host Microbe 20 (5):606–617. https://doi.org/10.1016/j.chom.2016.10.001

Crespo-Piazuelo D, Estelle J, Revilla M, Criado-Mesas L, Ramayo-Caldas Y, Ovilo C, Fernandez AI, Ballester M, Folch JM (2018) Characterization of bacterial microbiota compositions along the intestinal tract in pigs and their interactions and functions. Sci Rep 8(1):12727. https://doi.org/10.1038/s41598-018-30932-6

Crhanova M, Hradecka H, Faldynova M, Matulova M, Havlickova H, Sisak F, Rychlik I (2011) Immune response of chicken gut to natural colonization by gut microflora and to *Salmonella enterica* serovar enteritidis infection. Infect Immun 79(7):2755–2763. https://doi.org/10.1128/IAI.01375-10

Cugini C, Calfee MW, Farrow JM 3rd, Morales DK, Pesci EC, Hogan DA (2007) Farnesol, a common sesquiterpene, inhibits PQS production in *Pseudomonas aeruginosa*. Mol Microbiol 65(4):896–906. https://doi.org/10.1111/j.1365-2958.2007.05840.x

Curbete MM, Salgado HR (2016) A critical review of the properties of fusidic acid and analytical methods for its determination. Crit Rev Anal Chem 46(4):352–360. https://doi.org/10.1080/10408347.2015.1084225

Czerucka D, Dahan S, Mograbi B, Rossi B, Rampal P (2000) *Saccharomyces boulardii* preserves the barrier function and modulates the signal transduction pathway induced in enteropathogenic *Escherichia coli*-infected T84 cells. Infect Immun 68(10):5998–6004. doi:https://doi.org/10.1128/iai.68.10.5998-6004.2000

D'Amore R, Ijaz UZ, Schirmer M, Kenny JG, Gregory R, Darby AC, Shakya M, Podar M, Quince C, Hall N (2016) A comprehensive benchmarking study of protocols and sequencing platforms for 16S rRNA community profiling. BMC Genomics 17:55. https://doi.org/10.1186/s12864-015-2194-9

Dagar SS, Kumar S, Mudgil P, Singh R, Puniya AK (2011) D1/D2 domain of large-subunit ribosomal DNA for differentiation of Orpinomyces spp. Appl Environ Microbiol 77 (18):6722–6725. https://doi.org/10.1128/AEM.05441-11

Dagar SS, Singh N, Goel N, Kumar S, Puniya AK (2015) Role of anaerobic fungi in wheat straw degradation and effects of plant feed additives on rumen fermentation parameters in vitro. Benef Microbes 6(3):353–360. https://doi.org/10.3920/BM2014.0071

De Luca A, Zelante T, D'Angelo C, Zagarella S, Fallarino F, Spreca A, Iannitti RG, Bonifazi P, Renauld JC, Bistoni F, Puccetti P, Romani L (2010) IL-22 defines a novel immune pathway of antifungal resistance. Mucosal Immunol 3(4):361–373. https://doi.org/10.1038/mi.2010.22

De Luca A, Carvalho A, Cunha C, Iannitti RG, Pitzurra L, Giovannini G, Mencacci A, Bartolommei L, Moretti S, Massi-Benedetti C, Fuchs D, De Bernardis F, Puccetti P, Romani L (2013) IL-22 and IDO1 affect immunity and tolerance to murine and human vaginal candidiasis. PLoS Pathog 9(7):e1003486. https://doi.org/10.1371/journal.ppat.1003486

Desnoyers M, Giger-Reverdin S, Bertin G, Duvaux-Ponter C, Sauvant D (2009) Meta-analysis of the influence of Saccharomyces cerevisiae supplementation on ruminal parameters and milk production of ruminants. J Dairy Sci 92(4):1620–1632. https://doi.org/10.3168/jds.2008-1414

DeVries TJ, Chevaux E (2014) Modification of the feeding behavior of dairy cows through live yeast supplementation. J Dairy Sci 97(10):6499–6510. https://doi.org/10.3168/jds.2014-8226

Dhama K, Chakraborty S, Verma AK, Tiwari R, Brathidasan R, Kumar A, Singh SD (2013) Fungal/mycotic diseases of poultry-diagnosis, treatment and control: a review. Pak J Biol Sci 16:1626–1640

Di Francia A, Masucci F, De Rosa G, Varricchio ML, Proto V (2008) Effects of Aspergillus oryzae extract and a *Saccharomyces cerevisiae* fermentation product on intake, body weight gain and digestibility in buffalo calves. Anim Feed Sci Technol 140:67–77

Donaldson GP, Lee SM, Mazmanian SK (2016) Gut biogeography of the bacterial microbiota. Nat Rev Microbiol 14(1):20–32. https://doi.org/10.1038/nrmicro3552

Dong TS, Gupta A (2019) Influence of early life, diet, and the environment on the microbiome. Clin Gastroenterol Hepatol 17(2):231–242. https://doi.org/10.1016/j.cgh.2018.08.067

dos Santos JD, de Oliveira Fugisaki LR, Medina RP, Scorzoni L, de Sa Alves M, de Barros PP, Ribeiro FC, Fuchs BB, Mylonakis E, Silva DHS, Junqueira JC (2020) Streptococcus mutans secreted products inhibit *Candida albicans* induced oral candidiasis. Front Microbiol 11

Dubos R, Schaedler RW, Costello R, Hoet P (1965) Indigenous, normal, and autochthonous flora of the gastrointestinal tract. J Exp Med 122:67–76

Elad D, Brenner J, Markovics A, Yakobson B, Shlomovitz S, Basan J (1998) Yeasts in the gastrointestinal tract of preweaned calves and possible involvement of *Candida glabrata* in neonatal calf diarrhea. Mycopathologia 141(1):7–14. https://doi.org/10.1023/a:1006860418915

Enaud R, Vandenborght LE, Coron N, Bazin T, Prevel R, Schaeverbeke T, Berger P, Fayon M, Lamireau T, Delhaes L (2018) The mycobiome: a neglected component in the microbiota-gut-brain axis. Microorganisms 6(1). https://doi.org/10.3390/microorganisms6010022

Erb Downward JR, Falkowski NR, Mason KL, Muraglia R, Huffnagle GB (2013) Modulation of post-antibiotic bacterial community reassembly and host response by *Candida albicans*. Sci Rep 3:2191. https://doi.org/10.1038/srep02191

Everard A, Matamoros S, Geurts L, Delzenne NM, Cani PD (2014) *Saccharomyces boulardii* administration changes gut microbiota and reduces hepatic steatosis, low-grade inflammation, and fat mass in obese and type 2 diabetic db/db mice. mBio 5(3):e01011–01014. https://doi.org/10.1128/mBio.01011-14

Fan D, Coughlin LA, Neubauer MM, Kim J, Kim MS, Zhan X, Simms-Waldrip TR, Xie Y, Hooper LV, Koh AY (2015) Activation of HIF-1alpha and LL-37 by commensal bacteria inhibits *Candida albicans* colonization. Nat Med 21(7):808–814. https://doi.org/10.1038/nm.3871

Fliegerova K, Mrazek J, Hoffmann K, Zabranska J, Voigt K (2010) Diversity of anaerobic fungi within cow manure determined by ITS1 analysis. Folia Microbiol (Praha) 55(4):319–325. https://doi.org/10.1007/s12223-010-0049-y

Fouts DE, Szpakowski S, Purushe J, Torralba M, Waterman RC, MacNeil MD, Alexander LJ, Nelson KE (2012) Next generation sequencing to define prokaryotic and fungal diversity in the bovine rumen. PLoS One 7(11):e48289. https://doi.org/10.1371/journal.pone.0048289

Frac M, Hannula SE, Belka M, Jedryczka M (2018) Fungal biodiversity and their role in soil health. Front Microbiol 9:707. https://doi.org/10.3389/fmicb.2018.00707

Frese SA, Parker K, Calvert CC, Mills DA (2015) Diet shapes the gut microbiome of pigs during nursing and weaning. Microbiome 3:28. https://doi.org/10.1186/s40168-015-0091-8

Frey-Klett P, Burlinson P, Deveau A, Barret M, Tarkka M, Sarniguet A (2011) Bacterial-fungal interactions: hyphens between agricultural, clinical, environmental, and food microbiologists. Microbiol Mol Biol Rev 75(4):583–609. https://doi.org/10.1128/MMBR.00020-11

Froslev TG, Kjoller R, Bruun HH, Ejrnaes R, Brunbjerg AK, Pietroni C, Hansen AJ (2017) Algorithm for post-clustering curation of DNA amplicon data yields reliable biodiversity estimates. Nat Commun 8(1):1188. https://doi.org/10.1038/s41467-017-01312-x

Galvao KN, Santos JE, Coscioni A, Villasenor M, Sischo WM, Berge AC (2005) Effect of feeding live yeast products to calves with failure of passive transfer on performance and patterns of antibiotic resistance in fecal *Escherichia coli*. Reprod Nutr Dev 45(4):427–440. https://doi.org/10.1051/rnd:2005040

Gantner BN, Simmons RM, Underhill DM (2005) Dectin-1 mediates macrophage recognition of *Candida albicans* yeast but not filaments. EMBO J 24(6):1277–1286. https://doi.org/10.1038/sj.emboj.7600594

Garsin DA, Lorenz MC (2013) Candida albicans and *Enterococcus faecalis* in the gut: synergy in commensalism? Gut Microbes 4(5):409–415. https://doi.org/10.4161/gmic.26040

Gessner MA, Werner JL, Lilly LM, Nelson MP, Metz AE, Dunaway CW, Chan YR, Ouyang W, Brown GD, Weaver CT, Steele C (2012) Dectin-1-dependent interleukin-22 contributes to early innate lung defense against *Aspergillus fumigatus*. Infect Immun 80(1):410–417. https://doi.org/10.1128/IAI.05939-11

Ghannoum MA, Jurevic RJ, Mukherjee PK, Cui F, Sikaroodi M, Naqvi A, Gillevet PM (2010) Characterization of the oral fungal microbiome (mycobiome) in healthy individuals. PLoS Pathog 6(1):e1000713. https://doi.org/10.1371/journal.ppat.1000713

Girard ID, Dawson KA (1995) Effect of a yeast culture on growth characteristics of representative ruminal bacteria. J Anim Sci 73(264)

Glockner FO, Yilmaz P, Quast C, Gerken J, Beccati A, Ciuprina A, Bruns G, Yarza P, Peplies J, Westram R, Ludwig W (2017) 25 years of serving the community with ribosomal RNA gene reference databases and tools. J Biotechnol 261:169–176. https://doi.org/10.1016/j.jbiotec.2017.06.1198

Gohl DM, Vangay P, Garbe J, MacLean A, Hauge A, Becker A, Gould TJ, Clayton JB, Johnson TJ, Hunter R, Knights D, Beckman KB (2016) Systematic improvement of amplicon marker gene methods for increased accuracy in microbiome studies. Nat Biotechnol 34(9):942–949. https://doi.org/10.1038/nbt.3601

Gordon GL, Phillips MW (1998) The role of anaerobic gut fungi in ruminants. Nutr Res Rev 11(1):133–168. https://doi.org/10.1079/NRR19980009

Gresse R, Chaucheyras-Durand F, Fleury MA, Van de Wiele T, Forano E, Blanquet-Diot S (2017) Gut microbiota dysbiosis in postweaning piglets: understanding the keys to health. Trends Microbiol 25(10):851–873. https://doi.org/10.1016/j.tim.2017.05.004

Griffith GW, Ozkose E, Theodorou MK, Davies DR (2009) Diversity of anaerobic fungal populations in cattle revealed by selective enrichment culture using different carbon sources. Fungal Ecol 2(2):87–97

Gropp K, Schild L, Schindler S, Hube B, Zipfel PF, Skerka C (2009) The yeast *Candida albicans* evades human complement attack by secretion of aspartic proteases. Mol Immunol 47(2–3):465–475. https://doi.org/10.1016/j.molimm.2009.08.019

Gruninger RJ, Puniya AK, Callaghan TM, Edwards JE, Youssef N, Dagar SS, Fliegerova K, Griffith GW, Forster R, Tsang A, McAllister T, Elshahed MS (2014) Anaerobic fungi (phylum Neocallimastigomycota): advances in understanding their taxonomy, life cycle, ecology, role

and biotechnological potential. FEMS Microbiol Ecol 90(1):1–17. https://doi.org/10.1111/1574-6941.12383

Guedes C, Goncalves D, Rodrigues M, Dias-da-Silva A (2008) Effects of a *Saccharomyces cerevisiae* yeast on ruminal fermentation and fibre degradation of maize silages in cows. Anim Feed Sci Technol 145:27–40

Guevarra RB, Hong SH, Cho JH, Kim BR, Shin J, Lee JH, Kang BN, Kim YH, Wattanaphansak S, Isaacson RE, Song M, Kim HB (2018) The dynamics of the piglet gut microbiome during the weaning transition in association with health and nutrition. J Anim Sci Biotechnol 9:54. https://doi.org/10.1186/s40104-018-0269-6

Guevarra RB, Lee JH, Lee SH, Seok MJ, Kim DW, Kang BN, Johnson TJ, Isaacson RE, Kim HB (2019) Piglet gut microbial shifts early in life: causes and effects. J Anim Sci Biotechnol 10:1. https://doi.org/10.1186/s40104-018-0308-3

Hallen-Adams HE, Suhr MJ (2017) Fungi in the healthy human gastrointestinal tract. Virulence 8 (3):352–358. https://doi.org/10.1080/21505594.2016.1247140

Hallen-Adams HE, Kachman SD, Kim J, Legge RM, Marinez I (2015) Fungi inhabiting the healthy human gastrointestinal tract: a diverse and dynamic community. Fungal Ecol 15:9–17

Halwachs B, Madhusudhan N, Krause R, Nilsson RH, Moissl-Eichinger C, Hogenauer C, Thallinger GG, Gorkiewicz G (2017) Critical issues in mycobiota analysis. Front Microbiol 8: 180. https://doi.org/10.3389/fmicb.2017.00180

Han GG, Lee JY, Jin GD, Park J, Choi YH, Chae BJ, Kim EB, Choi YJ (2017) Evaluating the association between body weight and the intestinal microbiota of weaned piglets via 16S rRNA sequencing. Appl Microbiol Biotechnol 101(14):5903–5911. https://doi.org/10.1007/s00253-017-8304-7

Han GG, Lee JY, Jin GD, Park J, Choi YH, Kang SK, Chae BJ, Kim EB, Choi YJ (2018) Tracing of the fecal microbiota of commercial pigs at five growth stages from birth to shipment. Sci Rep 8 (1):6012. https://doi.org/10.1038/s41598-018-24508-7

Heisel T, Montassier E, Johnson A, Al-Ghalith G, Lin YW, Wei LN, Knights D, Gale CA (2017) High-fat diet changes fungal microbiomes and interkingdom relationships in the murine gut. mSphere 2(5). https://doi.org/10.1128/mSphere.00351-17

Helstrom PB, Balish E (1979) Effect of oral tetracycline, the microbial flora, and the athymic state on gastrointestinal colonization and infection of BALB/c mice with Candida albicans. Infect Immun 23(3):764–774

Henderson G, Cox F, Kittelmann S, Miri VH, Zethof M, Noel SJ, Waghorn GC, Janssen PH (2013) Effect of DNA extraction methods and sampling techniques on the apparent structure of cow and sheep rumen microbial communities. PLoS One 8(9):e74787. https://doi.org/10.1371/journal.pone.0074787

Hernandez-Chavez MJ, Perez-Garcia LA, Nino-Vega GA, Mora-Montes HM (2017) Fungal strategies to evade the host immune recognition. J Fungi (Basel) 3(4). https://doi.org/10.3390/jof3040051

Hillman ET, Lu H, Yao T, Nakatsu CH (2017) Microbial ecology along the gastrointestinal tract. Microbes Environ 32(4):300–313. https://doi.org/10.1264/jsme2.ME17017

Hoffmann C, Dollive S, Grunberg S, Chen J, Li H, Wu GD, Lewis JD, Bushman FD (2013) Archaea and fungi of the human gut microbiome: correlations with diet and bacterial residents. PLoS One 8(6):e66019. https://doi.org/10.1371/journal.pone.0066019

Hogan DA, Vik A, Kolter R (2004) A *Pseudomonas aeruginosa* quorum-sensing molecule influences *Candida albicans* morphology. Mol Microbiol 54(5):1212–1223. https://doi.org/10.1111/j.1365-2958.2004.04349.x

Holman DB, Brunelle BW, Trachsel J, Allen HK (2017) Meta-analysis to define a core microbiota in the swine gut. mSystems 2(3). https://doi.org/10.1128/mSystems.00004-17

Hubka V, Kolarik M, Kubatova A, Peterson SW (2013) Taxonomic revision of Eurotium and transfer of species to Aspergillus. Mycologia 105(4):912–937. https://doi.org/10.3852/12-151

Huffnagle GB, Noverr MC (2013) The emerging world of the fungal microbiome. Trends Microbiol 21(7):334–341. https://doi.org/10.1016/j.tim.2013.04.002

Hume ME, Hernandez CA, Barbosa NA, Sakomura NK, Dowd SE, Oviedo-Rondon EO (2012) Molecular identification and characterization of ileal and cecal fungus communities in broilers given probiotics, specific essential oil blends, and under mixed Eimeria infection. Foodborne Pathog Dis 9(9):853–860. https://doi.org/10.1089/fpd.2011.1093

Huseyin CE, O'Toole PW, Cotter PD, Scanlan PD (2017a) Forgotten fungi-the gut mycobiome in human health and disease. FEMS Microbiol Rev 41(4):479–511. https://doi.org/10.1093/femsre/fuw047

Huseyin CE, Rubio RC, O'Sullivan O, Cotter PD, Scanlan PD (2017b) The fungal frontier: a comparative analysis of methods used in the study of the human gut mycobiome. Front Microbiol 8:1432. https://doi.org/10.3389/fmicb.2017.01432

Iliev ID, Leonardi I (2017) Fungal dysbiosis: immunity and interactions at mucosal barriers. Nat Rev Immunol 17(10):635–646. https://doi.org/10.1038/nri.2017.55

Iliev ID, Funari VA, Taylor KD, Nguyen Q, Reyes CN, Strom SP, Brown J, Becker CA, Fleshner PR, Dubinsky M, Rotter JI, Wang HL, McGovern DP, Brown GD, Underhill DM (2012) Interactions between commensal fungi and the C-type lectin receptor Dectin-1 influence colitis. Science 336(6086):1314–1317. https://doi.org/10.1126/science.1221789

Irinyi L, Serena C, Garcia-Hermoso D, Arabatzis M, Desnos-Ollivier M, Vu D, Cardinali G, Arthur I, Normand AC, Giraldo A, da Cunha KC, Sandoval-Denis M, Hendrickx M, Nishikaku AS, de Azevedo Melo AS, Merseguel KB, Khan A, Parente Rocha JA, Sampaio P, da Silva Briones MR, Ferreira RC, de Medeiros Muniz M, Castanon-Olivares LR, Estrada-Barcenas D, Cassagne C, Mary C, Duan SY, Kong F, Sun AY, Zeng X, Zhao Z, Gantois N, Botterel F, Robbertse B, Schoch C, Gams W, Ellis D, Halliday C, Chen S, Sorrell TC, Piarroux R, Colombo AL, Pais C, de Hoog S, Zancope-Oliveira RM, Taylor ML, Toriello C, de Almeida Soares CM, Delhaes L, Stubbe D, Dromer F, Ranque S, Guarro J, Cano-Lira JF, Robert V, Velegraki A, Meyer W (2015) International Society of Human and Animal Mycology (ISHAM)-ITS reference DNA barcoding database--the quality controlled standard tool for routine identification of human and animal pathogenic fungi. Med Mycol 53(4):313–337. https://doi.org/10.1093/mmy/myv008

Isaacson R, Kim HB (2012) The intestinal microbiome of the pig. Anim Health Res Rev 13 (1):100–109. https://doi.org/10.1017/S1466252312000084

Iwen PC, Schutte SD, Florescu DF, Noel-Hurst RK, Sigler L (2012) Invasive *Scopulariopsis brevicaulis* infection in an immunocompromised patient and review of prior cases caused by Scopulariopsis and Microascus species. Med Mycol 50(6):561–569. https://doi.org/10.3109/13693786.2012.675629

Jenkinson HF, Lala HC, Shepherd MG (1990) Coaggregation of *Streptococcus sanguis* and other streptococci with *Candida albicans*. Infect Immun 58(5):1429–1436

Jensen HE, Olsen SN, Aalbaek B (1994) Gastrointestinal aspergillosis and zygomycosis of cattle. Vet Pathol 31(1):28–36. https://doi.org/10.1177/030098589403100104

Joblin KN, Naylor GE (2010) Ruminal fungi for increasing forage intake and animal productivity. Sustainable Improvement of Animal Production and Health

Jouany JP (2006) Optimizing rumen functions in the close-up transition period and early lactation to drive dry matter intake and energy balance in cows. Anim Reprod Sci 96(3-4):250–264. https://doi.org/10.1016/j.anireprosci.2006.08.005

Kalmus P, Orro T, Waldmann A, Lindjarv R, Kask K (2009) Effect of yeast culture on milk production and metabolic and reproductive performance of early lactation dairy cows. Acta Vet Scand 51:32. https://doi.org/10.1186/1751-0147-51-32

Kamei K, Watanabe A (2005) Aspergillus mycotoxins and their effect on the host. Med Mycol 43 (Suppl 1):S95–S99. https://doi.org/10.1080/13693780500051547

Kang DW, Park JG, Ilhan ZE, Wallstrom G, Labaer J, Adams JB, Krajmalnik-Brown R (2013) Reduced incidence of Prevotella and other fermenters in intestinal microflora of autistic children. PLoS One 8(7):e68322. https://doi.org/10.1371/journal.pone.0068322

Kanora A, Maes D (2009) The role of mycotoxins in pig reproduction: a review. Vet Med 54:565–576

Kasfi K, Jafarpour B, Tarighi S (2018) Characterization of antagnostic microorganisms against Aspergillus spp. from grapevine leaf and berry surfaces. J Plant Pathol 100:179–190

Kaul S, Sumbali G (2000) Keratinophilic fungi from feathers of Indian poultry birds. Mycologist 14 (4):148–150

Kelly J, Daly K, Moran AW, Ryan S, Bravo D, Shirazi-Beechey SP (2017) Composition and diversity of mucosa-associated microbiota along the entire length of the pig gastrointestinal tract; dietary influences. Environ Microbiol 19(4):1425–1438. https://doi.org/10.1111/1462-2920.13619

Kers JG, Velkers FC, Fischer EAJ, Hermes GDA, Stegeman JA, Smidt H (2018) Host and environmental factors affecting the intestinal microbiota in chickens. Front Microbiol 9:235. https://doi.org/10.3389/fmicb.2018.00235

Khafipour E, Li S, Tun HM, Derakhshani H, Moossavi S, Plaizier JC (2016) Effects of grain feeding on microbiota in the digestive tract of cattle. Anim Front 6(2):13–19

Kim HB, Borewicz K, White BA, Singer RS, Sreevatsan S, Tu ZJ, Isaacson RE (2011) Longitudinal investigation of the age-related bacterial diversity in the feces of commercial pigs. Vet Microbiol 153(1–2):124–133

Kim D, Sengupta A, Niepa TH, Lee BH, Weljie A, Freitas-Blanco VS, Murata RM, Stebe KJ, Lee D, Koo H (2017) Candida albicans stimulates Streptococcus mutans microcolony development via cross-kingdom biofilm-derived metabolites. Sci Rep 7:41332. https://doi.org/10.1038/srep41332

Kiss EA, Vonarbourg C, Kopfmann S, Hobeika E, Finke D, Esser C, Diefenbach A (2011) Natural aryl hydrocarbon receptor ligands control organogenesis of intestinal lymphoid follicles. Science 334(6062):1561–1565. https://doi.org/10.1126/science.1214914

Kittelmann S, Naylor GE, Koolaard JP, Janssen PH (2012) A proposed taxonomy of anaerobic fungi (class neocallimastigomycetes) suitable for large-scale sequence-based community structure analysis. PLoS One 7(5):e36866. https://doi.org/10.1371/journal.pone.0036866

Kittelmann S, Seedorf H, Walter WA, Clemente JC, Knight R, Gordon JI, Janssen PH (2013) Simultaneous amplicon sequencing to explore co-occurrence patterns of bacterial, archaeal and eukaryotic microorganisms in rumen microbial communities. PLoS One 8

Krause DO, Denman SE, Mackie RI, Morrison M, Rae AL, Attwood GT, McSweeney CS (2003) Opportunities to improve fiber degradation in the rumen: microbiology, ecology, and genomics. FEMS Microbiol Rev 27(5):663–693

Krnjaja V, Stojanovic L, Trenkovski S, Bijelic Z (2009) The frequency of pathogenic fungi genera in animal feed. Lucrari Stiintifice, Seria Zootehnie 53:341–344

Kumar S, Dagar SS, Sirohi SK, Upadhyay RC, Puniya AK (2013) Microbial profiles, in vitro gas production and dry matter digestibility based on various ratios of roughage to concentrate. Ann Microbiol 63:541–545

Kumar A, Henrissat B, Arvas M, Syed MF, Thieme N, Benz JP, Sorensen JL, Record E, Poggeler S, Kempken F (2015a) De novo assembly and genome analyses of the marine-derived *Scopulariopsis brevicaulis* strain LF580 unravels life-style traits and anticancerous scopularide biosynthetic gene cluster. PLoS One 10(10):e0140398. https://doi.org/10.1371/journal.pone.0140398

Kumar S, Indugu N, Vecchiarelli B, Pitta DW (2015b) Associative patterns among anaerobic fungi, methanogenic archaea, and bacterial communities in response to changes in diet and age in the rumen of dairy cows. Front Microbiol 6:781. https://doi.org/10.3389/fmicb.2015.00781

Kurtzman C, Fell JW, Boekhout T (2011) The yeasts: a taxonomic study, 5th edn

Lamas B, Richard ML, Leducq V, Pham HP, Michel ML, Da Costa G, Bridonneau C, Jegou S, Hoffmann TW, Natividad JM, Brot L, Taleb S, Couturier-Maillard A, Nion-Larmurier I, Merabtene F, Seksik P, Bourrier A, Cosnes J, Ryffel B, Beaugerie L, Launay JM, Langella P, Xavier RJ, Sokol H (2016) CARD9 impacts colitis by altering gut microbiota metabolism of tryptophan into aryl hydrocarbon receptor ligands. Nat Med 22(6):598–605. https://doi.org/10.1038/nm.4102

Li S, Yoon I, Scott M, Khafipour E, Plaizier JC (2016) Impact of Saccharomyces cerevisiae fermentation product and subacute ruminal acidosis on production, inflammation, and fermentation in the rumen and hindgut of diary cows. Anim Feed Sci Technol 211:50–60

Li X, Leonardi I, Semon A, Doron I, Gao IH, Putzel GG, Kim Y, Kabata H, Artis D, Fiers WD, Ramer-Tait AE, Iliev ID (2018) Response to fungal dysbiosis by gut-resident CX3CR1(+) mononuclear phagocytes aggravates allergic airway disease. Cell Host Microbe 24(6):847–856. e844. https://doi.org/10.1016/j.chom.2018.11.003

Li XV, Leonardi I, Iliev ID (2019) Gut mycobiota in immunity and inflammatory disease. Immunity 50(6):1365–1379. https://doi.org/10.1016/j.immuni.2019.05.023

Liggenstoffer AS, Youssef NH, Couger MB, Elshahed MS (2010) Phylogenetic diversity and community structure of anaerobic gut fungi (phylum Neocallimastigomycota) in ruminant and non-ruminant herbivores. ISME J 4(10):1225–1235. https://doi.org/10.1038/ismej.2010.49

Lima FS, Oikonomou G, Lima SF, Bicalho ML, Ganda EK, Filho JC, Lorenzo G, Trojacanec P, Bicalhoa RC (2015) Prepartum and postpartum rumen fluid microbiomes: characterization and correlation with production traits in dairy cows. Appl Environ Microbiol 81(4):1327–1337. https://doi.org/10.1128/AEM.03138-14

Limon JJ, Skalski JH, Underhill DM (2017) Commensal fungi in health and disease. Cell Host Microbe 22(2):156–165. https://doi.org/10.1016/j.chom.2017.07.002

Liu M, Spellberg B, Phan QT, Fu Y, Fu Y, Lee AS, Edwards JE Jr, Filler SG, Ibrahim AS (2010) The endothelial cell receptor GRP78 is required for mucormycosis pathogenesis in diabetic mice. J Clin Invest 120(6):1914–1924. https://doi.org/10.1172/JCI42164

Liu Y, Zheng Z, Yu L, Wu S, Sun L, Wu S, Xu Q, Cai S, Qin N, Bao W (2019) Examination of the temporal and spatial dynamics of the gut microbiome in newborn piglets reveals distinct microbial communities in six intestinal segments. Sci Rep 9(1):3453. https://doi.org/10.1038/s41598-019-40235-z

Looft T, Johnson TA, Alleng HK, Bayles DO, Alt DP, Stedtfeld RD, Chai B, Cole JR, Hashsham SA, Tiedje JM, Stanton TB (2012) In-feed antibiotic effects on the swine intestinal microbiome. PNAS 109:1691–1696

Looft T, Allen HK, Cantarel BL, Levine UY, Bayles DO, Alt DP, Henrissat B, Stanton TB (2014) Bacteria, phages and pigs: the effects of in-feed antibiotics on the microbiome at different gut locations. ISME J 8(8):1566–1576. https://doi.org/10.1038/ismej.2014.12

Lowenthal JW, Connick TE, McWaters PG, York JJ (1994) Development of T cell immune responsiveness in the chicken. Immunol Cell Biol 72(2):115–122. https://doi.org/10.1038/icb.1994.18

Mach N, Berri M, Estelle J, Levenez F, Lemonnier G, Denis C, Leplat JJ, Chevaleyre C, Billon Y, Dore J, Rogel-Gaillard C, Lepage P (2015) Early-life establishment of the swine gut microbiome and impact on host phenotypes. Environ Microbiol Rep 7(3):554–569. https://doi.org/10.1111/1758-2229.12285

Mackie RI, Sghir A, Gaskins HR (1999) Developmental microbial ecology of the neonatal gastrointestinal tract. Am J Clin Nutr 69(5):1035S–1045S. https://doi.org/10.1093/ajcn/69.5.1035s

Malekkhahi M, Tahmasbi A, Naserian A, Danesh-Nesgaran M, Kleen J, AlZahal O, Ghaffari M (2016) Effects of supplementation of active dried yeast and malate during sub-acute ruminal acidosis on rumen fermentation, microbial pouplation, selected blood metabolites, and milk production in dairy cows. Anim Feed Sci Technol 213:29–43

Manzoni M, Rollini M (2002) Biosynthesis and biotechnological production of statins by filamentous fungi and application of these cholesterol-lowering drugs. Appl Microbiol Biotechnol 58 (5):555–564. https://doi.org/10.1007/s00253-002-0932-9

Mar Rodriguez M, Perez D, Javier Chaves F, Esteve E, Marin-Garcia P, Xifra G, Vendrell J, Jove M, Pamplona R, Ricart W, Portero-Otin M, Chacon MR, Fernandez Real JM (2015) Obesity changes the human gut mycobiome. Sci Rep 5:14600. https://doi.org/10.1038/srep14600

Martin AS, Nisbet DJ (1992) Effect of direct-feed microbial on rumen microbial fermentation. J Diary Sci 75:1736–1744

Martin KJ, Rygiewicz PT (2005) Fungal-specific PCR primers developed for analysis of the ITS region of environmental DNA extracts. BMC Microbiol 5:28. https://doi.org/10.1186/1471-2180-5-28

Martin WT, Zhang Y, Willson P, Archer TP, Kinahan C, Barber EM (1996) Bacterial and fungal flora of dust deposits in a pig building. Occup Environ Med 53(7):484–487. https://doi.org/10.1136/oem.53.7.484

Mason KL, Erb Downward JR, Falkowski NR, Young VB, Kao JY, Huffnagle GB (2012a) Interplay between the gastric bacterial microbiota and *Candida albicans* during postantibiotic recolonization and gastritis. Infect Immun 80(1):150–158. https://doi.org/10.1128/IAI.05162-11

Mason KL, Erb Downward JR, Mason KD, Falkowski NR, Eaton KA, Kao JY, Young VB, Huffnagle GB (2012b) Candida albicans and bacterial microbiota interactions in the cecum during recolonization following broad-spectrum antibiotic therapy. Infect Immun 80 (10):3371–3380. https://doi.org/10.1128/IAI.00449-12

Mbata T (2008) Dermatophytes and other skin mycoses found in featherless broiler toe webs. Internet J Dermatol 7(2):1–4

McAleer JP, Nguyen NL, Chen K, Kumar P, Ricks DM, Binnie M, Armentrout RA, Pociask DA, Hein A, Yu A, Vikram A, Bibby K, Umesaki Y, Rivera A, Sheppard D, Ouyang W, Hooper LV, Kolls JK (2016) Pulmonary Th17 antifungal immunity is regulated by the gut microbiome. J Immunol 197(1):97–107. https://doi.org/10.4049/jimmunol.1502566

McGranaghan P, Davies JC, Griffith GW, Davies DR, Theodorou MK (1999) The survival of anaerobic fungi in cattle faeces. FEMS Microbiol Ecol 29:293–300

McMurdie PJ, Holmes S (2014) Waste not, want not: why rarefying microbiome data is inadmissible. PLoS Comput Biol 10(4):e1003531. https://doi.org/10.1371/journal.pcbi.1003531

Meri T, Blom AM, Hartmann A, Lenk D, Meri S, Zipfel PF (2004) The hyphal and yeast forms of *Candida albicans* bind the complement regulator C4b-binding protein. Infect Immun 72 (11):6633–6641. https://doi.org/10.1128/IAI.72.11.6633-6641.2004

Methner U, Barrow PA, Martin G, Meyer H (1997) Comparative study of the protective effect against Salmonella colonisation in newly hatched SPF chickens using live, attenuated Salmonella vaccine strains, wild-type Salmonella strains or a competitive exclusion product. Int J Food Microbiol 35(3):223–230. https://doi.org/10.1016/s0168-1605(96)01236-6

Miljkovic B, Pavlovski Z, Jovicic D, Radanovic O, Kureljusic B (2011) Fungi on feathers of common clinically healthy birds in belgrade. Biotechnol Anim Husb 27(1):45–54

Mngadi PT, Govinden R, Odhav B (2008) Co-occurring mycotoxins in animal feeds. Afr J Biotechnol 7:2239–2243

Moyes DL, Naglik JR (2011) Mucosal immunity and *Candida albicans* infection. Clin Dev Immunol 2011:346307. https://doi.org/10.1155/2011/346307

Munkvold GP (2003) Cultural and genetic approaches to managing mycotoxins in maize. Annu Rev Phytopathol 41:99–116. https://doi.org/10.1146/annurev.phyto.41.052002.095510

Naglik JR, Fidel PL Jr, Odds FC (2008) Animal models of mucosal Candida infection. FEMS Microbiol Lett 283(2):129–139. https://doi.org/10.1111/j.1574-6968.2008.01160.x

Nash AK, Auchtung TA, Wong MC, Smith DP, Gesell JR, Ross MC, Stewart CJ, Metcalf GA, Muzny DM, Gibbs RA, Ajami NJ, Petrosino JF (2017) The gut mycobiome of the human microbiome project healthy cohort. Microbiome 5(1):153. https://doi.org/10.1186/s40168-017-0373-4

Netea MG, Warris A, Van der Meer JW, Fenton MJ, Verver-Janssen TJ, Jacobs LE, Andresen T, Verweij PE, Kullberg BJ (2003) Aspergillus fumigatus evades immune recognition during germination through loss of toll-like receptor-4-mediated signal transduction. J Infect Dis 188 (2):320–326. https://doi.org/10.1086/376456

Netea MG, Joosten LA, van der Meer JW, Kullberg BJ, van de Veerdonk FL (2015) Immune defence against Candida fungal infections. Nat Rev Immunol 15(10):630–642. https://doi.org/10.1038/nri3897

Newbold CJ, Olvera-Ramirez A (2006) The use of yeast-based probiotics to meet new challenges in ruminant production. J Anim Sci 84

Newbold CJ, McIntosh FM, Wallace RJ (1998) Changes in the microbial population of a rumen-simulating fermenter in response to yeast culture. Can J of Anim Sci 78:241–244

Neyrinck AM, Possemiers S, Verstraete W, De Backer F, Cani PD, Delzenne NM (2012) Dietary modulation of clostridial cluster XIVa gut bacteria (Roseburia spp.) by chitin-glucan fiber improves host metabolic alterations induced by high-fat diet in mice. J Nutr Biochem 23 (1):51–59. https://doi.org/10.1016/j.jnutbio.2010.10.008

Nguyen LN, Lopes LC, Cordero RJ, Nosanchuk JD (2011) Sodium butyrate inhibits pathogenic yeast growth and enhances the functions of macrophages. J Antimicrob Chemother 66 (11):2573–2580. https://doi.org/10.1093/jac/dkr358

Niederwerder MC (2018) Fecal microbiota transplantation as a tool to treat and reduce susceptibility to disease in animals. Vet Immunol Immunopathol 206:65–72. https://doi.org/10.1016/j.vetimm.2018.11.002

Niederwerder MC, Constance LA, Rowland RRR, Abbas W, Fernando SC, Potter ML, Sheahan MA, Burkey TE, Hesse RA, Cino-Ozuna AG (2018) Fecal microbiota transplantation is associated with reduced morbidity and mortality in porcine circovirus associated disease. Front Microbiol 9:1631. https://doi.org/10.3389/fmicb.2018.01631

Nilsson RH, Anslan S, Bahram M, Wurzbacher C, Baldrian P, Tedersoo L (2019) Mycobiome diversity: high-throughput sequencing and identification of fungi. Nat Rev Microbiol 17 (2):95–109. https://doi.org/10.1038/s41579-018-0116-y

Nisbet DJ, Martin SA (1991) Effect of a *Saccharomyces cerevisiae* culture on lactate utilization by the ruminal bacterium *Selenomonas ruminantium*. J Anim Sci 69(11):4628–4633. https://doi.org/10.2527/1991.69114628x

Noverr MC, Huffnagle GB (2004) Regulation of *Candida albicans* morphogenesis by fatty acid metabolites. Infect Immun 72(11):6206–6210. https://doi.org/10.1128/IAI.72.11.6206-6210.2004

Noverr MC, Phare SM, Toews GB, Coffey MJ, Huffnagle GB (2001) Pathogenic yeasts *Cryptococcus neoformans* and *Candida albicans* produce immunomodulatory prostaglandins. Infect Immun 69(5):2957–2963. https://doi.org/10.1128/IAI.69.5.2957-2963.2001

Noverr MC, Falkowski NR, McDonald RA, McKenzie AN, Huffnagle GB (2005) Development of allergic airway disease in mice following antibiotic therapy and fungal microbiota increase: role of host genetics, antigen, and interleukin-13. Infect Immun 73(1):30–38. https://doi.org/10.1128/IAI.73.1.30-38.2005

Nowland TL, Plush KJ, Barton M, Kirkwood RN (2019) Development and function of the intestinal microbiome and potential implications for pig production. Animals (Basel) 9(3). https://doi.org/10.3390/ani9030076

Ober RA, Thissen JB, Jaing CJ, Cino-Ozuna AG, Rowland RRR, Niederwerder MC (2017) Increased microbiome diversity at the time of infection is associated with improved growth rates of pigs after co-infection with porcine reproductive and respiratory syndrome virus (PRRSV) and porcine circovirus type 2 (PCV2). Vet Microbiol 208:203–211. https://doi.org/10.1016/j.vetmic.2017.06.023

O'Brien M, O'Kiely P, Forristal PD, Fuller HT (2007) Visible fungal growth on baled grass silage during the winter feeding season in Ireland and silage characteristics associated with the occurrence of fungi. Anim Feed Sci Technol 139(3–4):234–256

Odds FC (1994a) Candida albicans, the life and times of a pathogenic yeast. J Med Vet Mycol 32 (Suppl 1):1–8. https://doi.org/10.1080/02681219480000681

Odds FC (1994b) Pathogenesis of Candida infections. J Am Acad Dermatol 31(3 Pt 2):S2–S5. https://doi.org/10.1016/s0190-9622(08)81257-1

Oever JT, Netea MG (2014) The bacteriome-mycobiome interaction and antifungal host defense. Eur J Immunol 44(11):3182–3191. https://doi.org/10.1002/eji.201344405

Oh J, Byrd AL, Deming C, Conlan S, Program NCS, Kong HH, Segre JA (2014) Biogeography and individuality shape function in the human skin metagenome. Nature 514(7520):59–64. https://doi.org/10.1038/nature13786

Oikonomou G, Teixeira AG, Foditsch C, Bicalho ML, Machado VS, Bicalho RC (2013) Fecal microbial diversity in pre-weaned dairy calves as described by pyrosequencing of metagenomic 16S rDNA. Associations of Faecalibacterium species with health and growth. PLoS One 8(4): e63157. https://doi.org/10.1371/journal.pone.0063157

Otte JM, Zdebik AE, Brand S, Chromik AM, Strauss S, Schmitz F, Steinstraesser L, Schmidt WE (2009) Effects of the cathelicidin LL-37 on intestinal epithelial barrier integrity. Regul Pept 156 (1–3):104–117. https://doi.org/10.1016/j.regpep.2009.03.009

Pajarillo EA, Chae JP, Balolong MP, Kim HB, Seo KS, Kang DK (2014) Pyrosequencing-based analysis of fecal microbial communities in three purebred pig lines. J Microbiol 52(8):646–651. https://doi.org/10.1007/s12275-014-4270-2

Peleg AY, Hogan DA, Mylonakis E (2010) Medically important bacterial-fungal interactions. Nat Rev Microbiol 8(5):340–349. https://doi.org/10.1038/nrmicro2313

Pena GA, Pereyra CM, Armando MR, Chiacchiera SM, Magnoli CE, Orlando JL, Dalcero AM, Rosa CA, Cavaglieri LR (2010) Aspergillus fumigatus toxicity and gliotoxin levels in feedstuff for domestic animals and pets in Argentina. Lett Appl Microbiol 50(1):77–81. https://doi.org/10.1111/j.1472-765X.2009.02756.x

Phillips MW, Gordon GL (1988) Sugar and polysaccharide fermentation by rumen anaerobic fungi from Australia, Britain and New Zealand. Biosystems 21(3–4):377–383. https://doi.org/10.1016/0303-2647(88)90036-6

Piancastelli C, Ghidini F, Donofrio G, Jottini S, Taddei S, Cavirani S, Cabassi CS (2009) Isolation and characterization of a strain of Lichtheimia corymbifera (ex Absidia corymbifera) from a case of bovine abortion. Reprod Biol Endocrinol 7:138. https://doi.org/10.1186/1477-7827-7-138

Pitta DW, Kumar S, Vecchiarelli B, Shirley DJ, Bittinger K, Baker LD, Ferguson JD, Thomsen N (2014) Temporal dynamics in the ruminal microbiome of dairy cows during the transition period. J Anim Sci 92(9):4014–4022. https://doi.org/10.2527/jas.2014-7621

Plantinga TS, van der Velden WJ, Ferwerda B, van Spriel AB, Adema G, Feuth T, Donnelly JP, Brown GD, Kullberg BJ, Blijlevens NM, Netea MG (2009) Early stop polymorphism in human DECTIN-1 is associated with increased candida colonization in hematopoietic stem cell transplant recipients. Clin Infect Dis 49(5):724–732. https://doi.org/10.1086/604714

Plato A, Hardison SE, Brown GD (2015) Pattern recognition receptors in antifungal immunity. Semin Immunopathol 37(2):97–106. https://doi.org/10.1007/s00281-014-0462-4

Puel A, Doffinger R, Natividad A, Chrabieh M, Barcenas-Morales G, Picard C, Cobat A, Ouachee-Chardin M, Toulon A, Bustamante J, Al-Muhsen S, Al-Owain M, Arkwright PD, Costigan C, McConnell V, Cant AJ, Abinun M, Polak M, Bougneres PF, Kumararatne D, Marodi L, Nahum A, Roifman C, Blanche S, Fischer A, Bodemer C, Abel L, Lilic D, Casanova JL (2010) Autoantibodies against IL-17A, IL-17F, and IL-22 in patients with chronic mucocutaneous candidiasis and autoimmune polyendocrine syndrome type I. J Exp Med 207(2):291–297. https://doi.org/10.1084/jem.20091983

Qamar A, Aboudola S, Warny M, Michetti P, Pothoulakis C, LaMont JT, Kelly CP (2001) *Saccharomyces boulardii* stimulates intestinal immunoglobulin A immune response to *Clostridium difficile* toxin A in mice. Infect Immun 69(4):2762–2765. https://doi.org/10.1128/IAI.69.4.2762-2765.2001

Qiu X, Zhang F, Yang X, Wu N, Jiang W, Li X, Li X, Liu Y (2015) Changes in the composition of intestinal fungi and their role in mice with dextran sulfate sodium-induced colitis. Sci Rep 5: 10416. https://doi.org/10.1038/srep10416

Quan J, Cai G, Ye J, Yang M, Ding R, Wang X, Zheng E, Fu D, Li S, Zhou S, Liu D, Yang J, Wu Z (2018) A global comparison of the microbiome compositions of three gut locations in

commercial pigs with extreme feed conversion ratios. Sci Rep 8(1):4536. https://doi.org/10.1038/s41598-018-22692-0

Radostitis O, Gay C, Hinchcliff K, Constable P (2006) Veterinary medicine, 10th edn

Ramayo-Caldas Y, Prenafeta F, Zingaretti LM, Gonzales O, Dalmau A, Quintanilla R, Ballester M (2020) Gut eukaryotic communities in pigs: diversity, composition and host genetics contribution. bioRxiv. https://doi.org/10.1101/2020.02.18.941856

Reece E, Doyle S, Greally P, Renwick J, McClean S (2018) *Aspergillus fumigatus* inhibits *Pseudomonas aeruginosa* in co-culture: implications of a mutually antagonistic relationship on virulence and inflammation in the CF airway. Front Microbiol 9:1205. https://doi.org/10.3389/fmicb.2018.01205

Reese S, Dalamani G, Kaspers B (2006) The avian lung-associated immune system: a review. Vet Res 37(3):311–324. https://doi.org/10.1051/vetres:2006003

Reich M, Labes A (2017) How to boost marine fungal research: a first step towards a multidisciplinary approach by combining molecular fungal ecology and natural products chemistry. Mar Genomics 36:57–75. https://doi.org/10.1016/j.margen.2017.09.007

Reich M, Wichels A, Panzer K, Krause E, Gimenez L, Gerdts G (2017) Impacts of a reduction in seawater pH mimickingocean acidification on the structure and diversity of mycoplankton communities. Aquat Microbial Ecol 79:221–233

Richard ML, Sokol H (2019) The gut mycobiota: insights into analysis, environmental interactions and role in gastrointestinal diseases. Nat Rev Gastroenterol Hepatol 16(6):331–345. https://doi.org/10.1038/s41575-019-0121-2

Richard E, Heutte N, Bouchart V, Garon D (2009) Evaluation of fungal contamination and mycotoxin production in maize silage. Anim Feed Sci Technol 148(2-4):309–320

Rizzetto L, De Filippo C, Cavalieri D (2014) Richness and diversity of mammalian fungal communities shape innate and adaptive immunity in health and disease. Eur J Immunol 44 (11):3166–3181. https://doi.org/10.1002/eji.201344403

Robinson K, Xiao Y, Johnson TJ, Chen B, Yang Q, Lyu W, Wang J, Fansler N, Becker S, Liu J, Yang H, Zhang G (2020) Chicken intestinal mycobiome: initial characterization and its response to bacitracin methylene disalicylate. Appl Environ Microbiol. https://doi.org/10.1128/AEM.00304-20

Romani L (2011) Immunity to fungal infections. Nat Rev Immunol 11(4):275–288. https://doi.org/10.1038/nri2939

Romani L, Puccetti P (2006) Protective tolerance to fungi: the role of IL-10 and tryptophan catabolism. Trends Microbiol 14(4):183–189. https://doi.org/10.1016/j.tim.2006.02.003

Rossi F, Cocconcelli PS, Masoero F (1995) Effect of a *Saccharomyces cerevisiae* culture on growth and lactate utilization by the ruminal bacterium *Megasphaera elsdenii*. Ann Zootech 44:403–409

Rossi F, Luccia AD, VIncenti D, Cocconcelli PS (2004) Effects of peptidic fractions from *Saccharomyces cerevisiae* culture on growth and metabolism of the ruminal bacteria *Megasphaera elsdenii*. Anim Res 53(3):177–186

Round JL, Lee SM, Li J, Tran G, Jabri B, Chatila TA, Mazmanian SK (2011) The Toll-like receptor 2 pathway establishes colonization by a commensal of the human microbiota. Science 332 (6032):974–977. https://doi.org/10.1126/science.1206095

Sam QH, Chang MW, Chai LY (2017) The fungal mycobiome and its interaction with gut bacteria in the host. Int J Mol Sci 18(2). https://doi.org/10.3390/ijms18020330

Sandoval-Denis M, Sutton DA, Fothergill AW, Cano-Lira J, Gene J, Decock CA, de Hoog GS, Guarro J (2013) Scopulariopsis, a poorly known opportunistic fungus: spectrum of species in clinical samples and in vitro responses to antifungal drugs. J Clin Microbiol 51(12):3937–3943. https://doi.org/10.1128/JCM.01927-13

Satoh K, Makimura K, Hasumi Y, Nishiyama Y, Uchida K, Yamaguchi H (2009) Candida auris sp. nov., a novel ascomycetous yeast isolated from the external ear canal of an inpatient in a Japanese hospital. Microbiol Immunol 53(1):41–44. https://doi.org/10.1111/j.1348-0421.2008.00083.x

Savage DC (1969) Microbial interference between indigenous yeast and lactobacilli in the rodent stomach. J Bacteriol 98(3):1278–1283

Savage DC, Dubos RJ (1967) Localization of indigenous yeast in the murine stomach. J Bacteriol 94(6):1811–1816

Scanlan PD, Marchesi JR (2008) Micro-eukaryotic diversity of the human distal gut microbiota: qualitative assessment using culture-dependent and -independent analysis of faeces. ISME J 2 (12):1183–1193. https://doi.org/10.1038/ismej.2008.76

Schauber J, Svanholm C, Termen S, Iffland K, Menzel T, Scheppach W, Melcher R, Agerberth B, Luhrs H, Gudmundsson GH (2003) Expression of the cathelicidin LL-37 is modulated by short chain fatty acids in colonocytes: relevance of signalling pathways. Gut 52(5):735–741. https://doi.org/10.1136/gut.52.5.735

Schmidt JA, Albright S, Calza GM, Calza RE (2004) Characterization of Aspergillus oryzae fermentation extract effects on the rumen fungus Neocallimastix frontalis, EB 188. Part 2. Carbon source utilization and effects on zoospore production. Appl Microbiol Biotechnol 63 (4):431–437. https://doi.org/10.1007/s00253-003-1294-7

Schneider SM, Girard-Pipau F, Filippi J, Hebuterne X, Moyse D, Hinojosa GC, Pompei A, Rampal P (2005) Effects of *Saccharomyces boulardii* on fecal short-chain fatty acids and microflora in patients on long-term total enteral nutrition. World J Gastroenterol 11(39):6165–6169. https://doi.org/10.3748/wjg.v11.i39.6165

Schoch CL, Seifert KA, Huhndorf S, Robert V, Spouge JL, Levesque CA, Chen W, Fungal Barcoding C, Fungal Barcoding Consortium Author L (2012) Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. Proc Natl Acad Sci USA 109(16):6241–6246. https://doi.org/10.1073/pnas.1117018109

Schubert K, Groenewald JZ, Braun U, Dijksterhuis J, Starink M, Hill CF, Zalar P, de Hoog GS, Crous PW (2007) Biodiversity in the *Cladosporium herbarum* complex (Davidiellaceae, Capnodiales), with standardisation of methods for Cladosporium taxonomy and diagnostics. Stud Mycol 58:105–156. https://doi.org/10.3114/sim.2007.58.05

Shareck J, Belhumeur P (2011) Modulation of morphogenesis in *Candida albicans* by various small molecules. Eukaryot Cell 10(8):1004–1012. https://doi.org/10.1128/EC.05030-11

Shokri H, Khosravi AR, Nikaein D (2011) A comparative study of digestive tract mycoflora of broilers with layers. Int J Vet Res 5(1):1–4

Skalski JH, Limon JJ, Sharma P, Gargus MD, Nguyen C, Tang J, Coelho AL, Hogaboam CM, Crother TR, Underhill DM (2018) Expansion of commensal fungus *Wallemia mellicola* in the gastrointestinal mycobiota enhances the severity of allergic airway disease in mice. PLoS Pathog 14(9):e1007260. https://doi.org/10.1371/journal.ppat.1007260

Smith MG, Des Etages SG, Snyder M (2004) Microbial synergy via an ethanol-triggered pathway. Mol Cell Biol 24(9):3874–3884. https://doi.org/10.1128/mcb.24.9.3874-3884.2004

Sokol H, Conway KL, Zhang M, Choi M, Morin B, Cao Z, Villablanca EJ, Li C, Wijmenga C, Yun SH, Shi HN, Xavier RJ (2013) Card9 mediates intestinal epithelial cell restitution, T-helper 17 responses, and control of bacterial infection in mice. Gastroenterology 145(3):591–601. e593. https://doi.org/10.1053/j.gastro.2013.05.047

Sokol I, Gawel A, Bobrek K (2018) The prevalence of yeast and characteristics of the isolates from the digestive tract of clinically healthy Turkeys. Avian Dis 62(3):286–290. https://doi.org/10.1637/11780-121117-Reg.1

Sougioultzis S, Simeonidis S, Bhaskar KR, Chen X, Anton PM, Keates S, Pothoulakis C, Kelly CP (2006) Saccharomyces boulardii produces a soluble anti-inflammatory factor that inhibits NF-kappaB-mediated IL-8 gene expression. Biochem Biophys Res Commun 343(1):69–76. https://doi.org/10.1016/j.bbrc.2006.02.080

Spanamberg A, Sanches EMC, Santurio JM, Ferreiro L (2008) Mycotic mastitis in ruminants caused by yeast. Rural Sci 39(1). https://doi.org/10.1590/S0103-84782008005000045

Stensvold CR, Clark CG (2016) Current status of blastocystis: a personal view. Parasitol Int 65(6 Pt B):763–771. https://doi.org/10.1016/j.parint.2016.05.015

Strati F, Di Paola M, Stefanini I, Albanese D, Rizzetto L, Lionetti P, Calabro A, Jousson O, Donati C, Cavalieri D, De Filippo C (2016) Age and gender affect the composition of fungal population of the human gastrointestinal tract. Front Microbiol 7:1227. https://doi.org/10.3389/fmicb.2016.01227

Subramanya SH, Sharan NK, Baral BP, Hamal D, Nayak N, Prakash PY, Sathian B, Bairy I, Gokhale S (2017) Diversity, in-vitro virulence traits and antifungal susceptibility pattern of gastrointestinal yeast flora of healthy poultry, Gallus gallus domesticus. BMC Microbiol 17 (1):113. https://doi.org/10.1186/s12866-017-1024-4

Sugui JA, Kwon-Chung KJ, Juvvadi PR, Latge JP, Steinbach WJ (2014) *Aspergillus fumigatus* and related species. Cold Spring Harb Perspect Med 5 (2):a019786. https://doi.org/10.1101/cshperspect.a019786

Suhr MJ, Hallen-Adams HE (2015) The human gut mycobiome: pitfalls and potentials—a mycologist's perspective. Mycologia 107(6):1057–1073. https://doi.org/10.3852/15-147

Summers KL, Frey JF, Ramsay TG, Arfken AM (2019) The piglet mycobiome during the weaning transition: a pilot study. J Anim Sci 97(7):2889–2900. https://doi.org/10.1093/jas/skz182

Taghavi M, Ghorbani-Choboghlo H, Khosravi AR, Erfanmanesh A, Balal A (2014) Fungal flora of the combs and wattles of Iranian native chickens. Iran J Microbiol 6(1):46–50

Takino T, Kato-Mori Y, Motooka D, Nakamura S, Iida T, Hagiwara K (2017) Postnatal changes in the relative abundance of intestinal Lactobacillus spp. in newborn calves. J Vet Med Sci 79 (3):452–455. https://doi.org/10.1292/jvms.16-0406

Tekaia F, Latge JP (2005) Aspergillus fumigatus: saprophyte or pathogen? Curr Opin Microbiol 8 (4):385–392. https://doi.org/10.1016/j.mib.2005.06.017

Tell LA (2005) Aspergillosis in mammals and birds: impact on veterinary medicine. Med Mycol 43: 71–73

Tricario JM, Johnston JD, Dawson KA (2008) Dietary supplementation of ruminant diets with an *Aspergillus oryzae* alpha-amylase. Anim Feed Sci Technol 145:136–150

Tristant D, Moran CA (2015) The efficacy of efeding a live probiotic yeast, Yea-Sacc, on the performance of lactating dairy cows. J Appl Anim Nutr 3:1–6

Tso GHW, Reales-Calderon JA, Tan ASM, Sem X, Le GTT, Tan TG, Lai GC, Srinivasan KG, Yurieva M, Liao W, Poidinger M, Zolezzi F, Rancati G, Pavelka N (2018) Experimental evolution of a fungal pathogen into a gut symbiont. Science 362(6414):589–595. https://doi.org/10.1126/science.aat0537

Turnbaugh PJ, Hamady M, Yatsunenko T, Cantarel BL, Duncan A, Ley RE, Sogin ML, Jones WJ, Roe BA, Affourtit JP, Egholm M, Henrissat B, Heath AC, Knight R, Gordon JI (2009) A core gut microbiome in obese and lean twins. Nature 457(7228):480–484. https://doi.org/10.1038/nature07540

Underhill DM, Iliev ID (2014) The mycobiota: interactions between commensal fungi and the host immune system. Nat Rev Immunol 14(6):405–416. https://doi.org/10.1038/nri3684

Urubschurov V, Janczyk P, Pieper R, Souffrant WB (2008) Biological diversity of yeasts in the gastrointestinal tract of weaned piglets kept under different farm conditions. FEMS Yeast Res 8 (8):1349–1356. https://doi.org/10.1111/j.1567-1364.2008.00444.x

Urubschurov V, Janczyk P, Souffrant WB, Freyer G, Zeyner A (2011) Establishment of intestinal microbiota with focus on yeasts of unweaned and weaned piglets kept under different farm conditions. FEMS Microbiol Ecol 77(3):493–502. https://doi.org/10.1111/j.1574-6941.2011.01129.x

Urubschurov V, Busing K, Janczyk P, Souffrant WB, Zeyner A (2015) Development and evaluation of qPCR assay for quantitation of *Kazachstania slooffiae* and total yeasts occurring in the porcine gut. Curr Microbiol 71(3):373–381. https://doi.org/10.1007/s00284-015-0862-2

Urubschurov V, Busing K, Freyer G, Herlemann DP, Souffrant WB, Zeyner A (2017) New insights into the role of the porcine intestinal yeast, *Kazachstania slooffiae*, in intestinal environment of weaned piglets. FEMS Microbiol Ecol 93(2). https://doi.org/10.1093/femsec/fiw245

Urubschurov V, Busing K, Souffrant WB, Schauer N, Zeyner A (2018) Porcine intestinal yeast species, *Kazachstania slooffiae*, a new potential protein source with favourable amino acid

composition for animals. J Anim Physiol Anim Nutr (Berl) 102(2):e892–e901. https://doi.org/10.1111/jpn.12853

Usyk M, Zolnik CP, Patel H, Levi MH, Burk RD (2017) Novel ITS1 fungal primers for characterization of the mycobiome. mSphere 2(6). https://doi.org/10.1128/mSphere.00488-17

van de Veerdonk FL, Plantinga TS, Hoischen A, Smeekens SP, Joosten LA, Gilissen C, Arts P, Rosentul DC, Carmichael AJ, Smits-van der Graaf CA, Kullberg BJ, van der Meer JW, Lilic D, Veltman JA, Netea MG (2011) STAT1 mutations in autosomal dominant chronic mucocutaneous candidiasis. N Engl J Med 365(1):54–61. https://doi.org/10.1056/NEJMoa1100102

van der Velden WJ, Netea MG, de Haan AF, Huls GA, Donnelly JP, Blijlevens NM (2013) Role of the mycobiome in human acute graft-versus-host disease. Biol Blood Marrow Transplant 19 (2):329–332. https://doi.org/10.1016/j.bbmt.2012.11.008

Van Immerseel F, De Buck J, De Smet I, Mast J, Haesebrouck F, Ducatelle R (2002) Dynamics of immune cell infiltration in the caecal lamina propria of chickens after neonatal infection with a *Salmonella enteritidis* strain. Dev Comp Immunol 26(4):355–364. https://doi.org/10.1016/s0145-305x(01)00084-2

van Leeuwen PT, van der Peet JM, Bikker FJ, Hoogenkamp MA, Oliveira Paiva AM, Kostidis S, Mayboroda OA, Smits WK, Krom BP (2016) Interspecies interactions between *Clostridium difficile* and *Candida albicans*. mSphere 1(6). https://doi.org/10.1128/mSphere.00187-16

Vanderheyden N (1993) Aspergillosis in psittacine chicks. In: Proceedings of the annual conference of the association of avian veterinarians, Nashville, p 207

Vecchiarelli A (2007) Fungal capsular polysaccharide and T-cell suppression: the hidden nature of poor immunogenicity. Crit Rev Immunol 27(6):547–557. https://doi.org/10.1615/critrevimmunol.v27.i6.50

Villmones HC, Haug ES, Ulvestad E, Grude N, Stenstad T, Halland A, Kommedal O (2018) Species level description of the human ileal bacterial microbiota. Sci Rep 8(1):4736. https://doi.org/10.1038/s41598-018-23198-5

Vylkova S (2017) Environmental pH modulation by pathogenic fungi as a strategy to conquer the host. PLoS Pathog 13(2):e1006149. https://doi.org/10.1371/journal.ppat.1006149

Wallace RJ (1994) Rumen microbiology, biotechnology, and ruminant nutrition: progress and problems. J Anim Sci 72:2992–3003

Walter J, Ley R (2011) The human gut microbiome: ecology and recent evolutionary changes. Annu Rev Microbiol 65:411–429. https://doi.org/10.1146/annurev-micro-090110-102830

Ward TL, Dominguez-Bello MG, Heisel T, Al-Ghalith G, Knights D, Gale CA (2018) Development of the human mycobiome over the first month of life and across body sites. mSystems 3(3). https://doi.org/10.1128/mSystems.00140-17

Ward TL, Weber BP, Mendoza KM, Danzeisen JL, Llop K, Lang K, Clayton JB, Grace E, Brannon J, Radovic I, Beauclaire M, Heisel TJ, Knights D, Cardona C, Kogut M, Johnson C, Noll SL, Arsenault R, Reed KM, Johnson TJ (2019) Antibiotics and host-tailored probiotics similarly modulate effects on the developing avian microbiome, mycobiome, and host gene expression. mBio 10(5). https://doi.org/10.1128/mBio.02171-19

Weissenbacher-Lang C, Kureljusic B, Nedorost N, Matula B, Schiessl W, Stixenberger D, Weissenbock H (2016) Retrospective analysis of bacterial and viral co-infections in Pneumocystis spp. positive lung samples of Austrian pigs with pneumonia. PLoS One 11(7): e0158479. https://doi.org/10.1371/journal.pone.0158479

Wheeler ML, Limon JJ, Bar AS, Leal CA, Gargus M, Tang J, Brown J, Funari VA, Wang HL, Crother TR, Arditi M, Underhill DM, Iliev ID (2016) Immunological consequences of intestinal fungal dysbiosis. Cell Host Microbe 19(6):865–873. https://doi.org/10.1016/j.chom.2016.05.003

White TJ, Burns T, Lee S, Taylor J (1990) Amplification and sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) PCR protocols: a guide to methods and applications. Academic, San Diego, pp 315–322

White JK, Nielsen JL, Madsen AM (2019) Microbial species and biodiversity in settling dust within and between pig farms. Environ Res 171:558–567. https://doi.org/10.1016/j.envres.2019.01.008

Williams PE, Tait CA, Innes GM, Newbold CJ (1991) Effects of the inclusion of yeast culture (Saccharomyces cerevisiae plus growth medium) in the diet of dairy cows on milk yield and forage degradation and fermentation patterns in the rumen of steers. J Anim Sci 69 (7):3016–3026. https://doi.org/10.2527/1991.6973016x

Xiao Y, Kong F, Xiang Y, Zhou W, Wang J, Yang H, Zhang G, Zhao J (2018) Comparative biogeography of the gut microbiome between Jinhua and Landrace pigs. Sci Rep 8(1):5985. https://doi.org/10.1038/s41598-018-24289-z

Yang H, Yang M, Fang S, Huang X, He M, Ke S, Gao J, Wu J, Zhou Y, Fu H, Chen C, Huang L (2018) Evaluating the profound effect of gut microbiome on host appetite in pigs. BMC Microbiol 18(1):215. https://doi.org/10.1186/s12866-018-1364-8

Yarza P, Yilmaz P, Panzer K, Glockner FO, Reich M (2017) A phylogenetic framework for the kingdom Fungi based on 18S rRNA gene sequences. Mar Genomics 36:33–39. https://doi.org/10.1016/j.margen.2017.05.009

Zelante T, Iannitti RG, Cunha C, De Luca A, Giovannini G, Pieraccini G, Zecchi R, D'Angelo C, Massi-Benedetti C, Fallarino F, Carvalho A, Puccetti P, Romani L (2013) Tryptophan catabolites from microbiota engage aryl hydrocarbon receptor and balance mucosal reactivity via interleukin-22. Immunity 39(2):372–385. https://doi.org/10.1016/j.immuni.2013.08.003

Zhang Z, Li J, Zheng W, Zhao G, Zhang H, Wang X, Guo Y, Qin C, Shi Y (2016) Peripheral lymphoid volume expansion and maintenance are controlled by gut microbiota via RALDH+ dendritic cells. Immunity 44(2):330–342. https://doi.org/10.1016/j.immuni.2016.01.004

Zhao W, Wang Y, Liu S, Huang J, Zhai Z, He C, Ding J, Wang J, Wang H, Fan W, Zhao J, Meng H (2015) The dynamic distribution of porcine microbiota across different ages and gastrointestinal tract segments. PLoS One 10(2):e0117441. https://doi.org/10.1371/journal.pone.0117441

Zhou M, Hernandez-Sanabria E, Guan LL (2009) Assessment of the microbial ecology of ruminal methanogens in cattle with different feed efficiencies. Appl Environ Microbiol 75 (20):6524–6533. https://doi.org/10.1128/AEM.02815-08

Zlotowski P, Rozza DB, Pescador CA, Barcellos DE, Ferreiro L, Sanches EM, Driemeier D (2006) Muco-cutaneous candidiasis in two pigs with postweaning multisystemic wasting syndrome. Vet J 171(3):566–569. https://doi.org/10.1016/j.tvjl.2004.12.010

Influence of Sow Gut Microbiota on Colostrum and Piglet Performance

7

Shah Hasan and Claudio Oliviero

Abstract

Colostrum being the sole source of immunoglobulin and energy plays an essential role for piglet survival and growth. Studies have shown that colostrum and milk intake also influence the gut development and maturation of piglets. The early life colonization and development of the gut microbiota primes the development of the adult microbiome and has long-term impact on the health of the pigs. Growing number of evidences suggest that certain microbial species can exert beneficial effect on the sow and piglets, and thus improve production performances like colostrum yield, colostrum quality, sow physiology around farrowing, piglet weight gain, and health during lactation and weaning. The gut microbiota of pig which is unique at suckling stage, largely acquired from the mother, shifts over time. Multiple factors like age, environment, production system, diet can influence the gut microbiota of sow and piglets. The improvement of the sow and piglets microbiota toward beneficial bacteria can also be done by probiotic, prebiotic, and different feed additive applications.

Keywords

Sow · Piglets · Production performance · Colostrum · Gut microbiota

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7.1 Introduction: Colostrum Importance in Pig

Colostrum is essential for piglet survival and growth by providing essential immunoglobulins, and being source of energy. Among the main causes of piglets' mortality before weaning are: lower birth weight, inadequate colostrum intake, hypothermia, and hypoglycemia (Le Dividich et al. 2005). Newborn piglets lack globulins, relying on colostrum as the main source of antibody for the first weeks of age, until they become capable to produce it themselves (Salmon et al. 2009). The main reason is that piglets at birth don't have yet an active adaptive immune system, due to intrauterine placental barriers, therefore they are dependent on innate immune responses and passive uptake of immunoglobulins (Rooke and Bland 2002; Salmon et al. 2009). However, this passive intestinal absorption of large molecules like IgG is possible only for few hours after birth, until gut closure which occurs 24–36 h after birth (Quesnel et al. 2012). Failure of piglets to achieve an adequate intake of colostrum is the primary cause of piglet deaths occurring within the first days after birth (Quesnel et al. 2012). The concentration of IgG in the plasma of piglets shortly after birth is positively correlated with their survival and, in addition, dead piglets have lower serum IgG concentration than their surviving fellow piglets, indicating low colostrum intake (Vallet et al. 2013). There is clear evidence that colostrum and milk intake influence not only piglets' immune system, but also their gut development and maturation (Salmon et al. 2009; Turfkruyer and Verhasselt 2015). Other than immunoglobulins, colostrum contains many biologically active factors, including leukocytes, enzymes, hormones, growth factors but also bacteria (Hurley 2015; Chen et al. 2018). Some studies have found evidences that the development of the gut microbiota during early life primes the development of the adult microbiome and has long-term impacts on the health of the host (Turnbaugh et al. 2009; Han 2015). Colostrum and milk are indeed one of the largest sources of microbiota for the gut of neonate piglets. Chen et al. (2018) found that the composition and diversity of the milk microbiota changed significantly in colostrum but was relatively stable in transitional and mature milk. They found that *Corynebacterium* and *Streptococcus* were significantly higher in sow colostrum than in milk, while the other four most dominant bacterial taxa (*Lactobacillus*, two unclassified genera in the families Ruminococcaceae and Lachnospiraceae, and an unclassified genus in the order Clostridiales) had higher relative abundances in transitional and mature milk than in colostrum. Firmicutes and Proteobacteria were the most dominant phyla in sow milk (Chen et al. 2018). Another study revealed that the gut mucosa microbiota was different in high weight gain piglets and in low weight gain piglets (Morissette et al. 2018). The microbiota of high weight growth piglets had higher levels of *Bacteroidetes*, Bacteroides and Ruminococcaceae, and lower proportions of *Actinobacillus porcinus* and *Lactobacillus amylovorus* when compared with those of low weight growth piglets (Morissette et al. 2018). When looking to different studies' results, often the bacteria found in colostrum and milk are typical skin bacteria (like *Staphylococcus* and *Streptococcus*), indicating that the skin might be an important source of the milk microbiota (Urbaniak et al. 2016; Chen et al. 2018; Morissette et al. 2018). However, the presence of many obligate anaerobic

gut-associated genera such as *Bacteroides*, *Blautia*, *Lactobacillus*, *Ruminococcus*, and *Bifidobacterium*, indicates that bacterial communities in sow milk do not solely originate from the host skin or environmental sources (Hunt et al. 2011; Jost et al. 2013; Chen et al. 2018; Morissette et al. 2018). Rodríguez (2014) hypothesizes that milk bacterial community can originate from the maternal gastrointestinal tract through a bacterial entero-mammary pathway. The recent findings on the role of colostrum as source of energy, passive immunity, gut developing factors and bacteria for the neonate piglets, show how fundamental is the balance between sow's nutrition and health, with the environment, in relation to successful piglets' growth.

7.2 Gut Microbiota in Sow and Piglets

Pig intestine harbors a complex and diverse ecosystem of the microbial population. In a symbiotic relationship with the host, gut microbiota plays a significant role in the health and wellbeing of the pigs by providing energy, volatile fatty acids (VFA), vitamins, cellulose fermentation, immunological functions, and resistance to pathogens bacteria (Kim and Isaacson 2015; Fouhse et al. 2016; Stokes 2017; Yang et al. 2017; Guevarra et al. 2019). The gut microbiota in pigs is dynamic and varies with time, age, environments, production system, diet, and many other factors (Kubasova et al. 2017; Hasan et al. 2018; Niu et al. 2019). With the advent of the new technologies and consumer demand to produce antibiotic-free pigs, researches on this field have been continuously growing, trying to find the relationship between the gut microbiome and production performances of sow and piglets. During the last three decades, litter size has been significantly increased, and in the so-called hyper prolific sows, for example, the farrowing process can be affected by the sow gut microbiota (Hasan et al. 2018). The discussion on gut microbiota of this chapter will be on sow late gestation, farrowing and lactation, and on piglet pre-weaning stage. It is believed that gut microbiota at late pregnancy plays a significant role in the health and the production of sows, the nutrient metabolism, the immune stimulation, and the metabolic regulation. However, this is particularly important also for the piglets, as they acquire their first gut microbiota colonization from the mother and their immune system development depends on the acquisition of the microbiota and the immunoglobulins from the colostrum (Hasan et al. 2018).

The total number of the adult pig colon bacteria has been estimated to be 10^{10}–10^{11} per gram of gut content, with an average 500–1000 diversified species living in mammalian gastrointestinal tract (Gaskins et al. 2002; Isaacson and Kim 2012). Studies have shown that certain microbial species can exert beneficial effects on the sow and piglets, thus boosting production performance like colostrum yield, improving colostrum quality and sow physiology around farrowing (Tan et al. 2016; Hasan et al. 2018; Wang et al. 2019a, b). This study by Hasan et al. (2018) also showed that young piglets have a unique microbiome acquired either from the mother directly via suckling or from the farrowing environment.

7.2.1 Sow Gut Microbiota in Late Gestation, Farrowing and Lactation

The composition of gut microbiota is not static and shifts over time. In sows, at pregnancy both diversity and abundance of certain microbial population increased with progression of the pregnancy until weaning (Ji et al. 2019). A diverse gut microbiota provides many metabolic capacities and functional redundancy in sows, which ensures the sufficient supply of nutrients for fetal growth and development (Ji et al. 2019). In a recent study carried out by Hasan et al. (2018), during farrowing, from a phyla level perspective, most gut bacteria were classified in Firmicutes, Bacteroidetes, Proteobacteria, Actinobacteria, and Candidatus. The Firmicutes represent the most abundant proportion of the total population, followed by Bacteroides. These two phyla accounted for approximately 98% of all bacteria present (Fig. 7.1). Another comprehensive study published by Kim et al. (2011) reported the same, being 90% of total bacteria present in the sow gut Firmicutes and Bacteroidetes were the most abundant. On the other hand, however, the findings of the study by Ji et al. (2019) reported that Bacteroidetes increased linearly with the progression of the pregnancy and represent the most dominant (45.56%) on 110 days of pregnancy. Jost et al. (2014) reported that Firmicutes exhibited no detectable changes over perinatal period. Studies have demonstrated that gestational weight gain or increase in the back-fat thickness in the sow may be associated with an increase in the abundance of Firmicutes or an increase in the Firmicutes to Bacteroides ratio (Feng et al. 2015; Ji et al. 2019). In terms of phyla, the abundance of Tenericutes, Fibrobacteres, and Cyanobacteria has been shown to increase with the progression of the pregnancy (Ji et al. 2019). These phyla have some beneficial effects, for example Tenericutes increase intestinal cells' integrity and Fibrobacteres were characterized as having the potential to metabolize non-soluble polysaccharides, such as cellulose, hemicellulose, or pectin (Ji et al. 2019). During

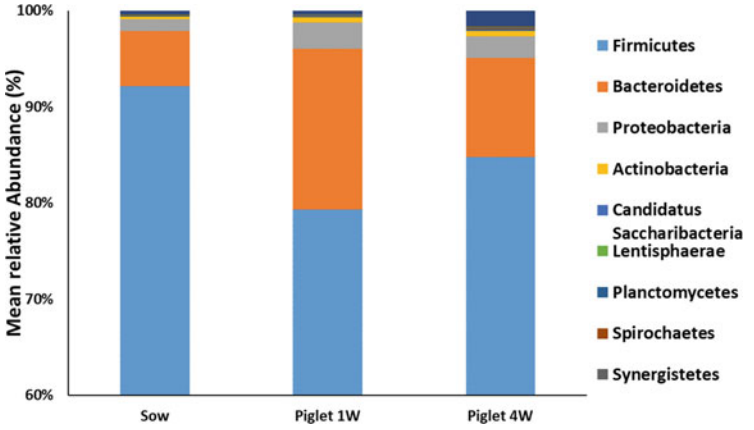

Fig. 7.1 The distribution of bacterial phyla in fecal samples of sows during farrowing, piglets 1 week (Piglet 1 W) and piglets 4 week (Piglet 4 W) ages. Figure adapted from Hasan et al. (2018)

late gestation *Romboutsia* was the dominant genus in sows which is from the phylum Firmicutes, followed by *Clostridium* sensu stricto, *Lactobacillus*, *Oscillibacter*, *Intestinimonas*, *Sporobacter*, *Christensenella*, *Barnesiella*, *Flavonifractor*, *Terrisporobacter*, *Acidaminobacter*, *Lachnospiracea incertae sedis*, and *Turicibacter*, other genera being much less 1% (Hasan et al. 2018). In a similar study sample collected at 109 days of gestation reported that *Clostridium* sensu stricto was the most dominant genus, also from the phylum Firmicutes. In the same study the nine most abundant genera, in more than 1% of the total DNA sequences, were *Treponema*, *Lactobacillus*, *Gemmatimonas*, *Prevotella*, *Barnesiella*, *Gp7*, *Lachnospiraceae incertae sedis*, *Flavisolibacter*, and *Clostridium* cluster XI (Tan et al. 2016). However, the findings of these studies differed from those of Ji et al. (2019), who reported an overall increase in abundance of *Prevotella* linearly with the progression of pregnancy being most dominant with 14.02% of the total microbiota followed by *Lactobacillus* (6.91%).

7.2.2 Piglets Gut Microbiota in Pre- and Post-Weaning

In recent years, due to increased attention on reduction or ban of the use of antimicrobials and zinc oxide, the intestinal microbiome of piglets received a lot of attention for its essential role in the immune system development and function. Recent studies report that the suckling piglet has a unique microbiota, largely acquired from the mother (Tan et al. 2016; Li et al. 2017; Hasan et al. 2018). The piglets acquire mainly fecal microbiota from the sow, but also microbial communities present in the birth canal, on the skin of the mother and from the environment. Furthermore, the chemical and microbial composition of colostrum and milk might also influence the intestinal microbiota of the progeny (Mach et al. 2015; Chen et al. 2018).

The diversity of the piglet gut microbiota increased over time with dietary changes from sow's milk to plant based starter diet. At phyla level, in pre-weaning piglets (at 1 week and 4 week of age) Firmicutes and Bacteroidetes accounted for more than 90% of the bacteria (Hasan et al. 2018). Even though Firmicutes is the most abundant in pre-weaning piglets but over the time the proportion of Bacteroidetes increased post-weaning (Pajarillo et al. 2014a). In suckling piglets *Bacteroides*, *Balutia*, *Dorea*, *Eschericia*, *Fusobacterium* were the most abundant genus. In several reports the dominance of *Bacteroides* in suckling piglets was mentioned as it is not common in adult piglets (Pajarillo et al. 2014a; Kim and Isaacson 2015; Kubasova et al. 2017). The greater amount of the Bacteroides in the pre-weaned piglets could be due to their ability to utilize monosaccharides and oligosaccharides from sow's milk. In post-weaning the most predominant genus was *Prevotella*, which is in the phylum Bacteroidetes and *Lactobacillus* from the phylum Firmicutes. The genus *Prevotella* in the post-weaning piglets justifies the ability to degrade plant derived cellulose and hemicellulose by producing specific enzymes. Kim et al. (2011) mentioned that *Prevotella* represented up to 30% of all classifiable bacteria when the pigs were 10 weeks of age. However, by the time these

pigs were 22 weeks of age, *Prevotella* accounted for only 3.5–4.0% of the bacteria. As the levels of *Prevotella* decreased, there was a pronounced increase in *Anaerobacter* (in the phylum Firmicutes). In another study, Looft et al. (2012) reported on gut microbiome of 18 and 20 weeks piglet, the majority of the bacteria were classified in the phyla Bacteroidetes and Firmicutes and most predominant genera were *Prevotella*, *Anaerovibrio*, *Succinivibrio*, *Oscillibacter*, *Parabacteroides, Hallella*, and *Coprococcus*.

7.3 Factors Affecting Sow and Piglet Gut Microbiota

7.3.1 Environment and Housing Effect

Even though piglets get the initial colonization during the birth, the management and production conditions, the maternal environment and the environmental microbiota sources complement the development. Source Tracker analysis showed that the microbiota from the slatted floor, sow's milk, and nipple surface were most likely the earliest to pass into the neonatal gastrointestinal tract, but did not have a long permanence during lactation. The sow's fecal microbiota were the easier to colonize in newborn piglet's guts by the cooccurrence with former colonized microbial communities (Chen et al. 2017). This study suggests that microbes from the maternal and surrounding environments may play an important role in the microbial succession of newborn piglets after birth. In pig production, the cleaning procedures applied in the farrowing unit prior to entrance of the sows decrease the occurrence of environmental microbiota. PCA analysis revealed that piglets at 1 week of age have unique microbiota (Hasan et al. 2018). Many studies, however, report similar results that the suckling piglet has unique microbiota acquired from the mother. The maternal dietary treatment had an impact on the composition of the microbiota in piglets, which was distinct from the sow's fecal microbial alterations. This was also observed when feeding sows with inulin, prebiotics, or probiotics (Tan et al. 2016; Hasan et al. 2018; Li et al. 2020). Nevertheless, piglets cohabiting the same pen have similar microbiota composition differently than separated siblings, proving the environmental effects (Thompson et al. 2008). Alternative sow enriched rearing systems in deep straw bedding are getting popular in pig production, since these systems reduce stress and straw provides a non-digestible fiber source. Microbiota of sows from enriched rearing system contained significantly more *Prevotella*, *Parabacteroides*, *CF231*, *Phascolarctobacterium*, *Fibrobacter*, *Anaerovibrio,* and *YRC22* (Kubasova et al. 2017).

7.3.2 Diet Effect

The changes in the diet can result in differences in the composition of the microbiome and in its potential functionality, which are linked with feed efficiency in the pigs. Recent research demonstrates the importance of dietary microbial

modulation. Dietary supplementation of hydrolyzed yeast (Hasan et al. 2018), resin acid-enriched composition (Hasan et al. 2019a, b), probiotics (Menegat et al. 2019), and prebiotics (Tan et al. 2016; Li et al. 2020) in sow's late gestation diet significantly changes microbial populations. Different levels and types of protein and fiber in the diet are also modulating the gut microbial population both in gestating sows and weaning piglets. Due to the various physicochemical properties of dietary fiber and its physiological effects, the supplementation of pregnancy diet with soluble fiber effectively enhances the stability of gut microbiota structure and greatly changes the composition of gut microbiota in sow (Li et al. 2020). The representative changes in the composition of gut microbiota include a decrease in *Proteobacteria* and an increase in *Ruminococcaceae*, *Oscillospira*, and *Eubacterium*. Moreover, the increase of genus *Eubacterium,* after dietary soluble fiber supplementation during pregnancy, promotes propionate and plasma fatty acid production, which may be one of the potential mechanisms by which dietary fiber improves insulin sensitivity and systemic inflammation in perinatal sow (Xu et al. 2020). Studies were also conducted to investigate impacts of dietary protein levels on the gut bacterial community. A moderate dietary protein restriction (13% CP) could alter the bacterial community and metabolites, promote colonization of beneficial bacteria in both ileum and colon, and improve gut barrier function (Fan et al. 2017).

7.3.3 Genetic Effect

Genetics of the pig can play a role in shaping the gut microbiota. A study conducted by Pajarillo et al. (2014b), with 15 weeks piglets from purebred pig lines Duroc, Landrace, and Yorkshire, demonstrated that Landrace breed had the most diverse bacterial community composition. *Prevotella*, *Blautia*, *Oscillibacter*, and *Clostridium* were detected in all samples regardless of breed. On the other hand, *Catenibacterium*, *Blautia*, *Dialister*, and *Sphaerochaeta* were differentially detected among breeds. These bacteria may be linked to functional genes or characteristics unique to the breeds with which they are associated. In another study by Bian et al. (2016), piglets from two different breeds Meishan and Yorkshire had bacterial taxa difference during the suckling period. Piglets from the Meishan sows had higher population of Fusobacteriaceae family with a lower abundances of Bacteroides compared to the piglet from the Yorkshire sows. Genetic factors can also determine the susceptibility of the pig to certain infection in the gut and resulting in microbial shifts. For example, enterotoxigenic *E. coli* (ETEC) expressing F4 fimbriae causes severe diarrhea in piglets carrying F4 specific intestinal receptor (Rhouma et al. 2017). Therefore, existence and function of these receptors are crucial for the susceptibility of pigs to ETEC infections.

7.3.4 Antibiotic Effect (Antibiotic Growth Promoter)

Antibiotics have been one of the most cost-effective tools to improve the feed efficiency and health of the pigs. The usage of antibiotics in pigs as growth promoters is banned in several countries, and it's becoming important because of the growing concern of antibiotic resistance of bacterial pathogens. It is likely that antibiotics enhance the growth by alteration of the composition of gastrointestinal microbiome in pigs, especially in sub-therapeutic levels. A study by Kim et al. (2012) showed that the use of tylosin shifts the microbial population in both abundant and less abundant species. In particular, *Lactobacillus*, *Sporacetigenium*, *Acetanaerobacterium*, and *Eggerthella* were detected more frequently in the group of pigs receiving the tylosin compared to the non-treated group. In another study, simultaneous administration of chlortetracycline, sulfamethazine, and penicillin for 14 days showed an increase in Proteobacteria compared to the non-medicated piglets. This shift was driven by an increase in *E. coli* population (Looft et al. 2012). Antibiotics are also frequently administered during the early life stages of piglets to control respiratory and gastrointestinal problems. This treatment may have an immediate effect on colonization of gut microbiota in piglets. In a study conducted in a commercial piggery by Hasan et al. (2019a), piglets were marked if they received antibiotic (amoxicillin or florfenicol) treatment within the first 3 days of their life and equal number of piglets were selected from non-treated nearest litters as control. Fecal samples collected at 1 week of age were assessed to check microbial composition by 16S rRNA gene sequencing. The diversity (Shannon index) and Richness were significantly lower in antibiotic treated piglets compared to the non-treated piglets. Overall, the antibiotic treatment at an early age not only decreased the relative abundance of some opportunistic pathogenic bacteria like *Campylobacter*, *Pasteurella*, but it also reduced some beneficial bacteria like *Prevotella* and *Butyricimonas*. Moreover, individual assessment of each of the antibiotic revealed that treatment at an early age in piglets significantly decreased the relative abundance of *Clostridium* sensu stricto, *Butyricimonas*, *Flavonifractor*, *Romboutsia*, *Bacteroides,* and *Roseburia*.

7.4 Improvement of Sow and Piglet Gut Microbiota

7.4.1 Probiotic and Prebiotic Concept

Probiotic and prebiotic or combination of both in swine diets stimulates the proliferation and metabolic activity of beneficial microbes, contributing to a stable microbial ecosystem. Probiotics are well-characterized bacteria, they can produce antimicrobial substances, modulate the host immune system, induce competitive exclusion of pathogenic bacteria, and modulate gut microbiota (Cammarota et al. 2014). From the perspective of probiotics in sow diets it is proposed to have a dual purpose, benefiting not only the sows but also their progeny. The probiotic application to sow's diet and the piglets' intimate maternal contact are important determinants of

gastrointestinal tract bacterial colonization in newborn piglets (Everaert et al. 2017). However, many obligate gut-associated genera in piglets such as *Bacteroides*, *Blautia*, *Lactobacillus*, *Ruminococcus*, and *Bifidobacterium* originate from sow milk (Hunt et al. 2011; Jost et al. 2013; Chen et al. 2018; Morissette et al. 2018). It has been speculated that these bacterial communities can be of maternal gastrointestinal tract through a bacterial entero-mammary pathway (Rodríguez 2014). Therefore, probiotic can have an effect on diet-driven modulation of milk bacterial population and influence the progeny intestinal microbiota during lactation. However, studies have demonstrated that provision of probiotics to sows can modify the sow fecal microbial population and carry over to progeny in pre-weaning and post-weaning stages (Silva et al. 2010; Baker et al. 2013; Starke et al. 2013). *Bacillus subtilis and Enterococcus faecium,* a common probiotic species in sow diet during late gestation and lactation, were found to improve the population of beneficial bacteria, primarily *Lactobacillus sp*., and to reduce the population of potentially harmful bacteria, including *C. perfringens* and *Escherichia coli* (Baker et al. 2013; Starke et al. 2013). Feeding piglets with *Lactobacillus salivarius,* a commonly found probiotic, decreased the relative proportion of the bacteria from the phylum Spirochaetes with genus *Treponema*, *Anaerostipes*, and *Lactonifactor* while proportions of *Subdoligranulum*, *Oribacterium*, and *Hallella* increased (Riboulet-Bisson et al. 2012). Several other probiotics *Lactobacillus* spp. like *Lactobacillus reuteri*, *Lactobacillus plantarum*, *Lactobacillus johnsonii* have been shown to improve piglet growth performance by regulating the gut microbiota and preventing diarrhea. Combination of probiotic and prebiotic, for example, lactulose with *Enterococcus faecium* NCIMB 11181 increases the relative proportion of *Lactobacillus* in the post-wean piglets (Chae et al. 2016). Prebiotics, for example, inulin, fructo-oligosaccharides, transgalacto-oligosaccharides, and lactulose are readily available fermentable source for the beneficial gut microbiota and protect the gut by lowering the pH and ensuring the cecal and colonic butyrate concentration (Fouhse et al. 2016).

7.4.2 Feeding Methods (Feed Additives Application)

Feeding sows with alternative compounds is a common practice in modern sow production with the hypothesis that feed additives are able to modulate gut microbiota of sow and piglets, improving their production performances and health. Although dietary components like fat, protein, and carbohydrate are explored for their impact on gut microbiota, however, to date, little attention has been paid to the studies related to supplementation of gestating sow diets with specific compounds, consequent gut microbiota modulation and their effect on sow and piglet performances. Sow and piglet feeding strategies mostly include functional fibers, yeast fractions and derivatives, essential oils, organic acids, medium chain and short chain fatty acids at different stages of productions. The mode of action of these feed ingredients relies on their ability to modify favorably the microbiota of the gut, which is of importance for sow's health and consequently piglets' health. Beneficial

bacteria can act as a barrier against pathogenic bacteria, having the ability to lower the pH of the gastrointestinal tract and produce antimicrobial compounds (Lallès et al. 2009). Microbiota fermenting indigestible carbohydrates produce short chain fatty acids (SCFA) that are an important energy source for the sow. Butyrate, in particular, is a gut health-promoting compound that acts as the main energy source for colonocytes and exerts anti-inflammatory properties (Sassone-Corsi and Raffatellu 2015). It is thus of interest to modify favorably the microbiota toward fermentative butyrate-producing and anti-pathogenic bacteria. Studies show that the reduction in the number of pathogenic bacteria in response to dietary supplementation is associated with an increase in beneficial microbiota, which in turn may modify the substrate availability and physiological conditions of the gastrointestinal tract (e.g., fermentation products, luminal pH, and bile acid concentration) (Liu et al. 2008). In a study, dietary supplementation of yeast hydrolysate in the pregnancy influences beneficial and fermentative bacteria (*Roseburia*, *Paraprevotella*, *Eubacterium*), while some opportunistic pathogens, including Proteobacteria, especially the genera *Desulfovibrio*, *Escherichia/Shigella,* and *Helicobacter*, were suppressed. In the same study, piglets at 1 week of age from sows fed the yeast product had more beneficial microbial populations with significant diversity and fewer opportunistic pathogens (Hasan et al. 2018). Yeast hydrolysate can bind and inhibit pathogen bacteria like *Salmonella* spp., *Clostridium* spp., and *Escherichia coli*, thereby promoting growth of beneficial gut bacteria, better utilization of feed nutrients, and reduced spread of pathogens to piglets (White et al. 2002; Burkey et al. 2004; Castillo et al. 2008; Liu et al. 2008). In another study, resin acid-enriched composition (RAC) has been used in feed as a novel additive to improve performance in sow (Hasan et al. 2019b). RAC, a novel dietary product, typically comprises resin acids (RA) (~8%) and free fatty acids (~90%), and 2 to 3% neutral components naturally occurring in coniferous trees. RAC modulates the microbial population in the small intestine, changes the microbial digestion, and improves the feed conversion ratio and gut microbiota in monogastric species (Kettunen et al. 2017; Vienola et al. 2018; Hasan et al. 2019b). Feeding RAC in late gestation significantly increased Firmicutes, and conversely Bacteroidetes and Proteobacteria were less abundant. RAC in sow diet increases the abundances of genus *Romboutsia* and *Clostridium* sensu stricto and decreases the abundances of *Barnesiella*, *Sporobacter*, *Intestinimonas*, and *Campylobacter* (Hasan et al. 2019b).

7.5 Sow Gut Microbiota Influence on Production

Understanding the sow gut microbiota in the modern swine production is of interest as it is an important issue in improving feed efficiency, reducing oxidative stress, it helps in farrowing, and colonizing microbes to neonates (Hasan et al. 2018; Wang et al. 2019a, b). In hyper prolific sow line pregnancy and lactation often lead to substantial metabolic and physiological changes which resulted in lower feed intake. However, hyper prolific sows often have a longer farrowing duration, high number of stillborn piglets, and oxidative stress (Hasan et al. 2019a, b). Therefore,

production efficiencies of the sow quite often can be affected by those mentioned factors. Growing evidence suggests that the gut microbiota plays a vital role in sow reproductive and production performance.

7.5.1 Feed Efficiency

Modern sows are often characterized with large litter size, and during pregnancy and lactation the sow undergoes substantial hormonal and metabolic changes (Algers and Uvnäs-Moberg 2007). However, raising a large litter also requires higher feed intake by sow. Alteration in the gut microbiota during the pregnancy plays a significant role in maternal pregnancy-induced metabolic changes. Recent researches suggest that during pregnancy up to lactation, the sow undergoes a decrease in insulin sensitivity and increase in systemic inflammation, resulted in lower feed intake of sow (Mosnier et al. 2010; Xu et al. 2020). Bacterial species like Eubacterium (e.g., *Eubacterium hallii*) can help to improve insulin sensitivity and systemic inflammation by producing propionate in the intestine. This has been shown in a recent study where sows were fed with soluble fiber (Guar gum and maize starch) during the pregnancy, resulting in a remarkable increase in *Eubacterium* (Xu et al. 2020). The *Eubacterium* spp., which is a common genus of adult pig gut microbiota, plays a crucial role in intestinal metabolic balance due to its ability to produce butyrate from the fermentation intermediates lactate and acetate and utilizes 1,2-propanediol to form propionate (Duncan et al. 2004; Engels et al. 2016). Insulin sensitivity and higher feed intake in late gestation and at farrowing are also correlated with higher abundance of *Akkermansia* and *Roseburia*. These are also short chain fatty acids (SCFA) producing bacteria, which can modulate insulin sensitivity by reducing fatty acid flux (Tan et al. 2016). However, feed efficiency in piglet especially after weaning is very crucial, as feed accounts for approximately 70% of the total cost of production. In a study while piglets being divided into high and low feed efficiency based on residual feed intake (RFI), microbes associated with a leaner and healthier host (e.g., *Christensenellaceae*, *Oscillibacter*, and *Cellulosilyticum*) were enriched in low RFI (more feed-efficient) pigs. However, more feed-efficient piglets also had notably lower abundance of *Nocardiaceae* (*Rhodococcus*) in the ileum with higher ileal iso-butyric acid concentrations (McCormack et al. 2017). The potential relevance of higher feed efficiency includes positive feedback between certain microbes and mucin production, goblet cells along the villi, and upregulation of butyric acid production. Therefore, it has been suggested that the porcine intestinal microbiota could potentially be targeted to improve feed efficiency in piglet. This will increase profitability while also reducing the environmental impact of pig production.

7.5.2 Oxidative Stress, Longer Farrowing Duration and Stillbirth

In hyper prolific sows gut microbiota and their functions can influence stillbirth rate, while stillbirth rate and farrowing duration are correlated with the gut microbiota composition and oxidative stress status of sow (Wang et al. 2019a, b). The dramatic raise in the total born piglets in the modern swine production, with the introduction of hyper prolific sow line, increases the incidence of stillborn piglets with longer farrowing duration and reduced sow reproductive performances (Hasan et al. 2019a, b). Elevated oxidative stress reported to be associated with farrowing and lactation complication in the hyper prolific sow. Researches have outlined a significant correlation of oxidative stress status and the level of several genera in the intestinal flora of sow (Tan et al. 2016; Wang et al. 2018, 2019a, b). A study by Wang et al. (2018) reported that *Ruminococcaceae* and *Coprococcus* might prolong the farrowing process and increase the stillbirth rate by regulating oxidative stress status of sow. In another report by the same author it was found that the relative abundances of *Blautia*, *Coproccus_3*, *Lachnospiraceae_UCG_001*, *Marvinbryantia,* and *Ruminococcaceae_UCG-004* were negatively correlated with the total antioxidant capacity (T-AOC) concentrations of sows and positively correlated with the stillbirth rate of sow. The relative abundances of *Prevotellaceae_UCG-001* and *Ruminococcaceae_UCG-014* were correlated with the farrowing duration of sow (Wang et al. 2019a, b). Whereas, *Prevotellaceae_NK3B31_group* might increase the antioxidant capacity and reduce the stillbirth of sows (Wang et al. 2018). In post parturition, *Bacteroides* can help sow to cope up with oxidative stress by improving the plasma concentration of T-AOC, while *Phascolarctobacterium* by preventing the production of reactive oxygen species (ROS), an inflammatory by-product (Wang et al. 2018). Sow suffer from oxidative stress in late gestation and early lactation found with an elevated level of reactive oxygen species (ROS), 8-hydroxy-deoxyguanosine (8-OHdG), and thiobarbituric acid reactive substances (TBARS). Increase in the abundances of butyrate-producing bacteria, butyrate being the main energy sources for colonocytes but also an anti-inflammatory compound, can help in this case to reduce oxidative stress in sows. For example, an increase in levels of *Clostridium cluster* XI was negatively correlated with 8-OHdG, which was resulted from feeding sow with soluble fiber konjac flour (Tan et al. 2016).

7.5.3 Colostrum Yield and Colostrum Quality

Feeding sows with alternative additives may modulate their natural ability to improve the colostrum yield. In hyper prolific sow some of the factors negatively influence the colostrum yield and quality are longer farrowing duration and higher blood progesterone at farrowing (Hasan et al. 2019a, b). Pearson's correlation analysis revealed that high colostrum yield, high colostrum proteins, high colostrum IgG, low blood progesterone level, and lower farrowing duration were positively correlated with the abundance of the bacterial families *Lacotobacillaceae*,

Ruminococcaceae, *Acidaminococcaceae*, *Planctomycetaceae*, *Marinilabiliaceae*, *Veillonellaceae,* and *Prevotellaceae* (Hasan et al. 2018). Improvement in beneficial gut microbiota increases the microbial protein synthesis in sow intestine and significantly altered the amino acid profile of intestinal digesta (McCormack et al. 2017). However, the beneficial fibrolytic bacteria increase the production of SCFA, which can influence the plasma concentration of acetic acid, butyric acid, and total SCFA (Tan et al. 2016). Thus, it can be speculated that the gut microbiota may contribute to the host metabolism, hydrolyze the feed, and promote nutrition absorption, which could have led to the increased colostrum yield and colostrum functional components and resulted in the positive correlation.

7.6 Piglet Gut Microbiota Influence on Growth and Health

During fetal life, piglets are believed to be devoid of microbes until their birth, when they will encounter microbial population by the contact with mother's external mucosae and skin, and with the environment itself (Isaacson and Kim 2012; Pajarillo et al. 2014a, b, c). During this early phase after birth, the gut microbiota gradually shape toward adult like population (Fig. 7.2) and it is influenced by different factors like diet, use of antibiotics, probiotics, or prebiotics (Bian et al. 2016; Chae et al. 2016; Hasan et al. 2018).

Different studies found that the alpha diversity of piglet gut microbiota increased from birth to after weaning age. At the family level, relative abundances of Bacteroidaceae and Enterobacteriaceae decline from birth over time, while those of Lactobacillaceae, Ruminococcaceae, Veillonellaceae, and Prevotellaceae increase in weaned piglets (Kim et al. 2011; Pajarillo et al. 2014a, b, c; Frese et al. 2015). During early life, the shaping of the gut microbiota in piglets will affect also their health and growth. Hasan et al. (2018) found that piglets growing faster and larger in

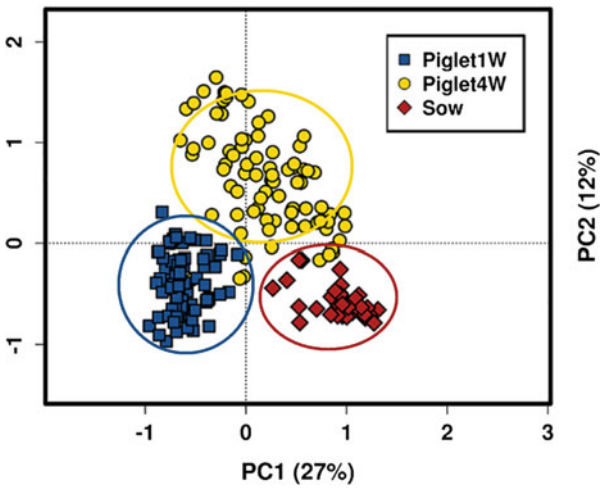

Fig. 7.2 Spatial description of gut microbiota of 37 sows and their piglets (1 and 4 weeks old). At 1 week of age, piglets have a very distinct gut microbiota population than their adult mothers, but already at 4 weeks of age the piglets' gut microbiota is switching toward adult sow alike population (adapted by Hasan et al. 2018, PlosOne)

size at 4 weeks of age had higher relative abundances of *Lactobacillus*, *Flavonifractor*, *Barnesiella*, *Gemmiger*, *Faecalibacterium*, *Roseburia* and *Anaerophaga* at 1 week of age. On the other hand, piglets growing more slowly and with poor average daily growth (ADG) hosted more *Desulfovibrio*, *Acidaminobacter*, *Dethiosulfatibacter*, *Fastiduisipila*, *Ruminnococcus,* and *Anaerotruncus* at 1 week of age. For instance, *Desulfovibrio* bacteria are responsible for inflammatory intestine syndrome in humans and animals, due to active hydrogen sulfide production, which has cytotoxic effects on the gut mucosa cells (Pitcher and Cummings 1996; Loubinoux et al. 2002). Hydrogen sulfite may act also through an inhibition of butyrate oxidation, the main energy source for colonocytes (Loubinoux et al. 2002). Impairing this energy function leads the intestinal epithelium cells to chronic inflammation and death (Loubinoux et al. 2002). Hasan et al. (2018) demonstrated that supplementation of a yeast hydrolysate to pregnancy diet in sows reduced the amount of *Desulfovibrio* bacteria in their feces. Zhang et al. (2016) showed that a diet rich in alfalfa during lactation period decreased the abundance of the pathogen known *Streptococcus suis* in feces of nursing piglets. In addition, the diet containing alfalfa increased the abundance of *Coprococcus eutactus*, a butyrate-producing microbe. Volatile fatty acids like acetate have been shown to be an anti-inflammatory metabolite maintaining gut homeostasis (Fukuda et al. 2011). Moreover, butyrate is beneficial for gut mucosal immunity and barrier function (Kelly et al. 2015). Suckling piglet showing diarrhea during lactation had lower abundance of Prevotellaceae, Lachnospiraceae, Ruminococcaceae, and Lactobacillaceae compared to healthy piglet of the same litter (Dou et al. 2017). Petri et al. (2010) found that colonization of the intestine by Lactobacillaceae species begins at 3 days of age and remaining the dominant group up to 20 days of age. Healthy piglets showed a steady decrease in *Lactobacillus* and *Escherichia*, as well as a gradual increase in *Prevotella* in the period from nursing to transition to solid food, therefore an altered relationship between *Prevotella* and *Escherichia* may be the main cause of diarrhea in pre-weaned piglets (Yang et al. 2019). The same authors indicate that a reduced number of *Bacteroides*, *Ruminococcus*, *Bulleidia*, and *Treponema*, which are responsible for the digestion and utilization of solid feeds, may be related to the onset of piglet diarrhea in the post-weaning phase. Recent findings report that high abundances of *Selenomonas* and *Moraxella* in ileum, and of *Lactobacillus* in both cecum and colon, were correlated with high weight gain in pre-weaned piglets (Ding et al. 2019). In conclusion, it is evident from many researches that different types of gut microbiota are correlated with piglets' growth. This correlation can be exploited because of the ability of certain gut microbiota to modulate intestinal homeostasis, nutrients digestion and absorption, production of energy sources from indigestible compounds (fiber), and ultimately protect gut mucosa from inflammatory processes. The focus of future research should be to identify specific microbiota correlated with health and growth, at different stages of the piglets' productive life. Subsequently, implement methods to improve the gut colonization of this particular microbiota, either with diet modulation or management and environmental conditions or by passive administration in form of pre- or probiotics.

References

Algers B, Uvnäs-Moberg K (2007) Maternal behavior in pigs. Horm Behav 52:78–85

Baker AA, Davis E, Spencer JD, Moser R, Rehberger T (2013) The effect of a bacillus-based directed microbial supplemented to sows on the gastrointestinal microbiota of their neonatal piglets. J Anim Sci 91:3390–3399. https://doi.org/10.2527/jas.2012-5821

Bian G, Ma S, Zhu Z, Su Y, Zoetendal EG, Mackie R, Lui J, Mu C, Huang R, Smidt H, Zhu W (2016) Age, introduction of solid feed and weaning are more important determinants of gut bacterial succession in piglets than breed and nursing mother as revealed by a reciprocal cross-fostering model. Environ Microbiol 18:1566–1577

Burkey TE, Dritz SS, Nietfeld JC, Johnson BJ, Minton JE (2004) Effect of dietary mannanoligosaccharide and sodium chlorate on the growth performance, acutephase response, and bacterial shedding of weaned pigs challenged with serotype typhimurium. J Anim Sci 82: 397–404

Cammarota G, Ianiro G, Gasbarrini A (2014) Fecal microbiota transplantation for the treatment of Clostridium difficile infection: a systematic review. J Clin Gastroenterol 48:693–702. https://doi.org/10.1097/MCG.0000000000000046

Castillo M, Martin-Orue SM, Taylor-Pickard JA, Perez JF, Gasa J (2008) Use of mannan-oligosaccharides and zinc chelate as growth promoters and diarrhea preventative in weaning pigs: effects on microbiota and gut function1. J Anim Sci 86:94

Chae JP, Pajarillo EA, Oh JK, Kim H, Kang DK (2016) Revealing the combined effects of lactulose and probiotic enterococci on the swine faecal microbiota using 454 pyrosequencing. Microb Biotechnol 9:486–495

Chen W, Mi J, Lv N, Gao J, Cheng J, Wu R, Ma J, Lan T, Liao X (2018) Lactation stage-dependency of the sow milk microbiota. Front Microbiol 9:945. https://doi.org/10.3389/fmicb.2018.00945

Chen X, Xu J, Ren E, Su Y, Zhu W (2017) Co-occurrence of early gut colonization in neonatal piglets with microbiota in the maternal and surrounding delivery environments. Anaerobe 49:30–40. https://doi.org/10.1016/j.anaerobe.2017.12.002

Ding X, Lan W, Liu G, Ni H, Gu J (2019) Exploring possible associations of the intestine bacterial microbiome with the pre-weaned weight gaining performance of piglets in intensive pig production. Sci Rep 9:15534. https://doi.org/10.1038/s41598-019-52045-4

Dou S, Gadonna-Widehem P, Rome V, Hamoudi D, Rhazi L, Lakhal L, Larcher T, Bahi-Jaber N, Pinon-Quintana A, Guyonvarch A, Huërou-Luron IL, Abdennebi-Najar L (2017) Characterisation of early-life fecal microbiota in susceptible and healthy pigs to post-weaning diarrhoea. PLoS One 12:e0169851. https://doi.org/10.1371/journal.pone.0169851

Duncan SH, Louis P, Flint HJ (2004) Lactate-utilizing bacteria, isolated from human feces, that produce butyrate as a major fermentation product. Appl Environ Microb 70:5810–5817

Engels C, Ruscheweyh HJ, Beerenwinkel N, Lacroix C, Schwab C (2016) The common gut microbe *Eubacterium hallii* also contributes to intestinal propionate formation. Front Microbiol 7:713

Everaert N, Van Cruchten S, Weström B, Bailey M, Van Ginneken C, Thymann T, Pieper R (2017) A review on early gut maturation and colonization in pigs, including biological and dietary factors affecting gut homeostasis. Anim Feed Sci Technol 233:89–103. https://doi.org/10.1016/j.anifeedsci.2017.06.011

Fan P, Liu P, Song P, Chen X, Ma X (2017) Moderate dietary protein restriction alters the composition of gut microbiota and improves ileal barrier function in adult pig model. Sci Rep 7:43412. https://doi.org/10.1038/srep43412

Feng ZM, Li TJ, Wu L, Xiao DF, Blachier F, Yin YL (2015) Monosodium L-glutamate and dietary fat differently modify the composition of the intestinal microbiota in growing pigs. Obes Facts 8:87–100

Fouhse JM, Zijlstra RT, Willing BP (2016) The role of gut microbiota in the health and disease of pigs. Anim Front 6:30–36. https://doi.org/10.2527/af.2016-0031

Frese SA, Parker K, Calvert CC, Mills DA (2015) Diet shapes the gut microbiome of pigs during nursing and weaning. Microbiome 3:28

Fukuda S, Toh H, Hase K, Oshima K, Nakanishi Y, Yoshimura K, Tobe T, Clarke JM, Topping DL, Suzuki T, Taylor TD, Itoh K, Kikuchi J, Morita H, Hattori M, Ohno H (2011) Bifidobacteria can protect from enteropathogenic infection through production of acetate. Nature 469:543–547

Gaskins HR, Collier CT, Anderson DB (2002) Antibiotics as growth promotants: mode of action. Anim Biotechnol 3:29–42

Guevarra RB, Lee JH, Lee SH et al (2019) Piglet gut microbial shifts early in life: causes and effects. J Anim Sci Biotechnol 10:1. https://doi.org/10.1186/s40104-018-0308-3

Han YW (2015) Fusobacterium nucleatum: a commensal-turned pathogen. Curr Opin Microbiol 23:141–147. https://doi.org/10.1016/j.mib.2014.11.013

Hasan S, Junnikkala S, Peltoniemi O, Paulin L, Lyyski A, Vuorenmaa J, Oliviero C (2018) Dietary supplementation with yeast hydrolysate in pregnancy influences colostrum yield and gut microbiota of sows and piglets after birth. PLoS One 13:e0197586. https://doi.org/10.1371/journal.pone.0197586

Hasan S, Peltoniemi O, Vuorenmaa J, Oliviero C (2019a) Use of antibiotics in early age can affect the gut microbiota composition and diversity in one-week age piglets. ESPHM Conference Abstract, 193

Hasan S, Saha S, Junnikkala S, Orro T, Peltoniemi O, Oliviero C (2019b) Late gestation diet supplementation of resin acid-enriched composition increases sow colostrum immunoglobulin G content, piglet colostrum intake and improve sow gut microbiota. Animal 13:1599–1606

Hunt KM, Foster JA, Forney LJ, Schütte UM, Beck DL, Abdo Z, Fox LK, Williams JE, McGuire MK, McGuire MA (2011) Characterization of the diversity and temporal stability of bacterial communities in human milk. PLoS One 6:e21313. https://doi.org/10.1371/journal.pone.0021313

Hurley WL (2015) Composition of sow colostrum and milk. In: Farmer C (ed) The gestating and lactating sow. Wageningen Academic, Wageningen, pp 193–229

Isaacson R, Kim HB (2012) The intestinal microbiome of the pig. Anim Health Res Rev 3:100–109

Ji YJ, Li H, Xie PF, Li ZH, Li HW, Yin YL, Blachier F, Kong XF (2019) Stages of pregnancy and weaning influence the gut microbiota diversity and function in sows. J Appl Microbiol 127:867–879. https://doi.org/10.1111/jam.14344

Jost T, Lacroix C, Braegger C, Chassard C (2013) Assessment of bacterial diversity in breast milk using culture-dependent and culture-independent approaches. Br J Nutr 110:1253–1262. https://doi.org/10.1017/S0007114513000597

Jost T, Lacroix C, Braegger C, Chassard C (2014) Stability of the maternal gut microbiota during late pregnancy and early lactation. Curr Microbiol 68:419–427

Kelly CJ, Zheng L, Campbell EL, Saeedi B, Scholz CC, Bayless AJ, Wilson KE, Glover LE, Kominsky DJ, Magnuson A, Weir TL, Ehrentraut SF, Pickel C, Kuhn KA, Lanis JM, Nguyen V, Taylor CT, Colgan SP (2015) Crosstalk between microbiota-derived short-chain fatty acids and intestinal epithelial HIF augments tissue barrier function. Cell Host Microbe 17:662–671

Kettunen H, Eerden E, Lipiński K, Rinttilä T, Valkonen E, Vuorenmaa J (2017) Dietary resin acid composition as a performance enhancer for broiler chickens. J Appl Anim Nutr 5:e3

Kim HB, Isaacson RE (2015) The pig gut microbial diversity: understanding the pig gut microbial ecology through the next generation high throughput sequencing. Vet Microbiol 177:242–251

Kim HB, Borewicz K, White BA, Singer RS, Sreevatsan S, Tu ZJ, Isaacson RE (2011) Longitudinal investigation of the age-related bacterial diversity in the feces of commercial pigs. Vet Microbiol 153:124–133

Kim HB, Borewicz K, White BA, Singer RS, Sreevatsan S, Tu ZJ, Isaacson RE (2012) Microbial shifts in the swine distal gut in response to the treatment with antimicrobial growth promoter, tylosin. Proc Natl Acad Sci U S A 109:15485–15490. https://doi.org/10.1073/pnas.1205147109

Kubasova T, Davidova-Gerzova L, Merlot E, Medvecky M, Polansky MO, Gardan-Salmon D, Quesnel H, Rychlik I (2017) Housing systems influence gut microbiota composition of sows but not of their piglets. PLoS One 12:e0170051. https://doi.org/10.1371/journal.pone.0170051

Mach N, Berri M, Estellé J, Levenez F, Lemonnier G, Denis C, Leplat JJ, Chevaleyre C, Billon Y, Doré J, Rogel-Gaillard C, Lepage P (2015) Early-life establishment of the swine gut microbiome and impact on host phenotypes. Environ Microbiol Rep 7:554–569. https://doi.org/10.1111/1758-2229.12285

Lallès JP, Bosi P, Janczyk P, Koopmans SJ, Torrallardona D (2009) Impact of bioactive substances on the gastrointestinal tract and performance of weaned piglets: a review. Animal 12:1625–1643

Le Dividich J, Rooke JA, Herpin P (2005) Nutritional and immunological importance of colostrum for the new-born pig. J Agric Sci 143:469–485

Li H, Liu Z, Lyu H, Gu X, Song Z, He X, Fan Z (2020) Effects of dietary inulin during late gestation on sow physiology, farrowing duration and piglet performance. Anim Reprod Sci 219:106531

Liu P, Piao XS, Kim SW, Wang L, Shen YB, Lee HS, Li SY (2008) Effects of chito-oligosaccharide supplementation on the growth performance, nutrient digestibility, intestinal morphology, and fecal shedding of and in weaning pigs. J Anim Sci 86:2609–2618

Looft T, Johnson TA, Allen HK, Bayles DO, Alt DP, Stedtfeld RD, Sul WJ, Stedtfeld TM, Chai B, Cole JR, Hashsham SA, Tiedje JM, Stanton TB (2012) In-feed antibiotic effects on the swine intestinal microbiome. Proc Natl Acad Sci 109:1691–1696. https://doi.org/10.1073/pnas.1120238109

Loubinoux J, Bronowicki J, Pereira IA, Mougenel J, Le Faou AE (2002) Sulfate-reducing bacteria in human feces and their association with inflammatory bowel diseases. FEMS Microbiol Ecol 40:107–112. https://doi.org/10.1111/j.1574-6941.2002.tb00942.x

McCormack UM, Curião T, Buzoianu SG, Prieto ML, Ryan T, Varley P, Crispie F, Magowan E, Metzler-Zebeli BU, Berry D, O'Sullivan O, Cotter PD, Gardiner GE, Lawlor PG (2017) Exploring a possible link between the intestinal microbiota and feed efficiency in pigs. Appl Environ Microbiol 83:e00380–e00317. https://doi.org/10.1128/AEM.00380-17

Menegat MB, JM DR, Woodworth JC, Dritz SS, Tokach MD, Goodband RD (2019) Effects of Bacillus subtilis C-3102 on sow and progeny performance, fecal consistency, and fecal microbes during gestation, lactation, and nursery periods. J Anim Sci 97:3920–3937. https://doi.org/10.1093/jas/skz236

Morissette B, Talbot G, Beaulieu C, Lessard M (2018) Growth performance of piglets during the first two weeks of lactation affects the development of the intestinal microbiota. J Anim Physiol Anim Nutr 102:525–532. https://doi.org/10.1111/jpn.12784

Mosnier E, Le Floc'h N, Etienne M, Ramaekers P, Seve B, Pere MC (2010) Reduced feed intake of lactating primiparous sows is associated with increased insulin resistance during the peripartum period and is not modified through supplementation with dietary tryptophan. J Anim Sci 88:612–625

Niu Q, Li P, Hao S, Kim SW, Du T, Hua J, Huang R (2019) Characteristics of gut microbiota in sows and their relationship with apparent nutrient digestibility. Int J Mol Sci 20:870

Pajarillo EA, Chae J, Balolong MP, Kim HB, Kang D (2014a) Assessment of fecal bacterial diversity among healthy piglets during the weaning transition. J Gen Appl Microbiol 60:140–146

Pajarillo EA, Chae JP, Balolong MP, Bum Kim H, Kang DK (2014b) Assessment of fecal bacterial diversity among healthy piglets during the weaning transition. J Gen Appl Microbiol 60:140–146

Pajarillo EA, Chae JP, Balolong MP, Kim HB, Seo KS, Kang DK (2014c) Pyrosequencing-based analysis of fecal microbial communities in three purebred pig lines. J Microbiol 52:646–651

Petri D, Hill JE, Van Kessel AG (2010) Microbial succession in the gastrointestinal tract (GIT) of the preweaned pig. Livest Sci 1–3:107–109. https://doi.org/10.1016/j.livsci.2010.06.037

Pitcher MC, Cummings JH (1996) Hydrogen sulphide: a bacterial toxin in ulcerative colitis? Gut 39:1–4

Quesnel H, Farmer C, Devillers N (2012) Colostrum intake: influence on piglet performance and factors of variation. Livest Sci 146:105–114

Rhouma M, Fairbrother JM, Beaudry F, Letellier A (2017) Post weaning diarrhea in pigs: risk factors and non-colistin-based control strategies. Acta Vet Scand 59:31. https://doi.org/10.1186/s13028-017-0299-7

Riboulet-Bisson E, Sturme MH, Jeffery IB, O'Donnell MM, Neville BA, Forde BM, Claesson MJ, Harris H, Gardiner GE, Casey PG, Lawlor PG, O'Toole PW, Ross RP (2012) Effect of *Lactobacillus* salivarius bacteriocin Abp118 on the mouse and pig intestinal microbiota. PLoS One 7:e31113

Rodríguez JM (2014) The origin of human milk bacteria: is there a bacterial entero-mammary pathway during late pregnancy and lactation? Adv Nutr 5(6):779–784. https://doi.org/10.3945/an.114.007229

Rooke JA, Bland IM (2002) The acquisition of passive immunity in the new-born piglet. Livest Prod Sci 78:13–23

Salmon H, Berri M, Gerdts V, Meurens F (2009) Humoral and cellular factors of maternal immunity in swine. Dev Comp Immunol 33:384–393. https://doi.org/10.1016/j.dci.2008.07.007

Sassone-Corsi M, Raffatellu M (2015) No vacancy: how beneficial microbes cooperate with immunity to provide colonization resistance to pathogens. J Immunol 194:4081–4087

Silva MLF, Lima JAF, Cantarelli VS, Amaral NO, Zangerônimo MG, Fialho ET (2010) Probiotics and antibiotics as additives for sows and piglets during nursery phase. Rev Bras Zootec 39:2453–2459. https://doi.org/10.1590/S1516-35982010001100019

Starke IC, Pieper R, Neumann K, Zentek KJ, Vahjen W (2013) Individual responses of mother sows to a probiotic enterococcus faecium strain lead to different microbiota composition in their offspring. Benef Microbes 4:345–356. https://doi.org/10.3920/BM2013.0021

Stokes CR (2017) The development and role of microbial-host interactions in gut mucosal immune development. J Anim Sci Biotechnol 8:12

Tan C, Wei H, Ao J, Long G, Peng J (2016) Inclusion of Konjac flour in the gestation diet changes the gut microbiota, alleviates oxidative stress, and improves insulin sensitivity in sows. Appl Environ Microbiol 82:5899–5909

Thompson CL, Wang B, Holmes AJ (2008) The immediate environment during postnatal development has long-term impact on gut community structure in pigs. ISME J 2:739–748. https://doi.org/10.1093/jas/skz236

Turfkruyer M, Verhasselt V (2015) Breast milk and its impact on maturation of the neonatal immune system. Curr Opin Infect Dis 28:199–206. https://doi.org/10.1097/QCO.0000000000000165

Turnbaugh PJ, Hamady M, Yatsunenko T, Cantarel BL, Duncan A, Ley RE, Sogin ML, Jones WJ, Roe BA, Affourtit JP, Egholm M, Henrissat B, Heath AC, Knight R, Gordon JI (2009) A core gut microbiome in obese and lean twins. Nature 457:480–484. https://doi.org/10.1038/nature07540

Urbaniak C, Angelini M, Gloor GB, Reid G (2016) Human milk microbiota profiles in relation to birthing method, gestation and infant gender. Microbiome 4:1. https://doi.org/10.1186/s40168-015-0145-y

Vallet J, Miles J, Rempel L (2013) A simple novel measure of passive transfer of maternal immunoglobulin is predictive of preweaning mortality in piglets. Vet J 195:91–97

Vienola K, Jurgens G, Vuorenmaa J, Apajalahti J (2018) Tall oil fatty acid inclusion in the diet improves performance and increases ileal density of lactobacilli in broiler chickens. Br Poult Sci 59:349–355

Wang H, Yongcheng J, Cong Y, Ming D, Tianyue T, Baichuan D, Wenkai R, Jinping D, Yulong Y, Chengqua T (2018) Differential analysis of gut microbiota correlated with oxidative stress in sows with high or low litter performance during lactation. Front Microbiol 9:1665. https://doi.org/10.3389/fmicb.2018.01665

Wang H, Hu C, Cheng C, Cui J, Ji Y, Hao X, Li Q, Ren W, Deng B, Yin Y, Deng J, Tan C (2019a) Unraveling the association of fecal microbiota and oxidative stress with stillbirth rate of sows. Theriogenology 136:131–137

Wang H, Hu C, Cheng C, Cui J, Ji Y, Hao X, Li Q, Ren W, Deng B, Yin Y, Deng J, Tan C (2019b) Unraveling the association of fecal microbiota and oxidative stress with stillbirth rate of sows. Theriogenology 36:131–137

White LA, Newman MC, Cromwell GL, Lindemann MD (2002) Brewers dried yeast as a source of mannan oligosaccharides for weanling pigs. J Anim Sci 80:2619–2628

Xu C, Peng J, Zhang X, Peng J (2020) Inclusion of soluble fiber in the gestation diet changes the gut microbiota, affects plasma propionate and odd-chain fatty acids levels, and improves insulin sensitivity in sows. Int J Mol Sci 21:635

Yang H, Huang X, Fang S, He M, Zhao Y, Wu Z et al (2017) Unraveling the fecal microbiota and metagenomic functional capacity associated with feed efficiency in pigs. Front Microbiol 8: 1555

Yang Q, Huang X, Wang P, Yan Z, Sun W, Zhao S, Gun S (2019) Longitudinal development of the gut microbiota in healthy and diarrheic piglets induced by age-related dietary changes. Microbiology 8:e923. https://doi.org/10.1002/mbo3.923

Zhang L, Mu C, He X, Su Y, Mao S, Zhang J, Smidt H, Zhu W (2016) Effects of dietary fibre source on microbiota composition in the large intestine of suckling piglets. FEMS Microbiol Lett 363: 1–6

Porcine Gut Microbiota and Host Interactions During the Transition from the Suckling to Postweaning Phase

8

Barbara U. Metzler-Zebeli

Abstract

Gut maturation in piglets is a very dynamic process, orchestrated by genetic programming and by maternal, environmental, and microbial factors. This chapter summarizes the current knowledge of the developing gut microbiota and host mucosal mechanisms for recognition and control of microbial activity from birth to weaning. Microbial colonization of the porcine gut and the microbe-host dialog commences immediately after birth. This development is interrupted at weaning, often leaving the piglet vulnerable to gut dysbiosis and inflammation. While research interest focused on the gastrointestinal microbiota and function especially in the postweaning period in the past, the importance of the suckling phase for intestinal priming and the first build-up of immune tolerance toward the commensal microbiota is more and more recognized. Despite early gut training with creep feeding, the abrupt loss of specific bioactive compounds in sow milk may be critical for the disturbed microbe-host dialogue postweaning. Nevertheless, knowledge on the evolution of host-related microbial recognition in the neonatal phase is still in its infancy. Advances in optimized gut health may be expected by dietary interventions that mitigate the abrupt microbial changes by mimicking the natural weaning situation via prolonged feeding of bioactive porcine milk compounds postweaning.

Keywords

Fermentation · Gut microbiota development · Receptor-mediated recognition · Mucosal signaling · Weaning · Sow milk

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Abbreviations

ETEC	Enterotoxigenic *Escherichia coli*
FXR	Farnesoid X receptor
GALT	Gut-associated lymphoid tissue
GIT	Gastrointestinal tract
GLP-1	Glucagon-like peptide-1
GPR	G-protein receptors
Ig	Immunoglobulin
LPS	Lipopolysaccharide
MAMP	Microbiota-associated molecular pattern
MCT	Monocarboxylate transporter
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NLR	Nucleotide oligomerization domain like receptors
PRR	Pattern-recognition receptors
rRNA	Ribosomal RNA
SCFA	Short-chain fatty acids
TGR5	G-protein coupled bile acid receptor 1
TLR	Toll-like receptor

8.1 Introduction

The gastrointestinal maturation in newborns is a specific and very dynamic process. It is distinctly influenced by intrinsic (i.e., genotype) and extrinsic factors including postnatal nutrition and the developing gut microbiota. As a consequence, the maturing gut epithelium is continuously exposed to a constantly changing gut microbial composition as well as to alterations in nutritional, environmental, and physiological factors during the first weeks of life (Willing and Van Kessel 2010; Melo et al. 2016; Guevarra et al. 2019). Colonization of the porcine gut microbiota commences after birth and is directed by components in sow milk and environmental factors; however, this development is interrupted at weaning, rendering the piglet vulnerable to enteric disease. Although the dietary composition has been one of the main targets to prevent intestinal dysbiosis and foster gut homeostasis after weaning, explaining dietary effects on mucosal functioning via shifts in the gut microbiota-host mucosal dialog is still in its early stages (e.g., Frese et al. 2015; Mach et al. 2015; Schocker et al. 2015a, 2018). Research and commercial interests in pig nutrition focus on the gastrointestinal microbiota and functionality, especially focusing on the period postweaning (Lauridsen 2020). However, the critical role of the suckling phase for intestinal priming including digestive, barrier and immune functions, development of a stable microbiota and the first build-up of an immune tolerance toward the commensal microbiota is more and more recognized (Gomez de Agüero et al. 2016; Schokker et al. 2015b, 2018). Moreover, evidence emerges

about the priming role of the transfer of gut microbes from the mother sow for the bacterial community development in the neonatal gastrointestinal tract (GIT). This can be regarded as further critical stabilization factor for the gut homeostasis in suckling piglets as preparation to weaning (Paßlack et al. 2015). With this advancing understanding of the critical role in the first priming of the gut by the intestinal microbes, the aim of this literature review is to summarize the current knowledge of the maturing gut microbiota from birth to weaning and their interaction with the host, thereby addressing microbial modes of action and host mucosal mechanisms for recognition and control of microbial activity.

8.2 Gut Health in Piglets at Weaning

A conglomeration of factors contributes to gut homeostasis in suckling and weaned piglets, including effective digestion and absorption of food, host metabolism and energy generation, the absence of gut disorders, microbiota load and diversity, effective immune status including chemical and physical barriers and a general state of well-being (Celi et al. 2017; Broom and Kogut; 2018; Pluske et al. 2018). From this angle, measuring gut health in the young piglet seems to be a feasible task. However, the setting of specific limits for single parameters for categorization as healthy is often hard to accomplish. This is also true for older weaned pigs when maturational changes in the gut microbiota and gut physiology still continue but slowly diminish compared to the preweaning period (Guevarra et al. 2019; Metzler-Zebeli et al. 2020). The challenge begins with the definition of the normal composition of the porcine gut microbiome as one major criterion for gut health due to the many factors, influencing its composition pre- and postweaning (Kim and Isaacson 2015; Guevarra et al. 2019). Due to spatial and inter-individual variations, there is more than "one" healthy gut microbiota, both at taxonomic and metabolic level, being largely influenced by nutrition (i.e., prenatal nutrition of the sow, sow colostrum and milk intake, milk replacer, and access to creep feed) and feed intake level, segmental stage of digestion and absorption, medication, genetics, environmental microbes (e.g., farm, pen) and stress (hypothalamus-pituitary-adrenal-axis). In addition, each individual has a unique microbiome, challenging the concept of a ubiquitous porcine gut microbiome. Therefore, it is legitimate to ask whether it is still valid to aim to define the "one" healthy gut microbiota composition around weaning.

If a stable immune system, a functioning mucosal barrier and a balanced gut microbial ecosystem is considered as the primary basis for a homeostatic condition in the gut (Xie et al. 2013), it is obvious that these aspects are either immature or de-stabilized due to the changes and stress experienced by the piglets at weaning. The newly weaned pig is faced with a radical change in diet, environmental and social stressors, and an acquired immune system that is in the early stages of development. Due to this, the immediate postweaning period still represents a critical time point in the health, welfare and development of the young pig, causing considerable economic losses in pig production (Bauer et al. 2011; Radcliffe 2011; Celi et al. 2017; Pluske et al. 2018). The piglet often responds to the weaning process

with reduced feed intake and a lag of growth. Irrespective of any dietary measure used to promote postweaning health and gut homeostasis, it is absolutely critical that the piglet continues to eat after the separation from the sow at weaning (Bauer et al. 2011). Therefore, all strategies around weaning including nutritional and management strategies need to focus on maintaining a proper feed intake level. This involves the palatability and consistence of food but also social factors, like the dam calling her offspring for "mealtime" and "instructing" them to eat solid feed in the late suckling period. More or less abrupt, depending on the farm management, the diet shifts from liquid to solid and from milk-based to plant-based feed at weaning, with lower palatability and digestibility, also requiring different intestinal enzyme activities than milk (Heo et al. 2013). Hence, a high-quality, complex creep feed that matches the milk-oriented immature digestive system of the young piglet has been proposed as a "gut training and preparation" strategy for a successful weaning transition. Contrastingly, the composition of creep feed and (pre-)starter diets is mostly based on macro-nutrients, thereby considering economic aspects, while missing components with functional properties in sow milk, such as fatty acids, bioactive peptides, biogenic amines, and oligosaccharides which play critical roles in gut development (Salcedo et al. 2016; Zhang et al. 2018; Mu et al. 2019; Lauridsen 2020). Therefore, despite creep feeding and the early introduction of plant-based feed, the lack of sow milk and thus of bioactive sow milk components (which influence gut maturation and function and control mucosal immune responses directly or indirectly by stimulating the development of the species-specific gut microbiota (Salcedo et al. 2016)) may be a major factor in the gut distress observed postweaning. It may be argued that some piglets do not eat solid feed preweaning, especially those piglets drinking from the first teats, as the cause for the very drastic alterations in the gut homeostasis. However, research evidence indicates certain benefits for gut functioning postweaning when supplementing short-chain fructooligosaccharides or polygenic amines, both representing bioactive compounds in sow milk, during the suckling and/or postweaning period (e.g., Rao et al. 2006; Boudry et al. 2017; Schokker et al. 2018). As such, ingestion of short-chain fructooligosaccharides from the early suckling to postweaning period shows proved to be advantageous for gut development and structure after weaning (e.g., Boudry et al. 2017; Schokker et al. 2018). Also, polyamines are important regulators for postnatal cellular proliferation and differentiation and are necessary for normal integrity of the gut epithelium (Rao et al. 2006). The highest concentrations of polyamines, namely spermidine and spermine, in sow milk are found in week 7 of lactation (Kelly et al. 1991), which is 3 weeks after the weaning time point in many conventional farming systems worldwide. This, together with certain beneficial effects of supplementing polyamines immediately after weaning on epithelial restitution and barrier function (Wang et al. 2015), shows their importance for normal porcine gut maturation and homeostasis. Based on this reasoning, the lack of functional sow milk components from the fifth week of life may render some piglets more prone to develop gut disorders postweaning.

One of the major challenges in the early postweaning period of pigs is the degeneration of the gut epithelial structure as a result of the low feed intake, gut

inflammation, and psychological stressors in the first days after weaning (Heo et al. 2013). This additionally impacts the digestive and absorptive capacity, gut mucosal integrity and therefore animal health due to potential translocation of pathogens and toxins to the systemic circulation (Bauer et al. 2011). At the postweaning stage, the mucosal immune system is still immature, and when the piglet starts eating again, undigested material remains in the gut which provides good conditions for bacterial pathogens to proliferate (Heo et al. 2013). In this scenario, the early postweaning period is associated with an increased diarrhea incidence due to the intestinal proliferation and mucosal attachment of enteropathogenic *Escherichia coli* (Pié et al. 2004; Heo et al. 2013). A "leaky gut" allows the translocation of undigested food particles and bacterial toxins into the body, stimulating an elevated immune response. To alleviate the negative effects of weaning, it is important that nutrients are rapidly supplied to and efficiently absorbed by the GIT (Radcliffe 2011). Nutritional research aims therefore at enhancing feed intake, improving nutrient absorption and developing dietary strategies to reduce the opportunistic pathogen load in the gut in an attempt to reduce the negative impacts of weaning on gut health and development. In doing so, dietary feed additives with microbiota- and immune function-modulating capacity as well as the dietary protein and carbohydrate composition have been intensively studied (see reviews, e.g., Metzler et al. 2005; Pluske et al. 2018). The present advancements in molecular techniques thereby allow for an improved understanding of the mechanisms involved in enhancing feed intake, nutrient absorption, immune response, and gut microbial changes (Radcliffe 2011; Kim and Isaacson 2015; Guevarra et al. 2019).

8.3 Gut Microbiota Development During the Suckling Phase

The gut microbiota has evolved from having mostly a negative image as major risk factor for gut dysbiosis in weaned pigs to being considered a metabolic powerhouse that provides the functionally limited host with an extensive array of enzymes and substrates required for growth (Schokker et al. 2015b). Much research evidence is available for the taxonomic composition of the bacterial microbiota from 16S ribosomal RNA (rRNA) gene surveys, whereas information on successional changes in archaea, protozoa, and fungi from the suckling to postweaning period is still rudimentary (Guevarra et al. 2019; Summers et al. 2019). Moreover, little information on the functional potential of the gut microbiome in nursing pigs from birth through weaning and the early postweaning period exists (Frese et al. 2015). The taxonomic composition and metabolic activity of the gut microbiota per se is shaped by a number of complex internal and external factors, including differences in inter-region conditions, principally related to function, such as available substrates for growth, pH, redox potential, digesta transit time, mucus production and composition and host antimicrobial secretions, including antimicrobial peptides, defensins, and immunoglobulin A (IgA), as well as age and inter-individual variation (see reviews of Guevarra et al. 2019; Kogut 2019). Gut microbes influence each other due to competition for substrates and niches, determining their gut environment by

secondary bacterial metabolites, such as antimicrobial and quorum-sensing molecules and metabolic cross-feeding (Louis et al. 2007; Flint et al. 2015). However, these factors are themselves under development after birth, while the GIT undergoes a remarkable shift from a more or less germ-free state to be populated by an extremely dense and diverse microbial community (Liu et al. 2019; Ruczizka et al. 2019; Shrestha et al. 2020). Postweaning, piglet's gut microbiota drastically changes again, fully developing from a milk-oriented to a plant-oriented bacterial community (Frese et al. 2015; Guevarra et al. 2019; Shrestha et al. 2020). Any perturbation of the gut microbiota composition which is typical after weaning due to the drastic dietary and environmental changes will lead to an unstable gut homeostasis (Pluske et al. 2018). As a result, gut disorders are still the most common causes of morbidity and mortality and reduced performance in weaned pigs. Traditionally, the large intestines were regarded as the most important gastrointestinal segments with respect to the gut microbiota. Since feces allow multiple samplings of the same animal and are easily accessible, most research evidence for the suckling phase has been gained for the fecal microbiota (Frese et al. 2015; Guevarra et al. 2019; Summers et al. 2019; Wang et al. 2019; Arnaud et al. 2020; Shrestha et al. 2020).

The fecal bacterial microbiome of pigs seems to be relatively stable before weaning, when piglets are fed solely on sow milk, and re-gains stability in the 3 weeks postweaning; but the preweaning and postweaning communities are clearly distinguishable based on the respective diets (Frese et al. 2015; Shrestha et al. 2020). Findings for the porcine fecal microbiome from weaning to 6 months of age support that maturational changes in the fecal bacterial composition continue throughout the fattening period (Zhao et al. 2015; Wang et al. 2019; Metzler-Zebeli et al. 2020). In contrast to the bacterial microbiota showing increasing species richness and diversity during the 28-day suckling phase (e.g., Frese et al. 2015; Shrestha et al. 2020), first data for piglet's fecal mycobiome show a low number of different species but a relatively stable fungal community throughout the suckling period. Numbers and diversity of fungi in feces drastically increase postweaning, which was associated with the higher fungal load of the postweaning diet and the lack of sow milk components with potential antifungal properties (Summers et al. 2019; Arfken et al. 2020).

8.3.1 Bacteriome Development

Targeted molecular and culturing approaches used in the past demonstrated that the *Lactobacillus* and *Bifidobacterium* communities are drastically affected by the diet shift at weaning (e.g., Konstantinov et al. 2004), thereby opening niches for the growth of opportunistic enteropathogens, such as *Escherichia coli*. More recent work using Illumina MiSeq 16S rRNA gene and shotgun sequencing showed drastic alterations in more or less all dominant bacterial taxa from the nursery to the postweaning period (Frese et al. 2015; Mach et al. 2015; Salcedo et al. 2016; Mu et al. 2019; Wang et al. 2019; Arnaud et al. 2020; Shrestha et al. 2020). Typically, *Enterobacteriaceae* and *Bacteroidaceae* are high abundant in piglet feces before

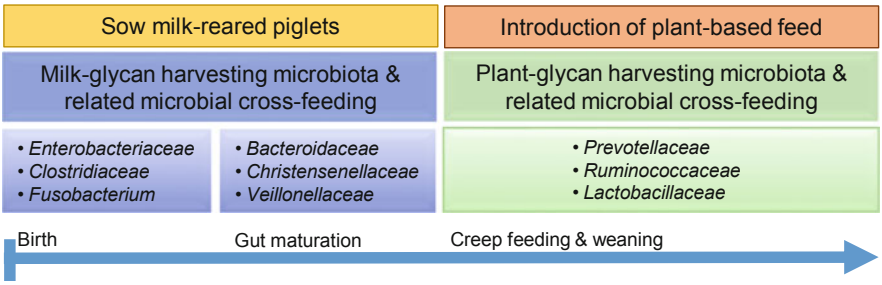

Fig. 8.1 Schematic of major bacterial taxa associated with the milk-glycan harvesting microbiota and taxa related to the plant-glycan harvesting microbiota after the introduction of plant-based feed detected in feces of suckling and weaned piglets

weaning and drop largely postweaning, whereas those of *Prevotellaceae*, *Lactobacillaceae,* and *Veillonellaceae* greatly increase from being low abundant in the suckling phase to becoming high abundant postweaning (Fig. 8.1; Frese et al. 2015). The very early gut colonizers, *Enterobacteriaceae* and *Clostridiaceae,* are characterized by their tolerance toward the aerobic condition in the neonatal gut (Ruczizka et al. 2019; Shrestha et al. 2020). *Enterobacteriaceae* lower the redox potential, thereby enabling growth of the first strict anaerobes, like the genus *Clostridium,* which often precedes other anaerobic bacteria, e.g. *Bacteroides* (Bezirtzoglou 1997). While their numbers decline in the following days, anaerobic *Fusobacterium* and *Bacteroides* predominate until about 2 weeks of life, whereas *Ruminococcaceae*, *Veillonellaceae*, and *Christensenellaceae* only appear in higher abundances in the later preweaning phase (Frese et al. 2015; Ruczizka et al. 2019; Arfken et al. 2020; Shrestha et al. 2020). In general, it is critical for the maturational successions in the taxonomic and functional composition whether the piglet is reared on sow milk alone or has access to milk replacer, creep or sow feed and other organic material (e.g., hay or straw) in their environment. Due to these and other host-related factors (e.g., genetic lines, mucosal glycosylation patterns), differences in the maturational bacterial successions and presence and dominance of species will always be present among studies. With the introduction of plant-based feed, bacterial genera increase that only would appear in higher numbers postweaning, such as *Prevotellaceae*, *Ruminococcaceae,* and *Lactobacillaceae* (Bian et al. 2016; Ruczizka et al. 2019; Wang et al. 2019; Arfken et al. 2020). Postweaning, the latter bacteria outnumber bacterial taxa whose functional abilities reflect their focus on the consumption of milk (Frese et al. 2015; Shrestha et al. 2020). Especially *Prevotella* has been associated with plant polysaccharide consumption; a genus that will remain dominant throughout the fattening period (Guevarra et al. 2019; Metzler-Zebeli et al. 2020). As soon as the appropriate substrate is lacking postweaning, *Prevotella* appears to supplant milk glycan-harvesting *Bacteroides* populations (Frese et al. 2015; Mach et al. 2015; Arfken et al. 2020; Shrestha et al. 2020). This typifies the dramatic taxonomic shifts associated with weaning which are also reflected in the glycan-degrading metagenome of the nursery and postweaning bacterial

communities (Frese et al. 2015; Mudd et al. 2016; Salcedo et al. 2016; Zhang et al. 2018). In this respect, porcine milk oligosaccharides are composed primarily of N-acetylglucosamine, sialic acids (N-acetylneuraminic or N-glycolneuraminic acid), galactose and glucose monomers, and less abundantly, fucose (Mudd et al. 2016; Salcedo et al. 2016; Zhang et al. 2018), thereby shaping the gut microbiota to a "milk-oriented microbiome" which contributes to neonatal health (Zivkovic et al. 2011, Frese et al. 2015) and being an underlying mechanism for the development of species-specific gut microbiota (Salcedo et al. 2016). The number of oligosaccharides reported for porcine milk ranges from 22 to 33 (Tao et al. 2010; Albrecht et al. 2014; Salcedo et al. 2016; Wei et al. 2018; Mu et al. 2019). Some authors even reported about 90 different oligosaccharides in sow milk (Zhang et al. 2018). The microbial metagenome of milk-fed piglets is enriched by predicted enzymes active on milk-derived glycans, which are otherwise indigestible to the host animal (Frese et al. 2015; Salcedo et al. 2016). With the abrupt removal of sow milk at weaning, it is clear that this sialic acid-consuming microbiota is disadvantaged. This disadvantage also occurs when suckling piglets are removed from the sow and reared on milk replacer alone. Although bovine whey permeate, which is rich in milk oligosaccharides (Quinn et al. 2020), is a typical component in porcine milk replacers, sow milk glycans differ in their composition to other species including bovine milk (Albrecht et al. 2014; Salcedo et al. 2016). The importance of sow milk for microbial development is underlined by findings showing that formula-induced changes in the developing microbiota were less pronounced in piglets that could still suckle sow milk (Wang et al. 2019). Future research needs to elucidate whether piglets with no access to sow milk during the suckling phase are more prone for gut disorders due to an abnormal (taxonomic or functional) development of the gut microbiota and gut microbe–host interaction. In fact, piglets receiving milk replacer during the suckling phase were reported to be at greater risk for intestinal colonization with enterotoxigenic *Escherichia coli* compared to piglets that suckle sow milk continuously (Sugiharto et al. 2015). Low milk intake and hence of bioactive milk compounds may be also relevant for large litters with great variation in within-litter birth weights and sow colostrum and milk intake, where the undersized piglets are removed from the sow to be raised on milk replacer, to improve piglet survival (de Vos et al. 2014).

Even if functional capacities do seemingly not change from pre- to postweaning, carbohydrate preferences and cross-feeding dependencies likely offer competitive advantages for potential pathogens in the ecosystem after weaning (Ng et al. 2013; Salcedo et al. 2016). As such, the fucose degradation capacities were not very drastically changed from before to after weaning; however, the identity of fucose-consuming taxa changed (Frese et al. 2015; Salcedo et al. 2016). More than 50% of reads predicted to encode a fucose permease belonged to *Enterobacteriaceae* preweaning but decreased to only 0.6% after weaning in the study of Salcedo et al. (2016). *Enterobacteriaceae* encode genes related to the consumption of free fucose but lack the enzymatic capacity to liberate these monomers from milk glycans, which points toward microbial cross-feeding. As a consequence of these adaptations, temporal shifts in the fecal microbiota structure and stability occur

throughout the immediate postweaning period, including significant shifts in the relative levels of specific bacterial phylotypes until the microbiota stabilizes again (e.g., Yang et al. 2015; Pollock et al. 2018). Since the composition of bioactive milk compounds are species-specific, addition of bovine milk products to (pre-)starter diets, such as whey or skimmed milk powder, may only partially compensate the weaning-associated removal of sow milk.

A different approach to study the metabolic capacities and changes in the fecal microbiome during the suckling period was performed by Grześkowiak et al. (2020) who used the BIOLOG technique. This technique measures the capacity of the heterotrophic microbial community to utilize selected carbon substrates. Grześkowiak et al. (2020) could show that the suckling piglets clustered in two groups with respect to their catabolism of carbohydrates. This clustering was independent of the piglet's age but dependent on the litter, indicating that the individual microbiota and host immune responses of the sows (e.g., originating from milk, feces and skin) might have affected the microbial activity in their offspring, supporting the theory of the mother–offspring association and possibly early microbial programming in terms of metabolic activity. Moreover, the BIOLOG data showed that the fecal microbial metabolism was based on the utilization of fucose and *N*-acetyl-d-glucosamine (Grześkowiak et al. 2020), which are components of the intestinal mucus but also constituents of sow milk glycans. Postweaning, the 5-week-old piglets clustered together with the sows for the microbial catabolism of substrates, suggesting the development of adult-like metabolic capacities of the fecal microbiome which was related to pig's cereal-based diet.

Albeit information on the colonization of the neonatal gut is still limited, the temporal and spatial variation in the gut microbiota composition during the neonatal period provides important information for the changing gut microbial–host interactions. From the few studies available (e.g., Arnaud et al. 2020), the bacterial microbiota development in digesta of the small and large intestines show a distinctive temporal behavior, whereby the colonic microbiota showed a comparable increase in species richness and diversity over the course of the 28-day-long suckling period (Arnaud et al. 2020) as observed for the fecal microbiota (Shrestha et al. 2020). In jejunal and ileal digesta, the number of species and diversity remained relatively stable throughout the suckling period (Arnaud et al. 2020). Both, jejunal and ileal digesta, harbored predominantly *Firmicutes* and *Proteobacteria* and only low numbers of *Bacteroidetes* and *Fusobacteria* (Arnaud et al. 2020). Unfortunately, the bacterial microbiota composition was not presented at lower taxonomic levels in the latter study, rendering it difficult to deduce developmental alterations at family, genus and species level and hence predict changes in metabolic features. However, the drop of *Actinobacteria* in jejunal and ileal digesta within the first week of life and the ratio of differently abundant operational taxonomic units between weeks support that the major shifts in colonization of these segments occur in week 1 of life (Arnaud et al. 2020).

Due to their close proximity, the maturational shifts in the mucosa-associated microbiota can be expected to have an even greater impact on the developing crosstalk with the host. Nevertheless, only few data are available for the neonatal

period so far. By attaching to the outer mucus layer, the mucosal microbiota can be assumed to play a crucial role for priming of the mucosal immune tolerance and receptor-mediated signaling. The most dramatic shifts in the mucosal bacterial microbiota were observed on the first day after birth (Liu et al. 2019). Like for the luminal microbiota, the mucosa-associated microbiota in the small intestine (jejunum and ileum) was relatively stable between day 3 and 35 of life, whereas the cecal and colonic bacterial communities showed more fluctuations especially in the first 2 weeks of life (Liu et al. 2019). Correspondingly, species richness and diversity mainly increased in the first week of life at the small and large intestinal mucosa, which stabilized thereafter. The jejunal and ileal mucosal microbiota were dominated by *Halomonadaceae* and a small fraction of *Firmicutes* (*Bacillaceae*, *Enterococcaceae*, and *Streptococcaceae*), whereas in the large intestine the mucosal microbiota were more diverse with no obvious dominant bacterial taxa in the first 35 days of life (Liu et al. 2019). The fluctuations at the cecal and colonic mucosa were thereby characterized by a decline in *Bacillaceae* and *Enterococcaceae* in the first days of life, followed by a gradual increase in *Lactobacillaceae*, *Lachnospiraceae*, *Ruminococcaceae*, and *Veillonellaceae* as the piglets aged.

8.3.2 Mycobiome Development

Other microbes such as fungi, bacteriophages, protozoa, and archaea develop in parallel to the bacteriome; however, very little information on composition and interaction with the host has been published for the early neonatal phase in pigs. With respect to the fecal mycobiome, evidence shows a certain vertical transmission of fungi from the maternal mycobiota during birth and environmental exposure, explaining the low but steady abundance of fungi in feces throughout the suckling period including *Saccharomycetaceae* (increased postweaning), *Dipodascaceae* (increased postweaning), *Cladospriaceae* (increased on day 1 and 14 of life), *Aspergillaceae* (increased postweaning), *Malasseziaceae* (increased on day 1 and 14 of life), and *Nectriaceae* (increased on day 1 of life; Summers et al. 2019). Interestingly, the observed fungal families were similar to those reported for the human gut (Chin et al. 2020). Major genera that changed included *Mucor*, *Cladosporium*, *Trichosporon* which were higher during the suckling period and dropped postweaning (Summers et al. 2019; Arfken et al. 2020). By contrast, the two yeast genera *Kazachstania* and *Hyphopichia* were present in low abundances preweaning and largely increased postweaning, potentially driving down community evenness by outcompeting other fungal species in their study as assumed by Arfken et al. (2020). The largely greater abundance of fungi postweaning may be related to the fact that feed left for a certain period of time in the nursery was quickly colonized by fungi, becoming the major source for gut colonization of the piglet postweaning (Summers et al. 2019). Because this effect was also observed for the creep feed, it can be speculated whether components in sow milk may have suppressed directly or indirectly the intestinal proliferation of fungi. An in vitro assay did not confirm protective effects of sow milk or colostrum on fungal growth

(Summers et al. 2019). However, this in vitro approach could not account for protective effects mediated via bacterial–fungal interactions or the innate immune response. Arfken et al. (2020) proposed that the piglet fecal bacteriome follows a defined pattern of colonization and succession in healthy developing piglets, whereas in the mycobiome a large portion of the community may be transient and driven by environmental or host-related factors and therefore varying among piglets. This was based on their findings that the bacteriome but not the mycobiome demonstrated a reduced dispersion among communities over time (Arfken et al. 2020). With respect to the metabolic role of fungi in the neonatal gut, Li et al. (2020) suggested a probable interaction between the fungal composition and the bacterial degradation of dietary protein and complex carbohydrates in the cecum and colon, which was based on results of canonical correspondence analysis between the fungal composition and short-chain fatty acid (SCFA) concentrations. In their study, the low-abundance genera *Fusarium*, *Plectosphaerella* and *Metarhizium* were positively correlated with isobutyrate, while *Xeromyces* were negatively correlated with acetate and *Cornuvesica* negatively with acetate and propionate (Li et al. 2020).

8.3.3 Interferences in Early Life Microbiota Development

The complex and dynamic microbe-to-microbe interactions make the developing microbiota very susceptible to interferences during the suckling phase, with consequences for the networking with the host. Although some studies did not find changes in the taxonomic composition of the fecal microbiome in piglets treated with antibiotics on day 7 of life or receiving a fecal microbiota transplant on day 13 of life during the suckling phase (e.g., Nowland et al. 2020), other studies clearly showed interruptions in the maturational successions. For instance, antibiotic treatment on day 4 of life led to different mucosal (immune) response to the postweaning newly acquired microbiota, and the gut systems of the treatment groups developed into different homeostasis (Schokker et al. 2014, 2015b). These detrimental effects have been well established for oral administration of antibiotics; however, a single parenteral antibiotic injection on the first day of life as prophylactic measure against bacterial diseases caused similar damaging effects, long-lastingly impacting the diversity and composition of the fecal microbiota (Ruczizka et al. 2019). These findings emphasize the importance of the parenteral application of drugs and their hepatic metabolism and metabolite excretion via bile for the microbiota development and interaction with the host piglet. Moreover, sex-related antibiotic metabolism may affect females more than male piglets. As such, the loss of bacterial diversity and of certain taxa in females due to the parenteral antibiotic administration appeared to contribute to decreased body weight of these females on day 97 of life (Ruczizka et al. 2019). Similarly, common infections with intestinal parasites, such as the protozoan parasite *Cystoisospora suis*, and viruses may interfere in the maturation of the gut bacterial microbiota (Huang et al. 2019; Shrestha et al. 2020). Accordingly, *C. suis* infection on day 1 of life disrupted the fecal bacterial maturation in suckling piglets approximately 2 weeks after infection, causing dysbiosis,

characterized by increased diarrhea frequency, greater abundances of *Fusobacteriaceae* and *Veillonellaceae* but proportionally less *Ruminococcaceae*, *Lachnospiraceae*, *S24-7, Clostridiaceae,* and *Erysipelotrichaceae*, as well as depression in species richness and diversity (Shrestha et al. 2020). However, this study suggested a certain plasticity of the piglet gut microbiota as bacterial changes were mainly visible on day 11 of life, partly having recovered on day 15 of life. Likewise, infection with the porcine rotavirus in the first 3 weeks of life resulted in the development of clearly distinguishable bacterial communities in feces (Huang et al. 2019), being characterized by less *Prevotellaceae*, *Ruminococcaceae*, *Rikenellaceae*, *Porphyromonadaceae* and *Lachnospiraceae*, while feces comprised more *Enterobacteriaceae* (especially *Escherichia*-like species). Corresponding to the impact on taxa abundances, "carbohydrate transport and metabolism and "amino acid transport and metabolism" pathways were the most depressed predicted metabolic function genes by the rotavirus in this study.

Even without external disturbances (e.g., medical treatments, infections or diet), litter-specific bacterial development during the suckling phase influences the host phenotype postweaning (Mach et al. 2015). Accordingly, litters with a fecal microbiota dominated by *Prevotella* positively correlated, whereas piglets from litters with *Ruminococcaceae* dominance negatively correlated with luminal secretory immunoglobulin A (IgA) concentrations and body weight postweaning (Mach et al. 2015). Moreover, findings emphasize the potential of early development of the microbiota diversity and composition during the suckling phase as indicative for piglet's susceptibility to postweaning diarrhea (Dou et al. 2017). For instance, piglets that did not develop diarrhea postweaning displayed a lower evenness and higher abundance of *Prevotellaceae*, *Lachnospiraceae*, *Ruminococcaceae,* and *Lactobacillaceae* in feces on day 7 of life compared to piglets that developed diarrhea postweaning (Dou et al. 2017). These bacterial families were linked to a higher fecal *Bacteroidetes* abundance in healthy piglets on day 30 of life 1 week before the onset of diarrhea in the diarrheic piglets. Understanding the maturational changes in the inter- and intra-segmental microbiota composition is therefore crucial from a prophylactic point of view to target specific bacterial communities in order to sustain gut homeostasis beyond weaning as well as efficient gathering of nutrients and nutrient metabolism of the growing animal.

8.4 Gut Microbial and Host-Related Mechanisms for Interaction from Birth to Weaning

Due to their presence and metabolic activity, the gut microbiota influences growth-related (i.e., digestibility, feed intake, carbohydrate and protein fermentation) and health-related traits such as immune competence and tolerance (Kim and Isaacson 2015; Mach et al. 2015; Schokker et al. 2015b). They regulate the mucosal immune system, not only educating the naïve infant immune system but also serving as an important source of immune stimulators throughout life. Therefore, an altered neonatal colonization and disturbed interactions between gut microbes and the

host during the neonatal period have a profound effect on the host phenotype later in life (Mach et al. 2015; Schokker et al. 2015b; Ruczizka et al. 2019) and may increase the susceptibility of the piglet to develop gut dysbiosis after weaning (Dou et al. 2017). The gut microbiota play a role in the renewal of gut epithelial cells and its barrier function, the breakdown of toxins, and the exclusion of pathogens. Concurrently, the gut microbiota are a rich source of molecules, such as lipopolysaccharide (LPS) and peptidoglycan, that may cause inflammation in peripheral tissues of the body (Broom and Kogut 2018). Microbial mechanisms involve the degradation of nutrients which are otherwise indigestible for the host (such as milk oligosaccharides and later dietary fiber), competition for easily digestible nutrients (especially in the small intestine), bile acid metabolism, production of primary and secondary fermentation metabolites (e.g., short-, medium and long-chain fatty acids, biogenic amines, vitamins and antimicrobials), synthesis of microbial-origin effector molecules, and production of neuroendocrine molecules (Broom and Kogut 2018; Lyte and Lyte 2019). The host gut, in turn, recognizes microbial activity via different routes, such as recognition of microbial metabolites via G-proteins (GPR) (McKenzie et al. 2017), receptor recognition of microbiota-associated molecular patterns (MAMP)—structural motifs that are highly conserved in microbes present on the outer bacterial cells (e.g., LPS; Broom and Kogut 2018), and neuroendocrine receptors (Lyte and Lyte 2019). Due to the many routes of interaction, the gut microbiota is capable to manipulate growth performance and feed efficiency in young pigs after weaning (McCormack et al. 2018, 2019). However, only few of these mechanistic pathways have been investigated in relation to the suckling phase so far. Anyway, the actual host mucosal response to the local (mucosa-associated) and "transient" (digesta-associated) microbiota needs to be understood as an aggregation of intermingled effects triggered via the different networking routes.

Fermentation metabolites, such as SCFA, biogenic amines, and other toxic microbial metabolites (e.g., ammonia and hydrogen sulfide), influence intestinal functioning and systemic energy acquisition (Willing and Van Kessel 2010; McKenzie et al. 2017; Lauridsen 2020). While SCFA contribute to pig's energy supply when absorbed and exert anti-inflammatory and antimicrobial properties, especially the activation of pattern-recognition receptors (PRR) expressed on the host mucosa and immune cells by microbial surface antigens can trigger a costly upregulation of the gut mucosal immune response, thereby diverting energy and nutrients from growth (Broom and Kogut 2018). Aside from pathogens, it can be assumed that drastic alterations in the microbiota composition due to dietary changes (e.g., at weaning) or medical treatments can lead to rapid activation of the gut immune response and loss of immune tolerance, by exposing the host to a different subset of MAMPs. After recognition by specialized PRR at the gut mucosa, MAMPs lead to the activation of cellular pro-inflammatory pathways, via key adaptor proteins (e.g., myeloid differentiation primary response 88) and transcription factors (e.g., nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB)), and production of various cytokines and chemokines, which signal to immune (including adaptive) cells in the underlying gut-associated lymphoid tissue (GALT) (Abreu 2010; Broom and Kogut 2018).

Certain evidence exists from rodent models that microbially produced catecholamines and acetylcholine play an important role in the crosstalk between microbes and the immune system (Villageliú et al. 2018; Lyte et al. 2019). Even less is known about the reverse scenario whether stress-related changes in host neuroendocrine production and gut physiology affect microbial viability, composition, and/or function in the young piglet immediately after weaning. There is some evidence for microbial endocrinological-based signaling by which the gut microbiome may modulate host food preference and appetite (Lyte et al. 2019). Albeit piglets usually have no influence on their choice of food, this relationship may contribute to the weaning-associated anorexia. Microbes seem to interfere largely in the production of key food intake-regulatory hormones, including somatostatin, ghrelin, leptin, insulin and glucagon-like peptide (GLP)-1 which partly signal via GPRs (for more details, see review of Lyte et al. 2019). Moreover, for humans and rodent models, it has been suggested that host-microbe neuroendocrine signaling within the GIT may signal via the vagus nerve, affecting feeding-regulatory regions in the brain (Lyte et al. 2019).

Likewise, little known about the contribution of fungi on the gut microbiota–host interactions in piglets (Summers et al. 2019; Arfken et al. 2020). Proposed mechanisms involve fungal secretion of prostaglandins and prostaglandin-like molecules with immunomodulatory capacities (Noverr et al. 2001; Erb-Downward and Huffnagle 2007; Erb-Downward and Noverr 2007) as well as direct interaction with the indigenous microbiota (Kang et al. 2018; Pepoyan et al. 2018). Although first data are available for changes in the fungal community from the neonatal to the postweaning period, fungal–host interactions have not been investigated sufficiently in suckling and weaned piglets (Summers et al. 2019; Arfken et al. 2019, 2020).

8.4.1 Neonatal Diet-Microbe-Host Networking

It is legitimate to assume that microbial modes of action in the neonatal phase are similar to the microbial signaling in older pigs but they are clearly at a developing stage, related to the actual species abundances and respective microbe-to-microbe interactions. The host response, in turn, is preprogrammed in neonatal tissues and driven by exposure to maternal, environmental, and microbial factors (Gomez de Agüero et al. 2016). In utero, the fetus is relatively microbe-free but quickly colonized at birth (Jiménez et al. 2005). Findings in humans emphasize the role of the amniotic fluid microbiota for the initial colonization and first microbe-host signaling in that the meconium microbiota resembled more the amniotic fluid microbiota than the vaginal or fecal microbiota (He et al. 2020). Moreover, experiments with germ-free pregnant mice that were transiently colonized with genetically engineered *Escherichia coli* HA107 evidenced that the maternal microbiota already shape the immune system of their offspring in utero (Gomez de Agüero et al. 2016). Colonization with *E. coli* HA107 altered the numbers of early postnatal intestinal innate leukocytes as such that the offspring of colonized dams had increased intestinal innate lymphoid cell proportions and total numbers

compared with germ-free controls. As an epitheliochorial and diffuse type of placenta, the porcine placenta may be less transmissible for microbes (Furukawa et al. 2014a, b). Nevertheless, pig research in relation to the "sow diet-piglet" axis let assume that a certain initial microbial priming of the naïve porcine GIT and immune system occurs in utero as well (e.g., Ferret-Bernard et al. 2020).

At birth, the gut mucosa becomes progressively colonized with microbes, directly exposing the immature neonatal immune system to potential pathogens (Gomez de Agüero et al. 2016). Colostrum is the first lactocrine signal, including a range of bioactive substances (e.g., proteins, growth factors, peptides, oligosaccharides, fatty acid-derived molecules, steroids and microRNAs), which are essential for thermoregulation and passive immunity against pathogens, and stimulation of intestinal maturation in the newborn piglet (Quesnel and Farmer 2019). Therefore, the early gut microbe–host interaction occurs under the continued protection from immunoglobulins (especially IgA and IgG) and antibacterial peptides in colostrum and later in mature milk (Møller et al. 2011; Quesnel and Farmer 2019), impeding the establishment of enteric pathogens. In terms of passive immunity, concentrations of IgG in colostrum are highly variable among sows (Quesnel 2011), providing different levels of humoral immunity and antimicrobial effects in the newborn piglet's gut. Moreover, dietary ingredients with immuno-modulating capacities, such as fish oil, prebiotics, and probiotics, have been reported to increase IgG, IgA, and/or IgM in sow colostrum when they were provided during the last weeks of gestation (Cao et al. 2019; Quesnel and Farmer 2019). By orchestrating the maturational successions in the gut microbiota of suckling piglets, these and other bioactive milk ingredients shape the gut maturation process in neonates (Quesnel and Farmer 2019; Ren et al. 2019). Aside from immunoglobulins, bioactive compounds in porcine mature milk with immune- and microbiota-modulatory properties include cytokines, growth factors, osteopontin, caseinoglycomacropeptides, gangliosides, lipids, and oligosaccharides (Møller et al. 2011; Salcedo et al. 2016; Ren et al. 2019; Lauridsen 2020). Albeit not being species-specific, feeding bovine colostrum reduced the incidence of necrotic colitis, improved gut maturation, and downregulated expression of intestinal genes related to inflammation in preterm pigs delivered by cesarean section after normal pregnancies or exposed to prenatal inflammation compared to formula fed piglets (Jensen et al. 2013; Ren et al. 2019). There is still a paucity of information available for the impact of the various functional compounds in milk on the neonatal development of gut mucosal functioning either directly or indirectly via orchestrating the successional alterations in the developing gut microbiota. Moreover, the role of the abrupt removal of sow milk and hence its bioactive compounds on intestinal inflammation and the microbial-host mucosal crosstalk has not been sufficiently elucidated yet. Feeding bovine colostrum instead of milk replacer to undersized piglets during the suckling period may be an alternative strategy by keeping intestinal *Enterobacteriaceae* and enterotoxigenic *E. coli* levels low and leading to a modulated intestinal expression of *TLR4* and *IL2* (Sugiharto et al. 2015; Poulsen et al. 2017). Moreover, newly weaned piglets showed improved intestinal mucosal restoration when their standard weaner diet was supplemented with bovine

colostrum (Huguet et al. 2006). However, feeding bovine colostrum from day 23 of life led to a change in the lactic acid bacterial community in the ileum on day 30 of life (Poulsen et al. 2017), emphasizing the importance of the species-specific milk for intestinal development and the microbe-host dialogue. Despite progressing research in this area, we are only advancing our knowledge about the specificities in the gut microbial community structures in relation to the programming of the gut mucosal immune system and development of the gut barrier function in newborn piglets (Schokker et al. 2018).

Certain information is available from germ-free and gnotobiotic pig models, indicating the modulatory role of specific taxa or the whole gut microbiota for small intestinal development and immune response (Willing and van Kessel 2008, 2009). However, the interactions between specific bacterial species or complex microbial communities were mostly examined at a restricted number of time points during the suckling phase, limiting the conclusions that can be drawn for the microbial-host interactive programming from birth to early postweaning. Nevertheless, the available data clearly demonstrate the stimulatory action of the microbiota when cesarean born piglets were associated with specific bacterial strains or conventionalized with a complex microbial community. For instance, conventionalization of germ-free piglets with sow feces improved small intestinal development as indicated by increased crypt depth and reduced villous height (Willing and van Kessel 2008) and modification of enzymatic activity at the brush border (Willing and Van Kessel 2009). Enterocyte upregulation of aminopeptidase N expression was either the direct response to the microbial colonization or feedback mechanism in response to reduced enzyme activity through microbial degradation (Willing and Van Kessel 2009). Moreover, induction of inflammatory responses and activation of apoptosis through death receptors appeared to be one important mechanism in enterocyte turnover mediated by the commensal bacteria. Both the conventional gut microbiota and *E. coli* but not *Lactobacillus fermentum* stimulated the overall cell turnover by increasing apoptosis through the expression of Fas ligand and tumor-necrosis factor-α and by increasing cell proliferation via activation of TLR expression in 14 day-old piglets (Willing and van Kessel 2008). In the following three sections, examples for mechanistic routes by which the gut microbiota and the host interact will be presented, focusing on the networking via SCFA, bile acids, and pathogen-receptor recognition will be presented (Fig. 8.2).

8.4.1.1 Microbial Signaling via SCFA

The SCFA are detected in relevant concentrations in ileal, cecal, and colonic digesta and feces (meconium) of suckling piglets starting from birth (~10 to 40 μmol/g across gut sites; Nakatani et al. 2018; Arnaud et al. 2020; Metzler-Zebeli, personal communication). Total SCFA concentrations increase in ileal and colonic digesta from birth to weaning, with especially acetate raising from the third to the fourth week in ileal digesta, whereas the concentrations of all individual SCFA increased in the large intestine from week 2 to 3 to 4 (Nakatani et al. 2018; Arnaud et al. 2020). These data let assume that the individual SCFA play a significant role in early host mucosal nutrition and priming of gene expression. Notably, in the study of Arnaud

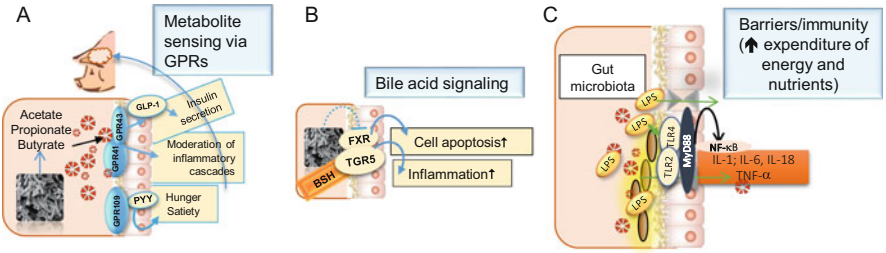

Fig. 8.2 Schematic of microbial and host mucosal mechanisms in the gut microbiota-host dialogue in piglets that undergo continuous alterations from the suckling and early postweaning period. (**a**) Activation of G-protein coupled receptor (GPR) via microbial metabolites trigger gut mucosal and systemic effects; (**b**) Bile-salt hydrolase (BSH) activity of gut microbes alter signaling via farnesoid X receptor (FXR) and G-protein coupled bile acid receptor 1 (TGR5); and (**c**) recognition of microbiota-associated molecular patterns, such as lipopolysaccharide (LPS), activate toll-like-receptor (TLR)-mediated pro-inflammatory signaling cascades. MyD88, myeloid differentiation primary response 88; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; IL, interleukin

et al. (2020), total SCFA and acetate levels were almost similar in ileal and colonic contents on day 28 of life, emphasizing the importance of SCFA for small intestinal development. After production, SCFA determine the gut environment, influencing pH, gut motility, inflammation, barrier function, nutrient uptake, microbial balance, and the fermentative activity (Louis et al. 2007; McKenzie et al. 2017). Due to the lactose content of milk, higher levels of lactate may be expected, which is commonly quickly converted into SCFA via cross-feeding. By contrast, fermentation of porcine milk oligosaccharides in vitro with fecal inoculum from 21-day-old piglets yielded acetate (49%), propionate (27%), butyrate (20%), lactate (2%), and succinate (1%; Difilippo et al. 2016), emphasizing the importance of milk oligosaccharides for gut mucosal SCFA signaling. Overall, the increase in SCFA concentrations and alterations in molar proportions in intestinal digesta with age can be directly related to gut microbiota maturation and changes in porcine milk composition during progressing lactation (Nakatani et al. 2018; Wei et al. 2018; Arnaud et al. 2020).

In the lumen, SCFA are major players in the microbe–microbe crosstalk, shaping the gut microbial composition and hence contributing to a high state of gut health when produced in adequate and balanced amounts (Nakanishi et al. 2009; Jacobson et al. 2018). In adequate concentrations, SCFAs have direct antimicrobial activity against pathogenic bacteria. For instance, *Bacteroides* spp. mediate resistance to *Salmonella* colonization by propionate production (Jacobson et al. 2018). The antimicrobial effect of SCFA is related to a decrease in intracellular pH and a specific effect of the anion affecting cell metabolism and replication in susceptible bacteria (Metzler et al. 2005). This inhibiting capacity is made use of when supplementing (pre-) starter diets with short- or medium-chain organic acids (e.g., formic acid, fumaric acid, lactic acid, or sorbic acid) (Metzler et al. 2005). Since the protective and antimicrobial effects of SCFAs are concentration dependent, dysregulation of intestinal SCFA production facilitates the intestinal colonization by pathogens

(Lamas et al. 2019). Below a certain threshold, the inhibitory effect of SCFA, such as butyrate, on the expression of virulence genes ceases, as shown, for instance, for enterohemorrhagic *Escherichia coli* and *Campylobacter jejuni* (Luethy et al. 2017). These concentration-dependent relationships still need to be investigated in more detail in suckling and newly weaned piglets.

The single SCFA has different binding affinities to mucosal receptors and transport proteins (Kimura et al. 2020), thereby guiding the mucosal signaling. At the mucosa, the SCFA bind to G-protein coupled receptors (GPR), such as GPR43, GPR41, and GPR109A, which mediate the regulatory action of SCFA on inflammatory processes at the gut mucosa and systemically (McKenzie et al. 2017). However, a scarcity of information about GPR signaling during the suckling period exists for the porcine gut. Suckling and weaned pigs have a great capacity to absorb and metabolize SCFA along the GIT (Metzler-Zebeli et al. 2012, 2017; Nakatani et al. 2018). Gut segment-specific transporter expression shows that the expression of sodium-dependent monocarboxylate transporter is generally higher in the small intestines, whereas in the large intestine SCFA transport seems to dominantly occur via the MCT-1 (e.g., Metzler-Zebeli et al. 2012, 2017; Newman et al. 2018). These differences may be explained via different affinities of the MCTs for the various SCFA and medium-chain fatty acids (e.g., lactate; Liu et al. 2019). In the suckling and early weaning phase, the paracellular absorption of SCFA from the lumen into the cecal vein of piglets appeared to be the major route for SCFA absorption (Nakatani et al. 2018). Transporter expression (MCT-1) and absorbed SCFA amounts increased postweaning, whereas cecal SCFA concentrations were not higher in the weaned piglets (Nakatani et al. 2018). Interestingly, weaning age appeared to affect SCFA absorption, with piglets weaned at day 21 having higher acetate and propionate concentrations in the cecal vein compared to piglets weaned on day 28 of life, which the authors related to increased cecal MCT-1 expression in the earlier weaned piglets (Nakatani et al. 2018).

Enterocytes use SCFA as respiratory fuel in a preferential order with butyrate being the most favored substrate. Nevertheless, other SCFA, such as valerate, caproate, propionate, and acetate, equally contribute to the ATP generation in the enterocytes (Jørgensen et al. 1997). Emerging evidence from mice suggests that the decline in the luminal provision of butyrate as fuel for the enterocytes and hence altered mucosal energy production may contribute to gut dysbiosis (Stecher and Jung 2018), which may be the case in piglets suffering from weaning-associated anorexia. Under normal conditions, SCFA are oxidized in the enterocytes via β-oxidation. When luminal butyrate levels decrease, the enterocyte switches to anaerobic ATP generation from lactate and consumes less oxygen as well as upregulates nitrate generation (Byndloss et al. 2017; Stecher and Jung 2018). Increased oxygen and nitrate levels may provide a growth-advantage to aero-tolerant and L-lactate utilizing anaerobes at the mucosa, such as *Escherichia coli* and *Salmonella* (Stecher and Jung 2018). Nowadays (pre-)starter diets comprise sufficient amounts of fermentable carbohydrates to support intestinal production of SCFA and butyrate. Therefore, the continuous delivery of fermentable substrate in form of an appropriate feed intake level in the first days postweaning is one of the

most critical factors for proper mucosal metabolism and intestinal microbe–microbe signaling (Bauer et al. 2011).

Especially butyrate exerts gut mucosal effects via manipulation of gene transcription, which are independent of toll-like receptor (TLR) and GPR signaling and due to the inhibition of histone deacetylases by butyrate. Findings in neonatal piglet models for human infant nutrition and weaned pigs support certain beneficial effects of butyrate on gut development and physiology (Kotunia et al. 2004; Manzanilla et al. 2006; Dong et al. 2016). Gastrointestinal development was more mature in neonatal piglets fed formula milk supplemented with sodium butyrate compared to piglets without butyrate supplementation (Kotunia et al. 2004). Using an intrauterine growth retardation model, Dong et al. (2016) showed that piglets receiving a 0.1% supplementation of the pro-drug of butyrate (tributyrin) in their milk exhibited better developed spleen and small intestines, improved intestinal villus morphology, enhanced digestive enzyme activities, and upregulated expression of IgG and GPR41 along the small intestine compared to those of the intrauterine grow retarded group. Moreover, medium-chain fatty acid coated butyrate exerted a certain trophic effect on the intestinal epithelium likely reinforcing the gut barrier in an enterotoxigenic *Escherichia coli* (ETEC) $F4^+$ model using early-weaned piglets (López-Colom et al. 2020). Although butyrate is normally available only in low concentrations in the small intestine, supplementation of butyrate to formulas for total parenteral nutrition of neonatal piglet models stimulated tissue regeneration after 80% jejuno-ileal resection by increasing proliferative pathways and decreasing apoptosis in the enterocytes (Bartholome et al. 2004). By contrast, inconclusive results exist for normal piglets in which supplementation of butyrate led either to an increase (Kotunia et al. 2004), decrease (Piva et al. 2002) or no effect on villus height (Manzanilla et al. 2006).

8.4.1.2 Microbial Signaling via Bile Acid Modification

Another example for the modulatory capacities of the gut microbiota is via modification of bile acid signaling (Tremaroli and Bäckhed 2012; Lin et al. 2019). Bile acids act as signaling molecules and bind to cellular receptors, including farnesoid X receptor (FXR) and G-protein coupled bile acid receptor 1 (TGR5), which both have been implicated in the modulation of glucose and lipid metabolism, inflammation, and cell proliferation (Sayin et al. 2013; Dossa et al. 2016; Yanguas-Casás et al. 2017; Lin et al. 2019). The different bile acids have different potencies to bind to these receptors. Therefore, degradation of primary and secondary bile acids by the gut microbiota via promoting deconjugation, dehydrogenation, and dihydroxylation of primary bile acids may have profound effects on bile acid metabolism. Glyco- and tauro-conjugated bile acids are deconjugated by bacteria with bile salt hydrolase activity (e.g., lactobacilli), and then the 7α-hydroxy group is removed by bacterial dehydroxylase activity to form the secondary bile acids, which have been linked to reduced cell apoptosis and intestinal inflammation (Sinha et al. 2020). Very little information about bile acid signaling exists for suckling and newly weaned pigs. Suckling piglets have smaller bile acid pools and lower lipid digestibility compared to weaned piglets (Harada et al. 1988; Cera et al. 1988; Lewis et al. 2000). Therefore,

differences in microbial colonization and hence bacterial degradation of bile acids in the upper GIT may have consequences for the utilization of dietary lipids (mostly from sow milk) during the suckling phase. After weaning, the lack of fat-rich sow milk and altered feed intake will lead to changes in bile acid secretion and bile acid signaling effects on and by the gut microbiota. Against this background, Lin et al. (2019) used an anorexic weaning piglet model with which they demonstrated accumulation of secondary bile acids in the ileum, partly due to an altered gut microbial bile acid deconjugation caused by a lower *Lactobacillus* abundance, leading to a downregulated FXR signaling at the ileal mucosa of undernourished piglets. The authors proposed that the increased secondary bile acids may contribute to the weaning-associated enteritis by exacerbating inflammatory responses in anorexic piglets.

8.4.1.3 Pathogen-Recognition Receptor-Mediated Signaling, Immune and Barrier Function

Aside from metabolite signaling at the gut mucosa, gut microbial–host interactions are mediated by recognition of bacterial conserved structures through PRR present on host cells, including, among others, TLRs, nucleotide oligomerization domain (NOD)-like receptors (NLR), and galectins (Gourbeyre et al. 2015). Expression of PRR varies along the GIT in weaned and growing pigs, which is an adaptation to the prevailing microbiota composition (Gourbeyre et al. 2015; Price et al. 2018). Information on the postnatal evolution of the PRR expression in relation to the developing microbiota along the GIT of neonatal piglets is still limited. Moreover, these studies focused mostly on the suckling (Schokker et al. 2018; Wang et al. 2019; Arnaud et al. 2020) or postweaning period (e.g., Tao et al. 2015), missing to show the drastic effect of the abrupt removal of sow milk for the microbial-mucosal interplay in the first days postweaning. In general, once there is recognition, the host activates several signaling cascades leading to the production of both immune-activating and immune-regulatory cytokines, crucial elements in the protection against GIT infections or in the induction of tolerance toward commensal bacteria (Broom and Kogut 2018). Although PRRs are traditionally known to recognize microbial molecules during infection to initiate inflammatory responses, they are also required to promote long-term tolerance toward the commensal microbiota (Chu and Mazmanian 2012) and respond to food antigens (Nakashima et al. 2018). Through PRR activity, the intestinal epithelium, together with intraepithelial and lamina propria immunocompetent cells, coordinates adequate local innate and adaptive immune responses to the luminal microbiota. This interplay has been described for older pigs, showing inter- and intra-segmental specific expression of PRR in the small intestine (Gourbeyre et al. 2015). However, limited information is available which PRR are expressed first (Arnaud et al. 2020) and how immune signaling is directed via the bioactive substances (e.g., immunoglobulins) in sow colostrum and milk and develops until after weaning.

In addition to the PRR expression, the interchange between bacteria and the host is dependent on gut barrier function properties, including epithelial permeability and secretion of electrolytes, mucus, and antimicrobial peptides (Broom and Kogut

2018). These mechanisms limit or control the presence of bacteria in the proximity of host cells and thus are important regulators in the bacteria-host mucosal networking. Similarly, epithelial permeability and goblet cell density varies along the GIT in older pigs and evolve during the maturation process (Schokker et al. 2018; Liu et al. 2019; Arnaud et al. 2020). While germ-free piglets show little or no secretory IgA, germ-free piglets colonized by a defined probiotic gut microbiota had increased IgA levels in the ileum at 5 days of life (Butler et al. 2016), indicating the importance of the exposure to luminal microbial antigens for immune development. The significance of the composition of the developing microbiota for gut barrier functionality upon interaction with pathogenic or beneficial bacteria was also demonstrated by Trevisi et al. (2018) after perfusing jejunal loops of cesarean-derived pigs with different microbial inocula. For this, piglets were neonatally colonized with differently complex microbiota. All piglets received pasteurized sow colostrum and a starter microbiota consisting of *Lactobacillus amylovorus*, *Clostridium glycolicum*, and *Parabacteroides* on days 1–3 of life. On days 3 and 4 of life, they were inoculated with a complex microbiota (diluted sow feces) or saline. With 4–5 weeks of life, jejunal loops were perfused for 8 h with either enterotoxigenic *Escherichia coli* F4 (ETEC), purified F4 fimbriae, *L. amylovorus* or saline as control. Results showed that an early association with a complex microbiota resulted in a modulation and activation of B and T lymphocytes, when compared to an association with a simple microbiota. Moreover, the early microbial colonization caused a balanced immune and inflammatory response as piglets early associated with a complex microbiota maintained a reduced activation of genes related to chemokine and cytokine activity after ETEC challenge. These findings clearly demonstrate the co-development of the gut microbiota and the gut mucosal response toward them, including receptor recognition and barrier function after birth, establishing a homeostatic state, whereby this postnatal co-development follows different timings depending on the gut site considered (Arnaud et al. 2020). Accordingly, expression of PRRs, such as *TLR1* to *TLR9*, *NOD2*, *CYCLOA,* and *RPL32*, was shown to exhibit different patterns on days 0, 2, 7, 14, and 28 of life in the jejunum, ileum, and colon of suckling piglets (Arnaud et al. 2020). As opposed to the microbiota, jejunal and ileal mucosa did not evolve drastically the first week of life but many changes started to occur between day 7 and 14 of life (Arnaud et al. 2020). When comparing the expression patterns across the three gut sites, the ileum was the gut site with the lowest expression of PRRs, except for *TLR5* which was mainly expressed in the ileum throughout the suckling phase. This low expression of TLRs is noteworthy due to the high density of Peyer's patches and higher expression levels in this area of the gut in older weaned pigs (Gourbeyre et al. 2015). The expression levels of *TLR6*, *TLR7,* and *TLR8* were higher in the jejunum than in the colon and ileum, whereas expression of *TLR1*, *TLR2*, *TLR3,* and *TLR4* was higher in the colon compared to the two small intestinal segments. The jejunal and ileal mucosa exhibited gradual changes in barrier and defense mechanisms including an increase in *TLR1*, *TLR7*, *TLR8*, and *TLR9* expression in both jejunum and ileum and in *TLR3*, *TLR4*, and *TLR6* expression in jejunum, beginning from day 7 until day 28 of life. Simultaneously, loosening of the small intestinal barrier function was indicated by an

increase in FD4 flux and gut-electrophysiological findings such as the decrease in short-circuit current. Loosening of the barrier function may be an adaptive mechanism toward the relatively stable microbiota in suckling animals, thereby allowing proper education of the immune system, including PRR maturation and built-up of immune tolerance (Arnaud et al. 2020). However, this topic has not been sufficiently explored so far. Principal component analysis suggested specific microbiota-mucosal relationships with clear clustering of colonic samples (*Micrococcaceae*), irrespective of the age, and clustering of jejunal (*Enterobacteriaceae*) and ileal samples (*Peptostreptococcaceae)*, with gradual changes with age in this study (Arnaud et al. 2020).

More evidence for the importance of early life programming for development of the intestinal mucosal immune system and development of the intestinal barrier function comes from observations in suckling piglets which received a daily oral administration of fructooligosaccharides over a period of 12 days between day 2 and 14 of life (Schokker et al. 2018). However, the microbiota composition and mucosal host response were only studied at two time points in the suckling phase on days 14 and 25 of life. Due to the use of fructooligosaccharides, this study highlights the importance of available nutrients for orchestrating that specific microbial community structures expand during the suckling period (Schokker et al. 2018). As short-chain fructooligosaccharides can be found in sow milk (Salcedo et al. 2016), this also emphasizes the importance of milk glycans for the proper development of the gut microbiota and host mucosal response. At the microbiota community level, fructooligosaccharide administration showed a clear "bifidogenic" effect and raised lactobacilli but it came along with less *Bacteroidia* in colonic digesta at day 14 of life. However, this did not translate into significant changes in the local mucosal transcriptome (Schokker et al. 2018). For the jejunum, significant changes were observed for microbiota composition and differentially expressed gene sets in mucosal tissues of the jejunum at both days 14 and 25 of life. Accordingly, piglets receiving the fructooligosaccharides had lower mucosal expression of cell cycle-related processes and higher expression of extracellular matrix genes on day 14, suggesting changes in jejunal mucosal barrier function, and less activity of immune related processes on day 25 in the jejunal mucosa compared to control piglets (Schokker et al. 2018). However, the authors missed to integrate the microbiota and host mucosal data to "pinpoint" influencing bacterial taxa. Wang et al. (2019) reported higher luminal SCFA concentrations (especially acetate and butyrate) in the colon of galactooligosaccharide-supplemented piglets (1 g/kg BW) on days 8 and 21 of life, which they could link with altered gene expression profiles of inflammatory cytokines (IL-8 and IL-10) and barrier proteins (zonula occludens-1 and claudin-1). According to their findings, the signaling occurred via regulation of the phosphorylation of the NF-κB and 5'-AMP-activated protein kinase pathways (Wang et al. 2019).

Due to the still immature adaptive immune system in piglets at weaning, it can be assumed that the mucosal non-specific innate immune response may be of similar importance as the developing adaptive immune response as well as sub-sampling and processing of bacterial antigens by the gut-associated lymphoid tissue (GALT).

Evidence for weaned pigs strongly supports the importance of alterations in receptor-mediated recognition of MAMPs around weaning as one major trigger for gut inflammatory processes observed postweaning. For instance, Tao et al. (2015) investigated the impact of weaning on alterations in the expression of intestinal TLRs and NODs on day 1 and 4 postweaning which were evidently linked to weaning-related shifts in the ileal microbiota. In considering the drastic changes only in the glycan-degrading bacterial community from the milk to the plant-based diet, it is easily feasible that the subsequent alterations in the exposed bacterial surface ligands (e.g., lipopolysaccharides, lipoteichoic acid, lipoprotein, flagellin, CpG-containing DNA as MAMPs for TLRs and diaminopimelic acid and muramyldipeptide containing peptidoglycans as MAMPs for NODs), lead to different activation of signal-transduction proteins which trigger pro-inflammatory pathways via expression of pro-inflammatory, anti-inflammatory, and apoptotic factors (Willing and Van Kessel 2010; Tremaroli and Bäckhed 2012; Chu and Mazmanian 2012).

8.4.2 Influencing the Gut Microbiota-Host Relationship in Pigs from Early Life on

Early-life conditions are critical for gut development and microbial colonization (Schokker et al. 2014, 2018). In considering the drastic alterations in the bacterial microbiome and functions and subsequently the expected changes in microbe-host mucosa networking, the transition from sow milk to a solely plant-based diet should be as "smooth" as possible for the piglet. It has become common practice to supplement combinations of feed additives with different bioactive functionalities to pig's creep feed and (pre-)starter diets to reduce postweaning diarrhea and growth-check, including probiotics, prebiotics, phytobiotics, organic acids, enzymes, and diatomaceous earth (Metzler et al. 2005; Pluske et al. 2018). Because prophylactic effects of dietary components can be established if only introduced before weaning, "gut friendly" feeding regimens generally should start before weaning. In semi-natural environmental settings, the piglets start following their dam on her foraging trips soon after birth, chewing on straw and showing rooting behavior from the second week of life (Petersen 1994). In commercial settings, suckling piglets are commonly offered supplemental feed in the form of milk replacers and creep feed aiming to adapt the GIT to solid feed before weaning (Pluske et al. 2018). Creep feed consumption increases especially in the fourth week of life and has been correlated to digestive maturity but also to poor milk yield of the sow (Pajor et al. 1991; de Greeff et al. 2016). Creep feed consumption during lactation increases feed intake early after weaning, suggesting an improved capacity of piglets to cope with weaning (Muns and Magowan 2018). This effect seemed thereby to be more related to the acceptance and intake of solid feed after weaning and not to a reduction in gut structure atrophy, which still occurred despite creep feeding (Muns and Magowan 2018). Since most piglets start eating greater amounts of solid feed in the days before weaning, these findings may imply the importance of specific bioactive components

occurring in sow milk to attenuate the alterations in the gut microbiota and mucosal PRR-signaling in the newly weaned pigs.

8.5 Conclusion and Perspectives

This literature overview provided insight into the substantial changes in the developing gut microbiota composition from birth to postweaning, formed by sow milk components and later by the intake of (solid) plant-based feed. Maturational alterations in host mucosal functions follow a genetically programmed sequence which is modulated by the current microbial profile and other environmental factors. The gut microbe–host interactions during the suckling phase occur under the continued protection from bioactive substances in colostrum and in mature milk which suppress pathogen growth and act as microbial substrate. The abrupt loss of bioactive porcine milk components due to the removal of the sow at weaning may be one important factor for the disturbed microbe-host dialogue postweaning. Relatively little is known so far with respect to the postnatal evolution of microbe-recognition at the gut mucosa. Against this background, it may be worth to elucidate whether newly weaned piglets would benefit from the dietary inclusion of bioactive porcine milk components in the first weeks postweaning—representing their patterns and concentrations in sow milk until the age when natural weaning occurs—and whether this dietary supplementation could potentially attenuate the drastic shifts in gut microbiota-host signaling, loss of mucosal immune tolerance and inflammation, thereby enhancing growth, well-being and gut function and health in piglets postweaning.

References

Abreu MT (2010) Toll-like receptor signalling in the intestinal epithelium: how bacterial recognition shapes intestinal function. Nat Rev Immunol 10(2):131–144. https://doi.org/10.1038/nri2707

Albrecht S, Lane JA, Mariño K, Al Busadah KA, Carrington SD, Hickey RM, Rudd PM (2014) A comparative study of free oligosaccharides in the milk of domestic animals. Br J Nutr 111(7):1313–1328. https://doi.org/10.1017/S0007114513003772

Arfken AM, Frey JF, Ramsay TG, Summers KL (2019) Yeasts of burden: exploring the mycobiome–bacteriome of the piglet GI tract. Front Microbiol 10:2286. https://doi.org/10.3389/fmicb.2019.02286

Arfken AM, Frey JF, Summers KL (2020) Temporal dynamics of the gut bacteriome and mycobiome in the weanling pig. Microorganisms 8(6):868. https://doi.org/10.3390/microorganisms8060868

Arnaud AP, Rome V, Richard M, Formal M, David-Le Gall S, Boudry G (2020) Post-natal co-development of the microbiota and gut barrier function follows different paths in the small and large intestine in piglets. FASEB J 34(1):1430–1446. https://doi.org/10.1096/fj.201902514R

Bartholome AL, Albin DM, Baker DH, Holst JJ, Tappenden KA (2004) Supplementation of total parenteral nutrition with butyrate acutely increases structural aspects of intestinal adaptation

after an 80% jejunoileal resection in neonatal piglets. JPEN J Parenter Enteral Nutr 28(4):210–223. https://doi.org/10.1177/0148607104028004210

Bauer E, Metzler-Zebeli BU, Verstegen MW, Mosenthin R (2011) (2011) Intestinal gene expression in pigs: effects of reduced feed intake during weaning and potential impact of dietary components. Nutr Res Rev 24(2):155–175. https://doi.org/10.1017/S0954422411000047

Bezirtzoglou E (1997) The intestinal microflora during the first weeks of life. Anaerobe 3:173–177

Bian G, Ma S, Zhu Z, Su Y, Zoetendal EG, Mackie R, Liu J, Mu C, Huang R, Smidt H, Zhu W (2016) Age, introduction of solid feed and weaning are more important determinants of gut bacterial succession in piglets than breed and nursing mother as revealed by a reciprocal cross-fostering model. Environ Microbiol 18(5):1566–1577. https://doi.org/10.1111/1462-2920.13272

Boudry G, Hamilton MK, Chichlowski M, Wickramasinghe S, Barile D, Kalanetra KM, Mills DA, Raybould HE (2017) Bovine milk oligosaccharides decrease gut permeability and improve inflammation and microbial dysbiosis in diet-induced obese mice. J Dairy Sci 100(4):2471–2481. https://doi.org/10.3168/jds.2016-11890

Broom LJ, Kogut MH (2018) Gut immunity: its development and reasons and opportunities for modulation in monogastric production animals. Anim Health Res Rev 19(1):46–52. https://doi.org/10.1017/S1466252318000026

Butler JE, Santiago-Mateo K, Wertz N, Sun X, Sinkora M, Francis DL (2016) Antibody repertoire development in fetal and neonatal piglets. XXIV. Hypothesis: the ileal Peyer patches (IPP) are the major source of primary, undiversified IgA antibodies in newborn piglets. Dev Comp Immunol 65:340–351. https://doi.org/10.1016/j.dci.2016.07.020

Byndloss MX, Olsan EE, Rivera-Chávez F, Tiffany CR, Cevallos SA, Lokken KL, Torres TP, Byndloss AJ, Faber F, Gao Y (2017) Microbiota-activated PPAR-g signaling inhibits dysbiotic Enterobacteriaceae expansion. Science 357:570–575. https://doi.org/10.1126/science.aam9949

Cao M, Li Y, Wu QJ, Zhang P, Li WT, Mao ZY, Wu DM, Jiang XM, Zhuo Y, Fang ZF, Che LQ, Xu SY, Feng B, Li J, Lin Y, Wu D (2019) Effects of dietary *Clostridium butyricum* addition to sows in late gestation and lactation on reproductive performance and intestinal microbiota1. J Anim Sci 97(8):3426–3439. https://doi.org/10.1093/jas/skz186

Celi P, Cowieson AJ, Fru-Nji F (2017) Gastrointestinal functionality in animal nutrition and health: new opportunities for sustainable animal production. Anim Feed Sci Technol 234:88–100. https://doi.org/10.1016/j.anifeedsci.2017.09.012

Cera KR, Mahan DC, Reinhart GA (1988) Weekly digestibilities of diets supplemented with corn oil, lard or tallow by weanling swine. J Anim Sci 66(6):1430–1437. https://doi.org/10.2527/jas1988.6661430x

Chin VK, Yong VC, Chong PP (2020) Mycobiome in the gut: a multiperspective review. Mediators Inflamm 2020:9560684. https://doi.org/10.1155/2020/9560684

Chu H, Mazmanian SK (2012) Innate immune recognition of the microbiota promotes host-microbial symbiosis. Nat Immunol 14(7):668–675. https://doi.org/10.1038/ni.2635

de Greeff A, Resink JW, van Hees HM, Ruuls L, Klaassen GJ, Rouwers SM, Stockhofe-Zurwieden N (2016) Supplementation of piglets with nutrient-dense complex milk replacer improves intestinal development and microbial fermentation. J Anim Sci 94(3):1012–1019. https://doi.org/10.2527/jas.2015-9481

De Vos M, Che L, Huygelen V, Willemen S, Michiels J, Van Cruchten S, Van Ginneken C (2014) Nutritional interventions to prevent and rear low-birthweight piglets. J Anim Physiol Anim Nutr (Berl) 98(4):609–619. https://doi.org/10.1111/jpn.12133

Difilippo E, Pan F, Logtenberg M, Willems RH, Braber S, Fink-Gremmels J, Schols HA, Gruppen H (2016) In vitro fermentation of porcine milk oligosaccharides and galacto-oligosaccharides using piglet fecal inoculum. J Agric Food Chem 64(10):2127–2133. https://doi.org/10.1021/acs.jafc.5b05384

Dong L, Zhong X, He J, Zhang L, Bai K, Xu W, Wang T, Huang X (2016) Supplementation of tributyrin improves the growth and intestinal digestive and barrier functions in intrauterine growth-restricted piglets. Clin Nutr 35(2):399–407. https://doi.org/10.1016/j.clnu.2015.03.002

Dossa AY, Escobar O, Golden J, Frey MR, Ford HR, Gayer CP (2016) Bile acids regulate intestinal cell proliferation by modulating EGFR and FXR signaling. Am J Physiol Gastrointest Liver Physiol 310(2):G81–G92. https://doi.org/10.1152/ajpgi.00065.2015

Dou S, Gadonna-Widehem P, Rome V, Hamoudi D, Rhazi L, Lakhal L, Larcher T, Bahi-Jaber N, Pinon-Quintana A, Guyonvarch A, Huërou-Luron IL, Abdennebi-Najar L (2017) Characterisation of early-life fecal microbiota in susceptible and healthy pigs to post-weaning diarrhoea. PLoS One 12(1):e0169851. https://doi.org/10.1371/journal.pone.0169851

Erb-Downward JR, Huffnagle GB (2007) *Cryptococcus neoformans* produces authentic prostaglandin E2 without a cyclooxygenase. Eukaryot Cell 6(2):346–350. https://doi.org/10.1128/EC.00336-06

Erb-Downward JR, Noverr MC (2007) Characterization of prostaglandin E2 production by Candida albicans. Infect Immun 75(7):3498–3505. https://doi.org/10.1128/IAI.00232-07

Ferret-Bernard S, Le Normand L, Romé V, Le Bourgot C, Seeboth J, Savary G, Laurent F, Le Huërou-Luron I, Guzylack-Piriou L (2020) Maternal supplementation of food ingredient (prebiotic) or food contaminant (mycotoxin) influences mucosal immune system in piglets. Nutrients 12(7):2115. https://doi.org/10.3390/nu12072115

Flint HJ, Duncan SH, Scott KP, Louis P (2015) Links between diet, gut microbiota composition and gut metabolism. Proc Nutr Soc 74(1):13–22. https://doi.org/10.1017/S0029665114001463

Frese SA, Parker K, Calvert CC, Mills DA (2015) Diet shapes the gut microbiome of pigs during nursing and weaning. Microbiome 3:28. https://doi.org/10.1186/s40168-015-0091-8

Furukawa S, Kuroda Y, Sugiyama A (2014a) A comparison of the histological structure of the placenta in experimental animals. J Toxicol Pathol 27(1):11–18. Published correction appears in J Toxicol Pathol 29(1):74. https://doi.org/10.1293/tox.2013-0060

Furukawa S, Kuroda Y, Sugiyama A (2014b) A comparison of the histological structure of the placenta in experimental animals. J Toxicol Pathol 27(1):11–18. Published correction appears in J Toxicol Pathol 29(1):74. https://doi.org/10.1293/tox.2013-0060

Gomez de Agüero M, Ganal-Vonarburg SC, Fuhrer T (2016) The maternal microbiota drives early postnatal innate immune development. Science 351(6279):1296–1302. https://doi.org/10.1126/science.aad2571

Gourbeyre P, Berri M, Lippi Y, Meurens F, Vincent-Naulleau S, Laffitte J, Rogel-Gaillard C, Pinton P, Oswald IP (2015) Pattern recognition receptors in the gut: analysis of their expression along the intestinal tract and the crypt/villus axis. Physiol Rep 3(2):e12225. https://doi.org/10.14814/phy2.12225

Grześkowiak Ł, Teske J, Zentek J, Vahjen W (2020) Distinct patterns of microbial metabolic fingerprints in sows and their offspring: a pilot study. Arch Microbiol 202(3):511–517. https://doi.org/10.1007/s00203-019-01766-1

Guevarra RB, Lee JH, Lee SH, Seok MJ, Kim DW, Kang BN, Johnson TJ, Isaacson RE, Kim HB (2019) Piglet gut microbial shifts early in life: causes and effects. J Anim Sci Biotechnol 10:1. https://doi.org/10.1186/s40104-018-0308-3

Harada E, Kiriyama H, Kobayashi E, Tsuchita H (1988) Postnatal development of biliary and pancreatic exocrine secretion in piglets. Comp Biochem Physiol A Comp Physiol 91(1):43–51. https://doi.org/10.1016/0300-9629(88)91590-3

Heo JM, Opapeju FO, Pluske JR, Kim JC, Hampson DJ, Nyachoti CM (2013) Gastrointestinal health and function in weaned pigs: a review of feeding strategies to control post-weaning diarrhoea without using in-feed antimicrobial compounds. J Anim Physiol Anim Nutr (Berl) 97(2):207–237. https://doi.org/10.1111/j.1439-0396.2012.01284.x

Huang A, Cai R, Wang Q, Shi L, Li C, Yan H (2019) Dynamic change of gut microbiota during porcine epidemic diarrhea virus infection in suckling piglets. Front Microbiol 10:322. https://doi.org/10.3389/fmicb.2019.00322

Huguet A, Sève B, Le Dividich J, Le Huërou-Luron I (2006) Effects of a bovine colostrum-supplemented diet on some gut parameters in weaned piglets. Reprod Nutr Dev 46(2):167–178. https://doi.org/10.1051/rnd:2006006

Jacobson A, Lam L, Rajendram M, Tamburini F, Honeycutt J, Pham T, Van Treuren W, Pruss K, Stabler SR, Lugo K, Bouley DM, Vilches-Moure JG, Smith M, Sonnenburg JL, Bhatt AS, Huang KC, Monack D (2018) A gut commensal-produced metabolite mediates colonization resistance to salmonella infection. Cell Host Microbe 24(2):296–307.e7. https://doi.org/10.1016/j.chom.2018.07.002

Jensen ML, Sangild PT, Lykke M, Schmidt M, Boye M, Jensen BB, Thymann T (2013) Similar efficacy of human banked milk and bovine colostrum to decrease incidence of necrotizing enterocolitis in preterm piglets. Am J Physiol Regul Integr Comp Physiol 305(1):R4–R12. https://doi.org/10.1152/ajpregu.00094.2013

Jiménez E, Fernández L, Marín ML, Martín R, Odriozola JM, Nueno-Palop C, Narbad A, Olivares M, Xaus J, Rodríguez JM (2005) Isolation of commensal bacteria from umbilical cord blood of healthy neonates born by cesarean section. Curr Microbiol 51(4):270–274. https://doi.org/10.1007/s00284-005-0020-3

Jørgensen JR, Clausen MR, Mortensen PB (1997) Oxidation of short and medium chain C2-C8 fatty acids in Spraaue-Dawley rat colonocytes. Gut 40:400–405

Kang CH, Kim Y, Han SH, Kim JS, Paek NS, So JS (2018) In vitro probiotic properties of vaginal *Lactobacillus fermentum* MG901 and *Lactobacillus plantarum* MG989 against *Candida albicans*. Eur J Obstet Gynecol Reprod Biol 228:232–237. https://doi.org/10.1016/j.ejogrb.2018.07.005

Kelly D, King TP, Brown DS, McFadyen M (1991) Polyamide profiles of porcine milk and of intestinal tissue of pigs during suckling. Reprod Nutr Dev 31(1):73–80. https://doi.org/10.1051/rnd:19910107

Kim HB, Isaacson RE (2015) The pig gut microbial diversity: understanding the pig gut microbial ecology through the next generation high throughput sequencing. Vet Microbiol 177 (3–4):242–251. https://doi.org/10.1016/j.vetmic.2015.03.014

Kimura I, Ichimura A, Ohue-Kitano R, Igarashi M (2020) Free fatty acid receptors in health and disease. Physiol Rev 100(1):171–210. https://doi.org/10.1152/physrev.00041.2018

Kogut MH (2019) The effect of microbiome modulation on the intestinal health of poultry. Anim Feed Sci Technol 250:32–30. https://doi.org/10.1016/j.anifeedsci.2018.10.008

Konstantinov SR, Awati A, Smidt H, Williams BA, Akkermans AD, de Vos WM (2004) Specific response of a novel and abundant Lactobacillus amylovorus-like phylotype to dietary prebiotics in the guts of weaning piglets. Appl Environ Microbiol 70(7):3821–3830. https://doi.org/10.1128/AEM.70.7.3821-3830.2004

Kotunia A, Woliński J, Laubitz D, Jurkowska M, Romé V, Guilloteau P, Zabielski R (2004) Effect of sodium butyrate on the small intestine development in neonatal piglets fed [correction of feed] by artificial sow. J Physiol Pharmacol 55(Suppl. 2):59–68

Lamas A, Regal P, Vázquez B, Cepeda A, Franco CM (2019) Short-chain fatty acids commonly produced by gut microbiota influence *Salmonella enterica* motility, biofilm formation, and gene expression. Antibiotics (Basel) 8(4):265. https://doi.org/10.3390/antibiotics8040265

Lauridsen C (2020) Effects of dietary fatty acids on gut health and function of pigs pre- and post-weaning. J Anim Sci 98(4):skaa086. https://doi.org/10.1093/jas/skaa086

Lewis DS, Oren S, Wang X, Moyer ML, Beitz DC, Knight TJ, Mott GE (2000) Developmental changes in cholesterol 7alpha- and 27-hydroxylases in the piglet. J Anim Sci 78(4):943–951. https://doi.org/10.2527/2000.784943x

Li J, Luo Y, Chen D, Yu B, He J, Huang Z, Mao X, Zheng P, Yu J, Luo J, Tian G, Yan H, Wang Q, Wang H (2020) The fungal community and its interaction with the concentration of short-chain fatty acids in the caecum and colon of weaned piglets. J Anim Physiol Anim Nutr (Berl) 104(2):616–628. https://doi.org/10.1111/jpn.13300

Lin S, Yang X, Yuan P, Yang J, Wang P, Zhong H, Zhang X, Che L, Feng B, Li J, Zhuo Y, Lin Y, Xu S, Wu D, Burrin DG, Fang Z (2019) Undernutrition shapes the gut microbiota and bile acid profile in association with altered gut-liver FXR signaling in weaning pigs. J Agric Food Chem 67(13):3691–3701. https://doi.org/10.1021/acs.jafc.9b01332

Liu H, Zeng X, Zhang G, Hou C, Li N, Yu H, Shang L, Zhang X, Trevisi P, Yang F, Liu Z, Qiao S (2019) Maternal milk and fecal microbes guide the spatiotemporal development of mucosa-associated microbiota and barrier function in the porcine neonatal gut. BMC Biol 17(1):106. https://doi.org/10.1186/s12915-019-0729-2

López-Colom P, Castillejos L, Rodríguez-Sorrento A, Puyalto M, Mallo JJ, Martín-Orúe SM (2020) Impact of in-feed sodium butyrate or sodium heptanoate protected with medium-chain fatty acids on gut health in weaned piglets challenged with *Escherichia coli* F4. Arch Anim Nutr 74(4):271–295. https://doi.org/10.1080/1745039X.2020.1726719

Louis P, Scott KP, Duncan SH, Flint HJ (2007) Understanding the effects of diet on bacterial metabolism in the large intestine. J Appl Microbiol 102(5):1197–1208. https://doi.org/10.1111/j.1365-2672.2007.03322.x

Luethy PM, Huynh S, Ribardo DA, Winter SE, Parker CT, Hendrixson DR (2017) Microbiota-derived short-chain fatty acids modulate expression of *Campylobacter jejuni* determinants required for commensalism and virulence. MBio 8(3):e00407–e00417. https://doi.org/10.1128/mBio.00407-17

Lyte JM, Lyte M (2019) Review: microbial endocrinology: intersection of microbiology and neurobiology matters to swine health from infection to behavior. Animal 13(11):2689–2698. https://doi.org/10.1017/S1751731119000284

Mach N, Berri M, Estellé J, Levenez F, Lemonnier G, Denis C, Leplat JJ, Chevaleyre C, Billon Y, Doré J, Rogel-Gaillard C, Lepage P (2015) Early-life establishment of the swine gut microbiome and impact on host phenotypes. Environ Microbiol Rep 7(3):554–569. https://doi.org/10.1111/1758-2229.12285

Manzanilla EG, Nofrarías M, Anguita M, Castillo M, Perez JF, Martín-Orúe SM, Kamel C, Gasa J (2006) Effects of butyrate, avilamycin, and a plant extract combination on the intestinal equilibrium of early-weaned pigs. J Anim Sci 84(10):2743–2751. https://doi.org/10.2527/jas.2005-509

McCormack UM, Curião T, Wilkinson T, Metzler-Zebeli BU, Reyer H, Ryan T, Calderon-Diaz JA, Crispie F, Cotter PD, Creevey CJ, Gardiner GE, Lawlor PG (2018) Fecal microbiota transplantation in gestating sows and neonatal offspring alters lifetime intestinal microbiota and growth in offspring. mSystems 3(3):e00134–e00117. https://doi.org/10.1128/mSystems.00134-17

McCormack UM, Curião T, Metzler-Zebeli BU, Wilkinson T, Reyer H, Crispie F, Cotter PD, Creevey CJ, Gardiner GE, Lawlor PG (2019) Improvement of feed efficiency in pigs through microbial modulation via fecal microbiota transplantation in sows and dietary supplementation of inulin in offspring. Appl Environ Microbiol 85(22):e01255–e01219. https://doi.org/10.1128/AEM.01255-19

McKenzie C, Tan J, Macia L, Mackay CR (2017) The nutrition-gut microbiome-physiology axis and allergic diseases. Immunol Rev 278(1):277–295. https://doi.org/10.1111/imr.12556

Melo AD, Silveira H, Luciano FB, Andrade C, Costa LB, Rostagno MH (2016) Intestinal alkaline phosphatase: potential roles in promoting gut health in weanling piglets and its modulation by feed additives—a review. Asian-Australas J Anim Sci 29(1):16–22. https://doi.org/10.5713/ajas.15.0120

Metzler B, Bauer E, Mosenthin R (2005) Microflora management in the gastrointestinal tract of piglets. Asian-Australas J Anim Sci 18(9):1353–1362

Metzler-Zebeli BU, Gänzle MG, Mosenthin R, Zijlstra RT (2012) Oat β-glucan and dietary calcium and phosphorus differentially modify intestinal expression of proinflammatory cytokines and monocarboxylate transporter 1 and cecal morphology in weaned pigs. J Nutr 142(4):668–674. https://doi.org/10.3945/jn.111.153007

Metzler-Zebeli BU, Ertl R, Grüll D, Molnar T, Zebeli Q (2017) Enzymatically modified starch up-regulates expression of incretins and sodium-coupled monocarboxylate transporter in jejunum of growing pigs. Animal 11(7):1180–1188. https://doi.org/10.1017/S1751731116002615

Metzler-Zebeli BU, Klinsoda J, Vötterl JC, Verhovsek D (2020) Maturational changes alter effects of dietary phytase supplementation on the fecal microbiome in fattening pigs. Microorganisms 8(7):1073. https://doi.org/10.3390/microorganisms8071073

Møller HK, Thymann T, Fink LN, Frokiaer H, Kvistgaard AS, Sangild PT (2011) Bovine colostrum is superior to enriched formulas in stimulating intestinal function and necrotising enterocolitis resistance in preterm pigs. Br J Nutr 105(1):44–53. https://doi.org/10.1017/S0007114510003168

Mu C, Cai Z, Bian G, Du Y, Ma S, Su Y, Liu L, Voglmeir J, Huang R, Zhu W (2019) New insights into porcine milk N-glycome and the potential relation with offspring gut microbiome. J Proteome Res 18(3):1114–1124. https://doi.org/10.1021/acs.jproteome.8b00789

Mudd AT, Salcedo J, Alexander LS, Johnson SK, Getty CM, Chichlowski M, Berg BM, Barile D, Dilger RN (2016) Porcine milk oligosaccharides and sialic acid concentrations vary throughout lactation. Front Nutr 3:39. https://doi.org/10.3389/fnut.2016.00039

Muns R, Magowan E (2018) The effect of creep feed intake and starter diet allowance on piglets' gut structure and growth performance after weaning. J Anim Sci 96(9):3815–3823. https://doi.org/10.1093/jas/sky239

Nakanishi N, Tashiro K, Kuhara S, Hayashi T, Sugimoto N, Tobe T (2009) Regulation of virulence by butyrate sensing in enterohaemorrhagic *Escherichia coli*. Microbiology 155:521–530. https://doi.org/10.1099/mic.0.023499-0

Nakashima A, Yamada K, Iwata O, Sugimoto R, Atsuji K, Ogawa T, Ishibashi-Ohgo N, Suzuki K (2018) β-Glucan in foods and its physiological functions. J Nutr Sci Vitaminol (Tokyo) 64(1):8–17. https://doi.org/10.3177/jnsv.64.8

Nakatani M, Inoue R, Tomonaga S, Fukuta K, Tsukahara T (2018) Production, absorption, and blood flow dynamics of short-chain fatty acids produced by fermentation in piglet hindgut during the suckling-weaning period. Nutrients 10(9):1220. https://doi.org/10.3390/nu10091220

Newman MA, Petri RM, Grüll D, Zebeli Q, Metzler-Zebeli BU (2018) Transglycosylated starch modulates the gut microbiome and expression of genes related to lipid synthesis in liver and adipose tissue of pigs. Front Microbiol 9:224. https://doi.org/10.3389/fmicb.2018.00224

Ng KM, Ferreyra JA, Higginbottom SK, Lynch JB, Kashyap PC, Gopinath S, Naidu N, Choudhury B, Weimer BC, Monack DM, Sonnenburg JL (2013) Microbiota-liberated host sugars facilitate post-antibiotic expansion of enteric pathogens. Nature 502:96–99. https://doi.org/10.1038/nature12503

Noverr MC, Phare SM, Toews GB, Coffey MJ, Huffnagle GB (2001) Pathogenic yeasts *Cryptococcus neoformans* and *Candida albicans* produce immunomodulatory prostaglandins. Infect Immun 69(5):2957–2963. https://doi.org/10.1128/IAI.69.5.2957-2963.2001

Nowland TL, Torok VA, Low WY, Barton MD, Plush KJ, Kirkwood RN (2020) Faecal microbiota analysis of piglets during lactation. Animals (Basel) 10(5):762. https://doi.org/10.3390/ani10050762

Pajor EA, Fraser D, Kramer DL (1991) Consumption of solid food by suckling pigs: individual variation and relation to weight gain. Appl Anim Behav Sci 32:139–155

Paßlack N, Vahjen W, Zentek J (2015) Dietary inulin affects the intestinal microbiota in sows and their suckling piglets. BMC Vet Res 11:51. https://doi.org/10.1186/s12917-015-0351-7

Pepoyan A, Balayan M, Manvelyan A, Galstyan L, Pepoyan S, Petrosyan S, Tsaturyan V, Kamiya S, Torok T, Chikindas M (2018) Probiotic *Lactobacillus acidophilus* strain INMIA 9602 Er 317/402 administration reduces the numbers of *Candida albicans* and abundance of enterobacteria in the gut microbiota of familial mediterranean fever patients. Front Immunol 9:1426. https://doi.org/10.3389/fimmu.2018.01426

Petersen V (1994) The development of feeding and investigatory behaviour in free-ranging domestic pigs during their first 18 weeks of life. Appl Anim Behav Sci 42(2):87–98

Pié S, Lallès JP, Blazy F, Laffitte J, Sève B, Oswald IP (2004) Weaning is associated with an upregulation of expression of inflammatory cytokines in the intestine of piglets. J Nutr 134(3):641–647. https://doi.org/10.1093/jn/134.3.641

Piva A, Prandini A, Fiorentini L, Morlacchini M, Galvano F, Luchansky JB (2002) Tributyrin and lactitol synergistically enhanced the trophic status of the intestinal mucosa and reduced histamine levels in the gut of nursery pigs. J Anim Sci 80(3):670–680. https://doi.org/10.2527/2002.803670x

Pluske JR, Turpin DL, Kim JC (2018) Gastrointestinal tract (gut) health in the young pig. Anim Nutr 4(2):187–196. https://doi.org/10.1016/j.aninu.2017.12.004

Pollock J, Gally DL, Glendinning L, Tiwari R, Hutchings MR, Houdijk JGM (2018) Analysis of temporal fecal microbiota dynamics in weaner pigs with and without exposure to enterotoxigenic Escherichia coli. J Anim Sci 96(9):3777–3790. https://doi.org/10.1093/jas/sky260

Poulsen AR, de Jonge N, Sugiharto S, Nielsen JL, Lauridsen C, Canibe N (2017) The microbial community of the gut differs between piglets fed sow milk, milk replacer or bovine colostrum. Br J Nutr 117(7):964–978. https://doi.org/10.1017/S0007114517000216

Price AE, Shamardani K, Lugo KA, Deguine J, Roberts AW, Lee BL, Barton GM (2018) A map of toll-like receptor expression in the intestinal epithelium reveals distinct spatial, cell type-specific, and temporal patterns. Immunity 49:560–575.e6. https://doi.org/10.1016/j.immuni.2018.07.016

Quesnel H, Farmer C (2019) Review: nutritional and endocrine control of colostrogenesis in swine. Animal 13(S1):s26–s34. https://doi.org/10.1017/S1751731118003555

Quinn EM, Joshi L, Hickey RM (2020) Symposium review: dairy-derived oligosaccharides-their influence on host-microbe interactions in the gastrointestinal tract of infants. J Dairy Sci 103(4):3816–3827. https://doi.org/10.3168/jds.2019-17645

Radcliffe JS (2011) Nonruminant nutrition symposium: nutrient and non-nutrient sensing and signaling in the gastrointestinal tract. J Anim Sci 89(6):1853. https://doi.org/10.2527/jas.2011-4117

Rao JN, Platoshyn O, Golovina VA, Liu L, Zou T, Marasa BS, Turner DJ, Yuan JX, Wang JY (2006) TRPC1 functions as a store-operated Ca2+ channel in intestinal epithelial cells and regulates early mucosal restitution after wounding. Am J Physiol Gastrointest Liver Physiol 290:G782–G792. https://doi.org/10.1152/ajpgi.00441.2005

Ren S, Hui Y, Goericke-Pesch S, Pankratova S, Kot W, Pan X, Thymann T, Sangild PT, Nguyen DN (2019) Gut and immune effects of bioactive milk factors in preterm pigs exposed to prenatal inflammation. Am J Physiol Gastrointest Liver Physiol 317(1):G67–G77. https://doi.org/10.1152/ajpgi.00042.2019

Ruczizka U, Metzler-Zebeli B, Unterweger C, Mann E, Schwarz L, Knecht C, Hennig-Pauka I (2019) Early parenteral administration of ceftiofur has gender-specific short- and long-term effects on the fecal microbiota and growth in pigs from the suckling to growing phase. Animals (Basel) 10(1):17. https://doi.org/10.3390/ani10010017

Salcedo J, Frese SA, Mills DA, Barile D (2016) Characterization of porcine milk oligosaccharides during early lactation and their relation to the fecal microbiome. J Dairy Sci 99(10):7733–7743. https://doi.org/10.3168/jds.2016-10966

Sayin SI, Wahlström A, Felin J, Jäntti S, Marschall HU, Bamberg K, Angelin B, Hyötyläinen T, Orešič M, Bäckhed F (2013) Gut microbiota regulates bile acid metabolism by reducing the levels of tauro-beta-muricholic acid, a naturally occurring FXR antagonist. Cell Metab 17(2):225–235. https://doi.org/10.1016/j.cmet.2013.01.003

Schokker D, Zhang J, Zhang LL, Vastenhouw SA, Heilig HG, Smidt H, Rebel JM, Smits MA (2014) Early-life environmental variation affects intestinal microbiota and immune development in new-born piglets. PLoS One 9(6):e100040. https://doi.org/10.1371/journal.pone.0100040

Schokker D, Veninga G, Vastenhouw SA, Bossers A, de Bree FM, Kaal-Lansbergen LM, Rebel JM, Smits MA (2015a) Early life microbial colonization of the gut and intestinal development differ between genetically divergent broiler lines. BMC Genom 16:418. https://doi.org/10.1186/s12864-015-1646-6

Schokker D, Zhang J, Vastenhouw SA, Heilig HG, Smidt H, Rebel JM, Smits MA (2015b) Long-lasting effects of early-life antibiotic treatment and routine animal handling on gut microbiota composition and immune system in pigs. PLoS One 10(2):e0116523. https://doi.org/10.1371/journal.pone.0116523

Schokker D, Fledderus J, Jansen R, Vastenhouw SA, de Bree FM, Smits MA, Jansman AAJM (2018) Supplementation of fructooligosaccharides to suckling piglets affects intestinal

microbiota colonization and immune development. J Anim Sci 96(6):2139–2153. https://doi.org/10.1093/jas/sky110

Shrestha A, Metzler-Zebeli BU, Karembe H, Sperling D, Koger S, Joachim A (2020) Shifts in the fecal microbial community of *Cystoisospora suis* infected piglets in response to toltrazuril. Front Microbiol 11:983. https://doi.org/10.3389/fmicb.2020.00983

Sinha SR, Haileselassie Y, Nguyen LP, Tropini C, Wang M, Becker LS, Sim D, Jarr K, Spear ET, Singh G, Namkoong H, Bittinger K, Fischbach MA, Sonnenburg JL, Habtezion A (2020) Dysbiosis-induced secondary bile acid deficiency promotes intestinal inflammation. Cell Host Microbe 27(4):659–670.e5. https://doi.org/10.1016/j.chom.2020.01.021

Stecher B, Jung K (2018) LACTATEing salmonella: a host-derived fermentation product fuels pathogen growth. Cell Host Microbe 23(1):3–4. https://doi.org/10.1016/j.chom.2017.12.012

Sugiharto S, Poulsen AS, Canibe N, Lauridsen C (2015) Effect of bovine colostrum feeding in comparison with milk replacer and natural feeding on the immune responses and colonisation of enterotoxigenic *Escherichia coli* in the intestinal tissue of piglets. Br J Nutr 113(6):923–934. https://doi.org/10.1017/S0007114514003201

Summers KL, Frey JF, Ramsay TG, Arfken AM (2019) The piglet mycobiome during the weaning transition: a pilot study. J Anim Sci 97(7):2889–2900. https://doi.org/10.1093/jas/skz182

Tao N, Ochonicky KL, German JB, Donovan SM, Lebrilla CB (2010) Structural determination and daily variations of porcine milk oligosaccharides. J Agric Food Chem 58:4653–4659. https://doi.org/10.1021/jf100398u

Tao X, Xu Z, Men X (2015) Transient changes of enzyme activities and expression of stress proteins in the small intestine of piglets after weaning. Arch Anim Nutr 69(3):201–211. https://doi.org/10.1080/1745039X.2015.1034828

Tremaroli V, Bäckhed F (2012) Functional interactions between the gut microbiota and host metabolism. Nature 489(7415):242–249. https://doi.org/10.1038/nature11552

Trevisi P, Priori D, Jansman AJM, Luise D, Koopmans SJ, Hynönen U, Palva A, van der Meulen J, Bosi P (2018) Molecular networks affected by neonatal microbial colonization in porcine jejunum, luminally perfused with enterotoxigenic *Escherichia coli*, F4ac fimbria or *Lactobacillus amylovorus*. PLoS One 13(8):e0202160. https://doi.org/10.1371/journal.pone.0202160

Villageliú DN, Rasmussen S, Lyte M (2018) A microbial endocrinology-based simulated small intestinal medium for the evaluation of neurochemical production by gut microbiota. FEMS Microbiol Ecol 94(7). https://doi.org/10.1093/femsec/fiy096

Wang J, Li GR, Tan BE, Xiong X, Kong XF, Xiao DF, Xu LW, Wu MM, Huang B, Kim SW, Yin YL (2015) Oral administration of putrescine and proline during the suckling period improves epithelial restitution after early weaning in piglets. J Anim Sci 93(4):1679–1688. https://doi.org/10.2527/jas.2014-8230

Wang Q, Sun Q, Qi R, Wang J, Qiu X, Liu Z, Huang J (2019) Effects of *Lactobacillus plantarum* on the intestinal morphology, intestinal barrier function and microbiota composition of suckling piglets. J Anim Physiol Anim Nutr (Berl) 103(6):1908–1918. https://doi.org/10.1111/jpn.13198

Wei J, Wang ZA, Wang B, Jahan M, Wang Z, Wynn PC, Du Y (2018) Characterization of porcine milk oligosaccharides over lactation between primiparous and multiparous female pigs. Sci Rep 8(1):4688. https://doi.org/10.1038/s41598-018-23025-x

Willing BP, van Kessel AG (2008) Intestinal microbiota differentially affect brush border enzyme activity and gene expression in the neonatal gnotobiotic pig. J Anim Physiol Anim Nutr (Berl) 93(5):586–595. https://doi.org/10.1111/j.1439-0396.2008.00841.x

Willing BP, Van Kessel AG (2009) Intestinal microbiota differentially affect brush border enzyme activity and gene expression in the neonatal gnotobiotic pig. J Anim Physiol Anim Nutr (Berl) 93(5):586–595. https://doi.org/10.1111/j.1439-0396.2008.00841.x

Willing BP, Van Kessel AG (2010) Host pathways for recognition: establishing gastrointestinal microbiota as relevant in animal health and nutrition. Livest Sci 133(1):82–91. https://doi.org/10.1016/j.livsci.2010.06.031

Xie G, Li X, Li H, Jia W (2013) Toward personalized nutrition: comprehensive phytoprofiling and metabotyping. J Proteome Res 12(4):1547–1559. https://doi.org/10.1021/pr301222b

Yang Y, Galle S, Le MH, Zijlstra RT, Gänzle MG (2015) Feed fermentation with reuteran- and levan-producing *Lactobacillus reuteri* reduces colonization of weanling pigs by enterotoxigenic *Escherichia coli*. Appl Environ Microbiol 81(17):5743–5752. https://doi.org/10.1128/AEM.01525-15

Yanguas-Casás N, Barreda-Manso MA, Nieto-Sampedro M, Romero-Ramírez L (2017) TUDCA: an agonist of the bile acid receptor GPBAR1/TGR5 with anti-inflammatory effects in microglial cells. J Cell Physiol 232(8):2231–2245. https://doi.org/10.1002/jcp.25742

Zhang S, Chen F, Zhang Y, Lv Y, Heng J, Min T, Li L, Guan W (2018) Recent progress of porcine milk components and mammary gland function. J Anim Sci Biotechnol 9:77. https://doi.org/10.1186/s40104-018-0291-8

Zhao W, Wang Y, Liu S, Huang J, Zhai Z, He C et al (2015) The dynamic distribution of porcine microbiota across different ages and gastrointestinal tract segments. PLoS One 10(2):e0117441. https://doi.org/10.1371/journal.pone.0117441

Zivkovic AM, German JB, Lebrilla CB, Mills DA (2011) Human milk glycobiome and its impact on the infant gastrointestinal microbiota. Proc Natl Acad Sci U S A 108:4653–4658. https://doi.org/10.1073/pnas.1000083107

9 The Unseen Minority: Biogeographical Investigations of the Ruminant Gastrointestinal Microbiome Highlight the Importance of Frequently Ignored Anatomical Regions

Herlin Kadriu and Carl Yeoman

Abstract

The ruminant gastrointestinal tract includes the esophagus, rumen, reticulum, omasum, abomasum, duodenum, jejunum, ileum, cecum, and colon, yet two-thirds of research focusing on the "gastrointestinal tract" (GIT) microbiome of ruminants is limited to the reticulum and rumen (reticulorumen). The purpose of this article is to summarize what is presently known about these other "dark-regions" of the ruminant GIT, highlight why they are important to consider, and encourage the rumen microbiology field to explore their relationships to animal production and health.

Keywords

Microbiota · Ruminant · Biogeography · Nutrition · Feed efficiency · GALT · Acidosis

9.1 Introduction

As per the definition provided in Mackie's eco-evolutionary review of host–microbe symbioses in fermentative digestion, "the gastrointestinal tract is a specialized tube divided into various well-defined anatomical regions extending from the lips to the anus" (Mackie 1997). Therein, the primary role of the gastrointestinal tract (GIT) is to facilitate the digestion of feed stuffs and absorbance of nutrients necessary to sustain the host animal's metabolic requirements (Huntington et al. 2006; Kristensen et al. 1998; Yeoman and White 2014). Microbial communities (microbiota) that

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reside within the GIT make important, and in the case of mature ruminants, indispensable contributions to these digestive processes (Yeoman and White 2014). Ruminants, a group of around 200 or more mammalian species belonging to the families Antilocapridae, Bovidae, Cervidae, Giraffidae, Moschidae, and Tragulidae that have a characteristically unique GIT morphology wherein a large pre-gastric chamber, comprising the interconnected reticulum and rumen (reticulorumen), provides resident microbiota with first access to ingested feed in, what Mackie describes as the cooperation model (Mackie 1997; Hackmann and Spain 2010; Chen et al. 2019). Because the diets of ruminants typically comprise feed stuffs whose nutrients are mostly locked up in structural carbohydrates, the majority of which are not endogenously digestible, these animals depend on their GIT microbiota to collectively convert ingested herbivorous materials to utilizable nutrients (Flint and Bayer 2008; El Kaoutari et al. 2013). Microbes residing within the ruminant GIT collectively encode for a large repertoire of hydrolytic enzymes, including numerous different glycoside hydrolases, carbohydrate esterases, and polysaccharide lyases that collectively and synergistically degrade these structural carbohydrates sufficiently to enable their import into the microbial cell (Hess et al. 2011; El Kaoutari et al. 2013; Flint et al. 2012). Once inside the microbial cell, additional processing involving fermentative enzymes generates energy for the microbe and leads to terminal products, including short chain fatty acids (SCFAs) (Seshadri et al. 2018), which being of no further nutritive value to the anaerobic microbe, are excreted from the microbial cell so the cell can maintain physiological pH (Yeoman and White 2014). The ruminant host makes use of these SCFAs for de novo fatty acid biosynthesis (i.e., acetate), gluconeogenesis (i.e., propionate), and, in the case of butyrate, as the major source of energy for colonic and ruminal epithelia, which metabolize butyrate to β-hydroxybutyrate, a ketone body of further potential energetic value (Britton and Krehbiel 1993; Cahill 2006). The metabolism of SCFAs by ruminants collectively contributes approximately 70% of the animal's daily energy requirements (Armentano 1992; Van Soest 1994). While of limited relevance to this article, it should be noted that SCFAs, and in particular butyrate is recognized as having additional indirect nutritive and non-nutritive beneficial roles for the host as has been demonstrated and reviewed many times in recent years (Frampton et al. 2020; Gonçalves et al. 2018; Dalile et al. 2019; Sukkar et al. 2019). Within the GIT of mature ruminant animals approximately 80–90% of total SCFAs are produced by microbial fermentation in the rumen (Oh et al. 1972) and, likely due to this factor, more than two-thirds of all research on the ruminant GIT microbiota has focused exclusively on the rumen (Fig. 9.1) with the majority of all research examining non-ruminal regions of the GIT occurring in the last 10 years and most by just a handful of researchers. As Van Soest once noted "Judging from the emphasis, one would think ruminants had no lower tract" (Van Soest 1994), however, microbes reside throughout the GIT and studies in recent years have provided evidence that these more distal regions of the GIT deserve further exploration.

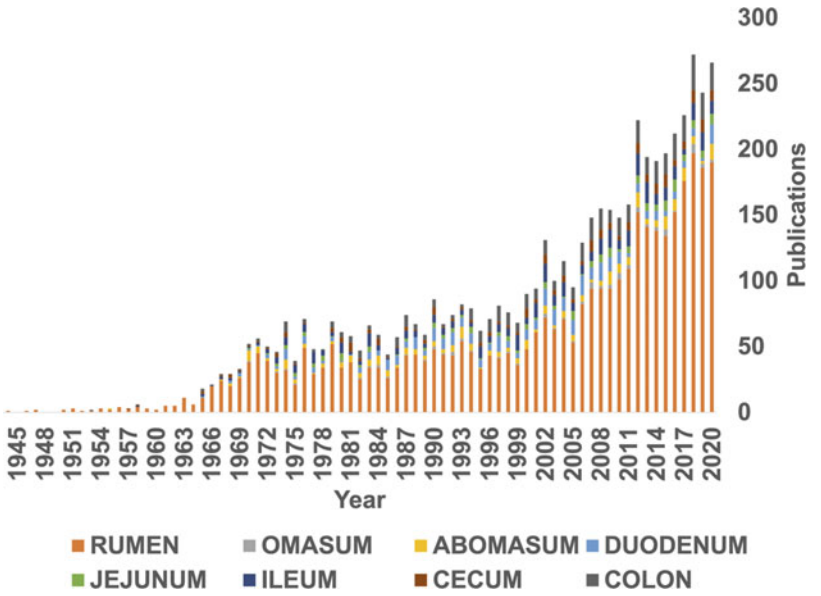

Fig. 9.1 Publications on various regions of the ruminant GIT by year. Publications were extracted from PubMed using the search strings [(Cattle OR bovine OR Sheep OR ovine OR ruminant) AND [GIT Region] AND (microbiota OR microbiome OR bacteriome OR 16S OR metagenome OR bacteria OR fungi OR archaea)] and manually curated to remove cross species comparisons that did not interrogate the microbiota of appropriate GIT region in the ruminant host

9.2 Nutritional Ecology of the Entire Ruminant GIT Microbiota

Following birth, all regions of the ruminant's GIT undergo a successional acquisition of microbial life with major contributions from maternal and environmental sources (Yeoman et al. 2018; Bi et al. 2019; Furman et al. 2020). While diet is firmly established as a major influence on the adult ruminant's GIT microbiota (Henderson et al. 2015), dietary transitions in early life also appear to correspond with large-scale restructuring events of, at least the rumen and colon (Yu et al. 2020; Furman et al. 2020). This is, at least in part likely reflective of the ruminant's unique esophageal groove that initially causes ingested feed to bypass the reticulorumen of neonatal animals passing feed directly into the abomasum (Comline and Titchen 1951).

Several studies have now shown that the density (Yeoman and White 2014), diversity, and composition of microbiota variy biospatially (among the different regions of the GIT) (Mao et al. 2015; Wang et al. 2017a; Perea et al. 2017; Thomas et al. 2017; Zhang et al. 2018; Chong et al. 2020) and biogeographically (through space and time) (Michelland et al. 2009; Yeoman et al. 2018; Bi et al. 2019; Zhuang et al. 2020). Analyses suggest the microbial composition differs more between more distantly separated regions of the GIT (e.g., between the jejunum and colon) than between GIT regions that are nearer one another (e.g., between the jejunum and

duodenum), perhaps reflecting a sink:source influence (Mao et al. 2015; Wang et al. 2017a; Perea et al. 2017; Chong et al. 2020). The colonic microbiota, alike that of the rumen, appear to be inhabited by common fibrolytic (fiber-degrading) microbial taxa (Forsberg et al. 1997; Perea et al. 2017), albeit with greater proportions of Firmicutes and lesser proportions of Bacteroidetes in the luminal portions than are seen in the reticulorumen (Mao et al. 2015; Perea et al. 2017; Yeoman et al. 2018). Metaproteomic analysis has identified proteins involved in starch- and fucose-degradation as being abundant in the distal GIT (Tanca et al. 2017) and studies on the disappearance of the predominant plant structural carbohydrates indicate 5–18% of cellulose and 9–24% of hemicelluloses are utilized in the colon and cecum (Beever et al. 1972), with the grinding of feedstuffs before feeding increasing these proportions up to as much as 31% of cellulose and 41% of hemicellulose fibers (Hogan and Weston 1967). The effect of grinding on the proportion of fiber degraded in the distal GIT is likely mediated by reductions in particle size which is a primary factor governing the time feed is retained in the rumen (Welch 1982), affecting its degradative and fermentative potential as well as the populations of bacteria in the rumen (Ishaq et al. 2019). Consistent, with the fibrolytic activity and observed disappearance of structural fiber in the colon, microbial activities in the distal GIT contribute to the total amount of SCFA produced in the ruminant GIT (Oh et al. 1972; Mao et al. 2015) with SCFA concentrations in this region being responsive to diet (Wang et al. 2017b). In addition, methanogenic archaea, whose hydrogenotrophic (hydrogen-consuming) activities are almost invariably tied to ruminal fiber-degradation (Janssen and Kirs 2008), wherein their removal of hydrogen ensures maximal fibrolytic efficiency (Forsberg et al. 1997; Piao et al. 2014) are also present in the distal GIT (Tanca et al. 2017). Despite these functional overlaps, the composition of the colonic microbiota is significantly different from that of the rumen (Mao et al. 2015; Perea et al. 2017). Both the rumen and colon do exhibit high microbial α-diversity and comparatively little between-animal variation in their β-diversity (microbial composition) (Mao et al. 2015; Perea et al. 2017). By contrast, progressively greater between animal variation is observed for microbiota of the duodenum, jejunum, and ileum (Perea et al. 2017), which may reflect more rapid turnover in these regions as has been reported for the human ileal microbiota (Zoetendal et al. 2012). Microbiota in the small intestine are often seen to comprise higher proportions of Proteobacteria than are observed elsewhere (Mao et al. 2015; Perea et al. 2017). Alike other regions of the GIT, the small intestinal microbiota appear responsive to diet and given microbial metabolism of bile acids occurs in this region may have an important, but as yet unknown role in the animal's metabolism (Liu et al. 2020).

9.3 Feed Efficiency is Associated with Differences in Small Intestinal and Colonic Microbes

Given the critical role GIT-residing microbiota play in ruminant nutrition, it is not surprising that their composition has been observed to co-vary with measures of feed efficiency. Feed efficiency broadly refers to how efficiently an animal utilizes its feed, typically combining measures of feed intake with animal productivity to identify animals that can produce more (or sustain) with less (high feed efficiency) or vice versa (low feed efficiency). One commonly applied metric for assessing feed efficiency is residual feed intake (RFI), a measure of the difference between how much food an animal consumes relative to how much it is expected to consume based on the animal's weight (Arthur and Herd 2008). Numerous studies have collectively shown that differences in RFI are associated with variations in the presence, relative abundances, gene content, and/or activities of select members of the rumen microbiome, including members of all three microbial domains (bacterial, archaeal, and eukaryotic) (Guan et al. 2008; Hernandez-Sanabria et al. 2012; McCann et al. 2014; Myer et al. 2015; Li and Guan 2017; Shabat et al. 2016; Zhang et al. 2020; McLoughlin et al. 2020). As might be reasonably hypothesized, some of these studies have shown these microbial variations also correspond to differences in the concentrations of various energy-yielding SCFAs (Guan et al. 2008; Shabat et al. 2016). It is, therefore, important to note that the concentrations of SCFAs in the distal GIT are similar to those in the reticulorumen (Mao et al. 2015) and only differ in their contributions to the total SCFA amounts due to differences in the total volumes of the two GIT regions (Oh et al. 1972). Regardless, 7–18% of total SCFAs are produced outside of the rumen, with most being produced in the more distally located cecum (~6–14%) and colon (~1–3%) of the mature ruminant (Oh et al. 1972). The proportion of SCFAs produced in the distal GIT is higher in a dry lot (Oh et al. 1972) and possibly higher still in a feed lot, where to the best of our knowledge it has not been examined. Furthermore, the proportionate contribution of these distal GIT regions to total GIT SCFA concentrations is higher still in younger animals, where distal GIT SCFA concentrations exceed those of the rumen microbiota through, at least the first 3 weeks of life (Oh et al. 1972). Therefore, it is perhaps not surprising that in 2017, Perea and colleagues also determined that RFI co-varied with the relative abundances of several taxa in the distal GIT, specifically the colon and feces (the cecum was not evaluated) and these differences involved taxa for which cultivated members have previously been characterized as having fibrolytic roles in the ruminant GIT (Perea et al. 2017). What perhaps was not expected was the finding that differences in RFI also corresponded to differences in microbes located in the small intestine (Perea et al. 2017), where SCFA concentrations have been reported as being much lower to near absent (Oh et al. 1972; Mao et al. 2015). A similar observation was recently reported by Freetly and colleagues who noted differences in 11 jejunal bacterial OTUs of cattle who differed in average daily gain (ADG) when provided ad libitum feed access over 84 days (Freetly et al. 2020). As noted by Perea and colleagues, these differences correspond to taxonomic groups with well-elaborated relationships to GIT health (i.e.,

Bifidobacteria, present only in low-RFI "efficient" animals) and dysbiosis and disease (i.e., Proteobacteria, higher relative abundance in high-RFI "inefficient" animals) (Mitsuoka 2005; Mukhopadhya et al. 2012; Tojo et al. 2014; Yeoman and White 2014; Shin et al. 2015; Arboleya et al. 2016; Rizzatti et al. 2017; Perea et al. 2017). These findings support the previously hypothesized relationship between GIT health and animal productivity (Yeoman and White 2014). Multiple hypotheses exist for the differential relationships of the Proteobacterial phylum and the *Bifidobacterium* genus to health and disease, but one common mediatory linkage is their differing relationships to immune function. Whereas, Bifidobacterium species have been linked to the stimulation and modulation of both systemic and GIT immunity (O'Mahony et al. 2005; Ménard et al. 2008; Savignac et al. 2014; Groeger et al. 2013; Singh et al. 2013), blooms in the Proteobacterial lineage have been linked to immunological dysregulation (Shin et al. 2015). It is, therefore, notable that the largest of all immunological tissues, the Gut-associated lymphatic tissue (GALT) resides in this region of the GIT (Pearson et al. 2012; Lopetuso et al. 2013).

9.4 Potential Contributions of the Distal GIT to Immunological Maturation

The GALT is perhaps one of the most important immunological tissues, representing approximately 70% of all lymphoid tissue (Lopetuso et al. 2013) and housing approximately 80% of all immunoglobulin-producing plasma cells (Lopetuso et al. 2013). The GALT includes both isolated and aggregated lymphoid follicles or tissues (Peyer's patches) that are located along the anti-mesenteric side of the intestine (Press et al. 1992; Pabst et al. 2005). Neonatal ruminants display two types of Peyer's patches: the ileo-cecal and the jejunal Peyer's patches that are regarded as the primary lymphoid tissues (Press et al. 1992). Both types of Peyer's patches have been shown to have high lymphopoiesis activity (generation of lymphocytes). The ileo-cecal Peyer's patches are noted as being the primary generators of plasma cell populations for ruminants (Reynaud et al. 1995), while jejunal Peyer's patches resemble those found in other mammalian species with high apparent T cell capabilities (Hein et al. 1989). For neonatal ruminant animals, the GALT is likely exposed to an increasing density and diversity of microbes acquired from both maternal (Yeoman et al. 2018) and environmental sources (Bi et al. 2019). It has been shown in non-ruminants that the interactions between the GALT and GIT microbiota affects the developing function of the mammalian immune system (Gray et al. 2017; Hu et al. 2018), with insufficient or restricted interactions resulting in smaller Peyer's patches, fewer antibody-producing plasma cells, fewer intraepithelial lymphocytes, impaired antimicrobial peptide and antibody secretion, differences in their rearrangements of immunoglobulin heavy and light chains, and an incomplete cytokine profile, among other immunologic deficiencies (Round and Mazmanian 2009; Ivanov et al. 2009; Wesemann et al. 2013). Complete maturation of the GALT and associated tissues is, therefore, dependent on microbial stimulation (Lopetuso et al. 2013). Although at the time of

writing, limited evidence has been published to show this interaction in the ruminant GIT, exposure to bacteria has been shown to increase IgA receptor expression and increase secretion of mucosal secretory IgA in the ruminant GIT (Taschuk and Griebel 2012). The jejunal and ileo-cecal location of the ruminant GALT provides further motivation for interrogation of these non-ruminal GIT locations. In fact, the rumen is primarily composed of stratified keratinized squamous epithelial cells which are advantageous for absorption, but lack the immunological properties of the mucosal epithelium observed in other regions of the gastrointestinal tract (Graham and Simmons 2005). Given this observation it is critical to assess the implications of dietary and other treatments on the non-ruminal GIT epithelium. Particularly given evidence already exists to show that high grain dietary regimens, which are conducive to ruminal acidosis can cause mucosal injury in the colon and are associated with elevated levels of pro-inflammatory cytokines (Wang et al. 2017b).

9.5 The Colon May Be More Susceptible to Acidosis

In the rearing of domestic ruminant livestock, a common practice is the feeding of high-grain diets, typically in a feed lot over the final portion of the animal's life prior to harvest. This husbandry practice is associated with greater feed efficiency, improved animal performance, increased marbling of meat, and reduced animal methane emissions that result in increased profits for animal producers and lower greenhouse gas emissions (McGeough et al. 2010; Schoonmaker et al. 2010). However, abrupt shifts from a low-quality forage to a high concentration of grains often results in adverse effects on the ruminal microbiota whose rapid fermentation of these easily digestible feed sources leads to unsustainable increases in SCFA and, lactate concentrations that mediate a reduction in ruminal pH. These reductions in ruminal pH and increases in lactic acid affect microbial attachment to fiber particles and inhibit the growth of acid-sensitive microbes limiting fiber degradation (Sung et al. 2007; Herrera et al. 2009) while also adversely affecting the rumen epithelium, including reductions in barrier function that lead to increased passive paracellular diffusion of microbes and microbial endotoxin that often results in septicemia and inflammation, and can ultimately lead to death (Howard 1981; Khafipour et al. 2009; Liu et al. 2013). Recent studies have found that, similar to the rumen, the distal GIT microbiota is affected by a high grain diet, which has been shown to lead to changes in the composition of the colonic microbiota, increases in SCFA and lactic acid concentrations, and corresponding decreases in luminal pH (Wang et al. 2017b; Lin et al. 2020), which are associated with mucosal epithelial damage (Wang et al. 2017b). These effects are especially concerning given monensin, an ionophore commonly used in feedlots that can reduce the risk of ruminal acidosis (Nagaraja et al. 1981; Newbold and Wallace 1988), does not appear to permeate to the colon (Thomas et al. 2017). Thus further research is needed to establish the propensity of the colonic microbiota and epithelium to adapt to high grain diets, the concordance of ruminal- and "colonic-acidosis," the relationship between colonic-acidosis and

feedlot morbidities, the relationship between colonic-acidosis and performance, and to identify nutritional or other preventative or therapeutic interventions.

9.6 Summary

To date, a limited body of research has focused on the non-ruminal portions of the ruminant GIT, yet these studies have exposed important nutritional, immunological, and physiological relationships that may underscore previously unrecognized contributions of the ruminants GIT microbiota to animal health and productivity. This review overviews our present knowledge of these GIT dark-regions and seeks to facilitate research that integrates these important GIT regions into studies involving the ruminant GIT microbiome.

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References

Arboleya S, Watkins C, Stanton C, Ross RP (2016) Gut Bifidobacteria populations in human health and aging. Front Microbiol 7:1204. https://doi.org/10.3389/fmicb.2016.01204

Armentano LE (1992) Ruminant hepatic metabolism of volatile fatty acids, lactate and pyruvate. J Nutr 122(3 Suppl):838–842. https://doi.org/10.1093/jn/122.suppl_3.838

Arthur JPF, Herd RM (2008) Residual feed intake in beef cattle. Rev Bras Zootec 37:269–279. https://doi.org/10.1590/S1516-35982008001300031

Beever DE, da Silva JFC, Prescott HHD, Armstrong DG (1972) The effect in sheep of physical form and stage of growth on the sites of digestion of a dried grass. Br J Nutr 28:347–356. https://doi.org/10.1079/bjn19720044

Bi Y, Cox MS, Zhang F, Suen G, Zhang N, Tu Y, Diao Q (2019) Feeding modes shape the acquisition and structure of the initial gut microbiota in newborn lambs. Environ Microbiol 21(7):2333–2346. https://doi.org/10.1111/1462-2920.14614

Britton R, Krehbiel C (1993) Nutrient metabolism by gut tissues. J Dairy Sci 76(7):2125–2131. https://doi.org/10.3168/jds.S0022-0302(93)77547-5

Cahill GF Jr (2006) Fuel metabolism in starvation. Annu Rev Nutr 26:1–22. https://doi.org/10.1146/annurev.nutr.26.061505.111258

Chen L, Qiu Q, Jiang Y, Wang K, Lin Z, Li Z, Bibi F, Yang Y, Wang J, Nie W, Su W, Liu G, Li Q, Fu W, Pan X, Liu C, Yang J, Zhang C, Yin Y, Wang Y, Zhao Y, Zhang C, Wang Z, Qin Y, Liu W, Wang B, Ren Y, Zhang R, Zeng Y, da Fonseca RR, Wei B, Li R, Wan W, Zhao R, Zhu W, Wang Y, Duan S, Gao Y, Zhang YE, Chen C, Hvilsom C, Epps CW, Chemnick LG, Dong Y, Mirarab S, Siegismund HR, Ryder OA, Gilbert MTP, Lewin HA, Zhang G, Heller R, Wang W (2019) Large-scale ruminant genome sequencing provides insights into their evolution and distinct traits. Science 364:eaav6202. https://doi.org/10.1126/science.aav6202

Chong CYL, Vatanen T, Oliver M, Bloomfield FH, O'Sullivan JM (2020) The microbial biogeography of the gastrointestinal tract of preterm and term lambs. Sci Rep 10:9113. https://doi.org/10.1038/s41598-020-66056-z

Comline RS, Titchen DA (1951) Reflex contraction of the oesophageal groove in young ruminants. J Physiol 115(2):210–226. https://doi.org/10.1113/jphysiol.1951.sp004666

Dalile B, Van Oudenhove L, Vervliet B, Verbeke K (2019) The role of short-chain fatty acids in microbiota-gut-brain communication. Nat Rev Gastroenterol Hepatol 16(8):461–478. https://doi.org/10.1038/s41575-019-0157-3

Flint HJ, Bayer EA (2008) Plant cell wall breakdown by anaerobic microorganisms from the Mammalian digestive tract. Ann N Y Acad Sci 1125:280–288. https://doi.org/10.1196/annals.1419.022

Flint HJ, Scott KP, Duncan SH, Louis P, Forano E (2012) Microbial degradation of complex carbohydrates in the gut. Gut Microbes 3(4):289–306. https://doi.org/10.4161/gmic.19897

Forsberg CW, Cheng KJ, White BA (1997) Polysaccharide degradation in the rumen and large intestine. In: Mackie RI, White BA (eds) Gastrointestinal microbiology, Chapman & Hall microbiology series. Springer, Boston, pp 319–379. https://doi.org/10.1007/978-1-4615-4111-0_10

Frampton J, Murphy KG, Frost G, Chambers ES (2020) Short-chain fatty acids as potential regulators of skeletal muscle metabolism and function. Nat Metab 2(9):840–848. https://doi.org/10.1038/s42255-020-0188-7

Freetly HC, Dickey A, Lindholm-Perry AK, Thallman RM, Keele JW, Foote AP, Wells JE (2020) Digestive tract microbiota of beef cattle that differed in feed efficiency. J Anim Sci 98(2):skaa008. https://doi.org/10.1093/jas/skaa008

Furman O, Shenhav L, Sasson G, Kokou F, Honig H, Jacoby S, Hertz T, Cordero OX, Halperin E, Mizrahi I (2020) Stochasticity constrained by deterministic effects of diet and age drive rumen microbiome assembly dynamics. Nat Commun 11(1):1904. https://doi.org/10.1038/s41467-020-15652-8

Gonçalves P, Araújo JR, Di Santo JP (2018) A cross-talk between microbiota-derived short-chain fatty acids and the host mucosal immune system regulates intestinal homeostasis and inflammatory bowel disease. Inflamm Bowel Dis 24(3):558–572. https://doi.org/10.1093/ibd/izx029

Graham C, Simmons NL (2005) Functional organization of the bovine rumen epithelium. Am J Physiol Regul Integr Comp Physiol 288(1):R173–R181. https://doi.org/10.1152/ajpregu.00425.2004

Gray J, Oehrle K, Worthen G, Alenghat T, Whitsett J, Deshmukh H (2017) Intestinal commensal bacteria mediate lung mucosal immunity and promote resistance of newborn mice to infection. Sci Transl Med 9(376):eaaf9412. https://doi.org/10.1126/scitranslmed.aaf9412

Groeger D, O'Mahony L, Murphy EF, Bourke JF, Dinan TG, Kiely B, Shanahan F, Quigley EM (2013) Bifidobacterium infantis 35624 modulates host inflammatory processes beyond the gut. Gut Microbes 4(4):325–339. https://doi.org/10.4161/gmic.25487

Guan LL, Nkrumah JD, Basarab JA, Moore SS (2008) Linkage of microbial ecology to phenotype: correlation of rumen microbial ecology to cattle's feed efficiency. FEMS Microbiol Lett 288(1):85–91. https://doi.org/10.1111/j.1574-6968.2008.01343.x

Hackmann TJ, Spain JN (2010) Invited review: ruminant ecology and evolution: perspectives useful to ruminant livestock research and production. J Dairy Sci 93(4):1320–1334. https://doi.org/10.3168/jds.2009-2071

Hein WR, Dudler L, Mackay CR (1989) Surface expression of differentiation antigens on lymphocytes in the ileal and jejunal Peyer's patches of lambs. Immunology 68(3):365–370

Henderson G, Cox F, Ganesh S, Jonker A, Young W, Global Rumen Census Collaborators, Janssen PH (2015) Rumen microbial community composition varies with diet and host, but a core microbiome is found across a wide geographical range. Sci Rep 5:14567. https://doi.org/10.1038/srep14567

Hernandez-Sanabria E, Goonewardene LA, Wang Z, Durunna ON, Moore SS, Guan LL (2012) Impact of feed efficiency and diet on adaptive variations in the bacterial community in the rumen fluid of cattle. Appl Environ Microbiol 78(4):1203–1214. https://doi.org/10.1128/AEM.05114-11

Herrera P, Kwon YM, Ricke SC (2009) Ecology and pathogenicity of gastrointestinal Streptococcus bovis. Anaerobe 15(1–2):44–54. https://doi.org/10.1016/j.anaerobe.2008.11.003

Hess M, Sczyrba A, Egan R, Kim TW, Chokhawala H, Schroth G, Luo S, Clark DS, Chen F, Zhang T, Mackie RI, Pennacchio LA, Tringe SG, Visel A, Woyke T, Wang Z, Rubin EM (2011) Metagenomic discovery of biomass-degrading genes and genomes from cow rumen. Science 331(6016):463–467. https://doi.org/10.1126/science.1200387

Hogan JP, Weston RH (1967) The digestion of chopped and ground roughages by sheep. II. The digestion of nitrogen and some carbohydrate fractions in the stomach and intestines. Aust J Agr Res 18:803. https://doi.org/10.1071/AR9670803

Howard JL (1981) Ruminal metabolic acidosis. Bovine Practitioner 1981(16):44–53. https://doi.org/10.21423/bovine-vol1981no16p44-53

Hu L, Geng S, Li Y, Cheng S, Fu X, Yue X, Han X (2018) Exogenous fecal microbiota transplantation from local adult pigs to crossbred newborn piglets. Front Microbiol 8:2663. https://doi.org/10.3389/fmicb.2017.02663

Huntington GB, Harmon DL, Richards CJ (2006) Sites, rates, and limits of starch digestion and glucose metabolism in growing cattle. J Anim Sci 84(Suppl):E14–E24. https://doi.org/10.2527/2006.8413_supple14x

Ishaq SL, Lachman MM, Wenner BA, Baeza A, Butler M, Gates E, Olivo S, Buono-Geddes J, Hatfield P, Yeoman CJ (2019) Pelleted-hay alfalfa increases sheep wether weight gain and rumen bacterial richness over loose-hay alfalfa feed. PLoS One 14(6):e0215797. https://doi.org/10.1371/journal.pone.0215797

Ivanov II, Atarashi K, Manel N, Brodie EL, Shima T, Karaoz U, Wei D, Goldfarb KC, Santee CA, Lynch SV, Tanoue T, Imaoka A, Itoh K, Takeda K, Umesaki Y, Honda K, Littman DR (2009) Induction of intestinal Th17 cells by segmented filamentous bacteria. Cell 139(3):485–498. https://doi.org/10.1016/j.cell.2009.09.033

Janssen PH, Kirs M (2008) Structure of the archaeal community of the rumen. Appl Environ Microbiol 74(12):3619–3625. https://doi.org/10.1128/AEM.02812-07

El Kaoutari A, Armougom F, Gordon JI, Raoult D, Henrissat B (2013) The abundance and variety of carbohydrate-active enzymes in the human gut microbiota. Nat Rev Microbiol 11(7):497–504. https://doi.org/10.1038/nrmicro3050

Khafipour E, Krause DO, Plaizier JC (2009) A grain-based subacute ruminal acidosis challenge causes translocation of lipopolysaccharide and triggers inflammation. J Dairy Sci 92(3):1060–1070. https://doi.org/10.3168/jds.2008-1389

Kristensen NB, Danfaer A, Agergaard N (1998) Absorption and metabolism of short-chain fatty acids in ruminants. Arch Tierernahr 51(2–3):165–175. https://doi.org/10.1080/17450399809381916

Li F, Guan LL (2017) Metatranscriptomic profiling reveals linkages between the active rumen microbiome and feed efficiency in beef cattle. Appl Environ Microbiol 83(9):e00061–e00017. https://doi.org/10.1128/AEM.00061-17

Lin L, Wang Y, Xu L, Liu J, Zhu W, Mao S (2020) Microbiome-host co-oscillation patterns in remodeling of colonic homeostasis during adaptation to a high-grain diet in a sheep model. Animal Microbiome 2(1):22. https://doi.org/10.1186/s42523-020-00041-9

Liu JH, Xu TT, Liu YJ, Zhu WY, Mao SY (2013) A high-grain diet causes massive disruption of ruminal epithelial tight junctions in goats. Am J Physiol Regul Integr Comp Physiol 305(3):R232–R241. https://doi.org/10.1152/ajpregu.00068.2013

Liu J, Liu F, Cai W, Jia C, Bai Y, He Y, Zhu W, Li RW, Song J (2020) Diet-induced changes in bacterial communities in the jejunum and their associations with bile acids in Angus beef cattle. Anim Microbiome 2:33. https://doi.org/10.1186/s42523-020-00051-7

Lopetuso LR, Scaldaferri F, Petito V, Gasbarrini A (2013) Commensal clostridia: leading players in the maintenance of gut homeostasis. Gut Pathog 5(1):23. https://doi.org/10.1186/1757-4749-5-23

Mackie RI (1997) Gut environment and evolution of mutualistic fermentative digestion. In: Mackie RI, White BA (eds) Gastrointestinal microbiology, Chapman & Hall microbiology series. Springer, Boston. https://doi.org/10.1007/978-1-4615-4111-0_2

Mao S, Zhang M, Liu J, Zhu W (2015) Characterizing the bacterial microbiota across the gastrointestinal tracts of dairy cattle: membership and potential function. Sci Rep 5:16116. https://doi.org/10.1038/srep16116

McGeough EJ, O'Kiely P, Hart KJ, Moloney AP, Boland TM, Kenny DA (2010) Methane emissions, feed intake, performance, digestibility, and rumen fermentation of finishing beef cattle offered whole-crop wheat silages differing in grain content. J Anim Sci 88(8):2703–2716. https://doi.org/10.2527/jas.2009-2750

McCann JC, Wiley LM, Forbes TD, Rouquette FM Jr, Tedeschi LO (2014) Relationship between the rumen microbiome and residual feed intake-efficiency of Brahman bulls stocked on bermudagrass pastures. PLoS One 9(3):e91864. https://doi.org/10.1371/journal.pone.0091864

McLoughlin S, Spillane C, Claffey N, Smith PE, O'Rourke T, Diskin MG, Waters SM (2020) Rumen microbiome composition is altered in sheep divergent in feed efficiency. Front Microbiol 11:1981. https://doi.org/10.3389/fmicb.2020.01981

Ménard O, Butel MJ, Gaboriau-Routhiau V, Waligora-Dupriet AJ (2008) Gnotobiotic mouse immune response induced by Bifidobacterium sp. strains isolated from infants. Appl Environ Microbiol 74(3):660–666. https://doi.org/10.1128/AEM.01261-07

Michelland RJ, Monteils V, Zened A, Combes S, Cauquil L, Gidenne T, Hamelin J, Fortun-Lamothe L (2009) Spatial and temporal variations of the bacterial community in the bovine digestive tract. J Appl Microbiol 107(5):1642–1650. https://doi.org/10.1111/j.1365-2672.2009.04346.x

Mitsuoka T (2005) Bifidobacteria and their role in human health. J Ind Microbiol 6:263–267. https://doi.org/10.3389/fmicb.2016.00925

Mukhopadhya I, Hansen R, El-Omar EM, Hold GL (2012) IBD-what role do Proteobacteria play? Nat Rev Gastroenterol Hepatol 9(4):219–230. https://doi.org/10.1038/nrgastro.2012.14

Myer PR, Smith TP, Wells JE, Kuehn LA, Freetly HC (2015) Rumen microbiome from steers differing in feed efficiency. PLoS One 10(6):e0129174. https://doi.org/10.1371/journal.pone.0129174

Nagaraja TG, Avery TB, Bartley EE, Galitzer SJ, Dayton AD (1981) Prevention of lactic acidosis in cattle by lasalocid or monensin. J Anim Sci 53(1):206–216. https://doi.org/10.2527/jas1981.531206x

Newbold CJ, Wallace RJ (1988) Effects of the ionophores monensin and tetronasin on simulated development of ruminal lactic acidosis in vitro. Appl Environ Microbiol 54(12):2981–2985. https://doi.org/10.1128/AEM.54.12.2981-2985.1988

Oh JH, Hume ID, Torell DT (1972) Development of microbial activity in the alimentary tract of lambs. J Anim Sci 35(2):450–459. https://doi.org/10.2527/jas1972.352450x

O'Mahony L, McCarthy J, Kelly P, Hurley G, Luo F, Chen K, O'Sullivan GC, Kiely B, Collins JK, Shanahan F, Quigley EM (2005) Lactobacillus and bifidobacterium in irritable bowel syndrome: symptom responses and relationship to cytokine profiles. Gastroenterology 128(3):541–551. https://doi.org/10.1053/j.gastro.2004.11.050

Pabst O, Herbrand H, Worbs T, Friedrichsen M, Yan S, Hoffmann MW, Körner H, Bernhardt G, Pabst R, Förster R (2005) Cryptopatches and isolated lymphoid follicles: dynamic lymphoid tissues dispensable for the generation of intraepithelial lymphocytes. Eur J Immunol 35(1):98–107. https://doi.org/10.1002/eji.200425432

Pearson C, Uhlig HH, Powrie F (2012) Lymphoid microenvironments and innate lymphoid cells in the gut. Trends Immunol 33(6):289–296. https://doi.org/10.1016/j.it.2012.04.004

Perea K, Perz K, Olivo SK, Williams A, Lachman M, Ishaq SL, Thomson J, Yeoman CJ (2017) Feed efficiency phenotypes in lambs involve changes in ruminal, colonic, and small-intestine-located microbiota. J Anim Sci 95(6):2585–2592. https://doi.org/10.2527/jas.2016.1222

Piao H, Lachman M, Malfatti S, Sczyrba A, Knierim B, Auer M, Tringe SG, Mackie RI, Yeoman CJ, Hess M (2014) Temporal dynamics of fibrolytic and methanogenic rumen microorganisms

during in situ incubation of switchgrass determined by 16S rRNA gene profiling. Front Microbiol 5:307. https://doi.org/10.3389/fmicb.2014.00307

Press CM, Halleraker M, Landsverk T (1992) Ontogeny of leukocyte populations in the ileal Peyer's patch of sheep. Dev Comp Immunol 16(2–3):229–241. https://doi.org/10.1016/0145-305x(92)90022-5

Reynaud CA, Garcia C, Hein WR, Weill JC (1995) Hypermutation generating the sheep immunoglobulin repertoire is an antigen-independent process. Cell 80(1):115–125. https://doi.org/10.1016/0092-8674(95)90456-5

Rizzatti G, Lopetuso LR, Gibiino G, Binda C, Gasbarrini A (2017) Proteobacteria: a common factor in human diseases. Biomed Res Int 2017:9351507. https://doi.org/10.1155/2017/9351507

Round JL, Mazmanian SK (2009) The gut microbiota shapes intestinal immune responses during health and disease. Nat Rev Immunol 9(5):313–323. https://doi.org/10.1038/nri2515

Savignac HM, Kiely B, Dinan TG, Cryan JF (2014) Bifidobacteria exert strain-specific effects on stress-related behavior and physiology in BALB/c mice. Neurogastroenterol Motil 26(11):1615–1627. https://doi.org/10.1111/nmo.12427

Schoonmaker JP, Trenkle AH, Beitz DC (2010) Effect of feeding wet distillers grains on performance, marbling deposition, and fatty acid content of beef from steers fed low- or high-forage diets. J Anim Sci 88(11):3657–3665. https://doi.org/10.2527/jas.2010-2896

Seshadri R, Leahy SC, Attwood GT, Teh KH, Lambie SC, Cookson AL, Eloe-Fadrosh EA, Pavlopoulos GA, Hadjithomas M, Varghese NJ, Paez-Espino D, Hungate 1000 Collaborators, Perry R, Henderson G, Creevey CJ, Terrapon N, Lapebie P, Drula E, Lombard V, Rubin E, Krypides NC, Henrissat B, Woyke T, Ivanova NN, Kelly WJ (2018) Cultivation and sequencing of rumen microbiome members from the Hungate1000 collection. Nat Biotechnol 36:359–367. https://doi.org/10.1038/nbt.4110

Shabat SK, Sasson G, Doron-Faigenboim A, Durman T, Yaacoby S, Berg Miller ME, White BA, Shterzer N, Mizrahi I (2016) Specific microbiome-dependent mechanisms underlie the energy harvest efficiency of ruminants. ISME J 10(12):2958–2972. https://doi.org/10.1038/ismej.2016.62

Shin NR, Whon TW, Bae JW (2015) Proteobacteria: microbial signature of dysbiosis in gut microbiota. Trends Biotechnol 33(9):496–503. https://doi.org/10.1016/j.tibtech.2015.06.011

Singh A, Hacini-Rachinel F, Gosoniu ML, Bourdeau T, Holvoet S, Doucet-Ladeveze R, Beaumont M, Mercenier A, Nutten S (2013) Immune-modulatory effect of probiotic Bifidobacterium lactis NCC2818 in individuals suffering from seasonal allergic rhinitis to grass pollen: an exploratory, randomized, placebo-controlled clinical trial. Eur J Clin Nutr 67(2):161–167. https://doi.org/10.1038/ejcn.2012.197

Sukkar AH, Lett AM, Frost G, Chambers ES (2019) Regulation of energy expenditure and substrate oxidation by short-chain fatty acids. J Endocrinol 242(2):R1–R8. https://doi.org/10.1530/JOE-19-0098

Sung HG, Kobayashi Y, Chang J, Ha A, Hwang IH, Ha JK (2007) Low ruminal pH reduces dietary fiber digestion via reduced microbial attachment. Asian-Australian J Anim Sci 20(2):200–207. https://doi.org/10.5713/ajas.2007.200

Tanca A, Fraumene C, Manghina V, Palomba A, Abbondio M, Deligios M, Pagnozzi D, Addis MF, Uzzau S (2017) Diversity and functions of the sheep faecal microbiota: a multi-omic characterization. J Microbial Biotechnol 10(3):541–554. https://doi.org/10.1111/1751-7915.12462

Taschuk R, Griebel PJ (2012) Commensal microbiome effects on mucosal immune system development in the ruminant gastrointestinal tract. Anim Health Res Rev 13(1):129–141. https://doi.org/10.1017/S1466252312000096

Thomas M, Webb M, Ghimire S, Blair A, Olson K, Fenske GJ, Fonder AT, Christopher-Hennings J, Brake D, Scaria J (2017) Metagenomic characterization of the effect of feed additives on the gut microbiome and antibiotic resistome of feedlot cattle. Sci Rep 7(1):12257. https://doi.org/10.1038/s41598-017-12481-6

Tojo R, Suárez A, Clemente MG, de los Reyes-Gavilán CG, Margolles A, Gueimonde M, Ruas-Madiedo P (2014) Intestinal microbiota in health and disease: role of bifidobacteria in gut

homeostasis. World J Gastroenterol 20(41):15163–15176. https://doi.org/10.3748/wjg.v20.i41.15163

Van Soest P (1994) Nutritional ecology of the ruminant. ITHACA. Cornell University Press, London. Retrieved from http://www.jstor.org/stable/10.7591/j.ctv5rf668

Wang J, Fan H, Han Y, Zhao J, Zhou Z (2017a) Characterization of the microbial communities along the gastrointestinal tract of sheep by 454 pyrosequencing analysis. Asian Australas J Anim Sci 30(1):100–110. https://doi.org/10.5713/ajas.16.0166

Wang Y, Xu L, Liu J, Zhu W, Mao S (2017b) A high grain diet dynamically shifted the composition of mucosa-associated microbiota and induced mucosal injuries in the colon of sheep. Front Microbiol 8:2080. https://doi.org/10.3389/fmicb.2017.02080

Welch JG (1982) Rumination, particle size and passage from the rumen. J Anim Sci 54:885–894. https://doi.org/10.2527/jas1982.544885x

Wesemann DR, Portuguese AJ, Meyers RM, Gallagher MP, Cluff-Jones K, Magee JM, Panchakshari RA, Rodig SJ, Kepler TB, Alt FW (2013) Microbial colonization influences early B-lineage development in the gut lamina propria. Nature 501(7465):112–115. https://doi.org/10.1038/nature12496

Yeoman CJ, Ishaq SL, Bichi E, Olivo S, Lowe J, Aldridge BM (2018) Biogeographical differences in the influence of maternal microbial sources on the early successional development of the bovine neonatal gastrointestinal tract. Sci Rep 8:3197. https://doi.org/10.1038/s41598-018-21440-8

Yeoman CJ, White BA (2014) Gastrointestinal tract microbiota and probiotics in production animals. Annu Rev Anim Biosci 2:469–486. https://doi.org/10.1146/annurev-animal-022513-114149

Yu S, Zhang G, Liu Z, Wu P, Yu Z, Wang J (2020) Repeated inoculation with fresh rumen fluid before or during weaning modulates the microbiota composition and co-occurrence of the rumen and colon of lambs. BMC Microbiol 20(1):29. https://doi.org/10.1186/s12866-020-1716-z

Zhang H, Shao M, Huang H, Wang S, Ma L, Wang H, Hu L, Wei K, Zhu R (2018) The dynamic distribution of small-tail Han sheep microbiota across different intestinal segments. Front Microbiol 9:32. https://doi.org/10.3389/fmicb.2018.00032

Zhang Y, Li F, Chen Y, Wu H, Meng Q, Guan LL (2020) Metatranscriptomic profiling reveals the effect of breed on active rumen eukaryotic composition in beef cattle with varied feed efficiency. Front Microbiol 11:367. https://doi.org/10.3389/fmicb.2020.00367

Zoetendal EG, Raes J, van den Bogert B, Arumugam M, Booijink CC, Troost FJ, Bork P, Wels M, de Vos WM, Kleerebezem M (2012) The human small intestinal microbiota is driven by rapid uptake and conversion of simple carbohydrates. ISME J 6(7):1415–1426. https://doi.org/10.1038/ismej.2011.212

Zhuang Y, Chai J, Cui K, Bi Y, Diao Q, Huang W, Usdrowski H, Zhang N (2020) Longitudinal investigation of the gut microbiota in goat kids from birth to postweaning. Microorganisms 24(8):1111. https://doi.org/10.3390/microorganisms8081111

The Role of Farm Environment and Management in Shaping the Gut Microbiota of Poultry

10

Juan María Díaz Carrasco, Leandro Martin Redondo, Natalia Andrea Casanova, and Mariano Enrique Fernández Miyakawa

Abstract

Gut microbiota of poultry assembles from environmental sources mainly determined by farm management decisions such as feed composition, litter handling and sanitation practices, or the use of different feed additives. In turn, there are key welfare features of conventional and alternative poultry systems for broiler and laying hens such as the stocking density and access to range which have a strong influence in the gut microbiota of birds. This chapter provides an analysis of how these farm environmental factors alter the development, structure and diversity of microbial communities in the gastrointestinal tract of birds. The use of early intervention strategies and feed additives is also discussed as tools to promote gut health by manipulation of intestinal microbiota development of birds toward a configuration with lower vulnerability to colonization by gut pathogens.

Keywords

Poultry · Gut health · Microbiota · Farm management · Poultry litter · Laying hens · Alternative poultry systems · Gut pathogens · Host–microbe interactions · Feed additives

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10.1 Introduction

Aviculture is currently one of the most efficient animal production systems as the result of a process of improvement carried out over the last 6 or 7 decades. The chicken efficiency in extracting energy and nutrients from food has been key for this success which involves a complex and dynamic interaction between the host and the rich microbial community present within the gastrointestinal tract (GIT) (Yeoman et al. 2012; Pan and Yu 2014). The microbial community, including commensal, symbiotic, and pathogenic microorganisms that colonize an area of an animal is referred to as the *microbiota*. Gut microbiota has a highly dynamic population structure whose members aid the host in several important functions including resistance to pathogens colonization, development of immune system, and nutrition; therefore, host–microbe interactions in the GIT play a key role to maintain gut health and promote efficient use of poultry feed (Dominguez-Bello et al. 2010; Fouhse et al. 2016; Díaz Carrasco et al. 2019).

10.1.1 Colonization and Filtering of Gut Microorganisms

The microbiota of the GIT consists of diverse species assemblages distributed in various discrete and temporary patches (individual hosts), being defined as a metacommunity (O'Dwyer et al. 2012). The species structure within any patch (the host) depends on two connected processes, according to metacommunities theory. One process is the microbial colonization from the external output (environment) which continually adds species to a local community (GIT). The other process involves the destiny of these microbes as the incoming colonists persist or are lost, which depends on ecological interactions within the patches (for example, between the microorganisms or between the host and microbiota). This is a within-patch dynamics which ecologists call "filtering." The mechanisms governing colonization and filtering of gut microbiota are very complex, but this knowledge is key to understand potential outcomes of different interventions (Haiser and Turnbaugh 2012; Wong and Rawls 2012; Atarashi et al. 2013; Smith et al. 2015). Considering domestic animals, and particularly those raised in agricultural setting, both processes (colonization, persistence), which are highly connected and mutually dependent, can be characterized within a context that is relatively well known as genetics, food, or environmental conditions. However, to explain differences among hosts and variation in gut microbiota, the processes of colonization and persistence regulating microbial community assembly need to be understood taking into consideration the specific features of each system. For example, broilers and layers are similar as host but different in their compositional dynamics of microbiota, mainly due to differences in the length of life cycles, flock management protocols such as housing systems, and sex of birds.

Once colonizing bacteria reach GIT of the host, certain microorganisms may decrease in abundance (for example, if they are excreted or cannot reproduce successfully) or may effectively create a self-perpetuating population locally.

Whether a colonizing species persists in the community depends on the relative rates of recurrent colonization versus local extinction (for example, the balance of colonization versus filtering dynamics). According to the metacommunity theory, the filtering process can depend on several factors. First, entering microbes must have an appropriate spatial and nutritional niche within the host gut (Nicholson et al. 2012). Feed composition affects the persistence of present microbes through nutritional inputs needed to sustain microbial reproduction (Hildebrandt et al. 2009; Cheng et al. 2019). Second, feed intake and digesta retention time affect the rate at which microbes are lost via excretion in feces. Third, persistence of a microorganism in the gut strongly depends on interactions with other microorganisms. All bacteria require an energy source although these energy sources may differ between species and between strains. Bacteria compete for nutrients, but also the product of one strain's metabolism may be utilized in the nutrition of another, in a process termed metabolic cross-feeding (Smith et al. 2019). Fourth, colonizing microbes must survive potential host immune responses (Bolnick et al. 2014; Zheng et al. 2020). As a general concept, it is important to consider that the characteristics of a community in an ecosystem are shaped by environmental factors, and ecological succession occurs when these factors are modified. Besides the dynamic evolution of these interactions, there are determined events in the GIT that result in changes within the system with important consequences for the animal fitness, making it extremely difficult to recognize the determinants of community assembly and composition variations. For example, postnatal development of gut microbiota is linked to healthy growth and the early events of colonization are important drivers for host health. Therefore, the sources of microbes introduced to newly hatched chicks affect the development of intestinal microbiota, but other drivers also modulate colonization and succession within the animal gut microbiota in a process that can produce completely divergent results. As animals deal with a great multitude of colonizing microbes and external conditions (i.e., temperature or feed contaminants), microbiota develops under a system that is highly sensitive to initial conditions for which long-term prediction would be impossible to predict according to chaos theory (Lorenz 1963). For example, small differences in the first microbes colonizing GIT would yield widely diverging outcomes for such dynamical systems, making detailed long-term prediction quite difficult. However, it is possible to directly relate the network structure to its dynamics and identifying the stable patterns of activity, i.e., the attractors of the system. Farm animals are an excellent model to study these complex interactions because of the large scale, the massive numbers of replicates, the controlled conditions of rearing and the accumulated knowledge about physiology, diseases, nutrition, and behavior.

A big effort has been done in recent years to understand and identify the changing patterns of microbiota composition and define attractors in the gut of farm animals, particularly after the massive use of next generation sequencing technology and several other *omics* technologies. The bacterial microbiota of the chicken gut has been studied extensively, mainly because of its association with weight gain in broilers (Torok et al. 2011b; Stanley et al. 2012a; Bae et al. 2017; Díaz Carrasco et al. 2018; Johnson et al. 2018) and egg production in layers (Lei et al. 2013;

Inatomi 2016; Yan et al. 2017). For example, some studies have described differences in bacterial species abundance for broilers with high vs. low growth and feed efficiency (Stanley et al. 2012a; Singh et al. 2014). The body site and age of the bird are important during the development of bacterial microbiota in different breeds of broiler and layer chickens (Glendinning et al. 2017; Kers et al. 2018; Johnson et al. 2018). The initial development of intestinal microbiota in poultry has been associated with productivity and overall health, as several studies have shown that bacteria acquired immediately after hatch are crucial for optimal performance and resistance to infectious diseases (Kogut and Arsenault 2016; Ballou et al. 2016; Baldwin et al. 2018). After hatching, the diversity of intestinal microbiota of birds increases rapidly during the first weeks of life, but colonization and species succession seem to differ between layer- and meat-type chickens, probably due to environmental features and farm management issues specific to each rearing system.

In their natural environment, birds would spend much of their time and energy searching for food. Therefore, birds are driven to follow their natural behaviors, such as foraging, pecking, scratching, and feather maintenance behaviors like preening and dust bathing, and protection at night to avoid predators. The life of chickens in modern aviculture has been quite different. Animals destined for meat production are born in a hatchery and moved to a grow-out farm at 1 day-old, remaining there until they are heavy enough to be slaughtered. The parent birds (breeder birds) used to produce meat chickens have their eggs separated and placed in an incubator where the eggs are kept under optimum atmosphere conditions. After 21 days, the chicks break out of eggs shell using their egg tooth. Chickens are precocial, so they are reasonably mature and can walk around immediately after hatching. Chickens at a day-old are transported in transport modules (boxes) from the hatchery to the farm. Chicks travel along a conveyor belt and are dropped into transport boxes or modules. During this process, the chicks are immunized with spray and/or subcutaneous vaccinations (Breytenbach 2005). Newborn birds are unable to regulate their body temperatures, becoming very susceptible to thermal stress during transport, affecting the physiology and animal condition. "One-day-old chicks" are initially sustained by reserves from the yolk sac of energy and water for up to 72 h after hatching, but chick survivability is greatly reduced as the time to first feed and water access increases (Mitchell and Kettlewell 2009).

Current chicken breeds are the result of an artificial selective process for commercial objectives. Besides changes induced by domestication, the greatest progress made in chicken has been generated in the latter years with the introduction of industrial size farming (Schmidt et al. 2009; Tallentire et al. 2016). Intensive genetic selection driven by economically advantageous production characters, as high body weight and rapid rate of growth for broilers and intensive egg production for layer hens, led to increased productivity (Janke et al. 2004). Today, layer hens produce more than 320 eggs during 52 weeks of egg production, while broiler breeders achieve 50- to 60-fold increases in body weight from hatch to marketing (Druyan 2010). However, this artificial selection process carried out for almost a century also brought metabolic changes associated with differential farm management and

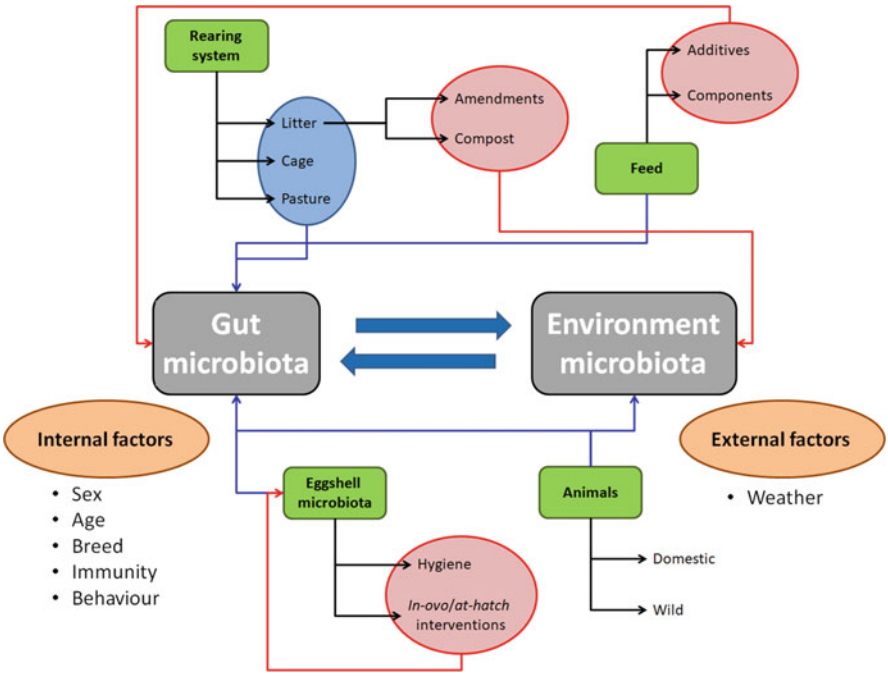

Fig. 10.1 Summary of host and environment contributions to GIT microbiota and potential interactions across diverse productive systems in poultry industry. Blue arrows represent sources of microorganisms, red arrows represent conditioning factors that influence colonization and persistence of different components of poultry microbiota

housing practices between broilers and layers. All these factors affect GIT microbiome and, therefore, gut microbiota of poultry.

This chapter provides an analysis of the dynamics of environmental and GIT microbiota in modern poultry productive setting and how most important drivers can modulate these interactions (Fig. 10.1).

10.2 Microbiota Sources Within Poultry Farms

Intestinal microbiota members can be acquired vertically, horizontally, and certainly from the environment. The gut microbial community of birds is highly interconnected with the environmental community of microorganisms that can share part of their species and show equally mutually dependent relationship: the environmental microbiota. Both microbiotas are altered by internal and external factors that shape their temporal evolution.

In poultry systems, direct contact with parents does not usually occur as in mammals (Dominguez-Bello et al. 2010; Pantoja-Feliciano et al. 2013). However, maternal and eggshell microbiotas are important for the initial colonization of the

GIT, as well as external environment could be considered as one of the main sources of initial GIT colonizers. Gut colonization continues throughout life as microorganisms are acquired from farm environment sources such as feed, water, air, litter, and soil.

10.2.1 Key Early Microbiota Acquisition Events

Contact with adult hens is naturally linked with development of gut microbiota of chicks as well as with behavioral and welfare aspects (Funkhouser and Bordenstein 2013; Edgar et al. 2016; Donaldson et al. 2017; Kubasova et al. 2019). However, since birds generally have no contact with mother hens, coupled with the strict hygiene protocols in poultry farms, the acquisition of intestinal microbiota after hatching has become an unnatural and stochastic process driven by the uptake of microorganisms present in food and water from the farm environment. Furthermore, it has been shown that interventions during the first days of life can have a lasting impact on gut microbiota, immune system, and intestinal morphology development, which in turn can alter gut susceptibility to infectious agents and bird productivity (Ballou et al. 2016; Simon et al. 2016; Kers et al. 2018).

10.2.1.1 Eggshell Microbiota

In nature, eggshell microbiota acts as a transgenerational carrier of maternal microbiota, and it's composed of microbes from maternal and environmental sources, mainly bacteria from female cloacae, skin, and feathers as well as soil and nest materials (Ding et al. 2017; Van Veelen et al. 2018; Trudeau et al. 2020). The first events of gut microbiota assembly occur before hatching of birds, by passing of microorganisms through the pores of the eggshell and egg white, including commensal and potentially pathogenic species (Roto et al. 2016; Lee et al. 2019).

In modern aviculture systems eggs are hatched away from the laying hens, within rigorously cleaned incubators, and eggshells are sanitized to avoid the transfer of potential gut pathogens, particularly those that are important from commercial and food safety viewpoints, such as *Salmonella* Enteritidis (Gantois et al. 2009; Sylte et al. 2017). Therefore, the maternal and environmental load of microorganisms on eggshells is artificially lowered in industrial hatcheries. A recent study showed that birds exposed to environmental-only or eggshells-associated microbiotas differ in their gut microbiota community structure and short-chain fatty acid profiles in the cecum (Maki et al. 2020). Interestingly, other authors showed that avian incubation of eggs has an inhibitory effect in the proliferation of microorganisms on the eggshell, reducing the risk of trans-shell infection by pathogenic bacteria (Cook et al. 2005; Shawkey et al. 2009).

10.2.1.2 In-Ovo and At-Hatch Interventions

After birth, chicks are maintained in a sterile environment until all birds have hatched. This time gap, known as hatching window, is a critical step on commercial production because birds are deprived of feed and water and then they are

transported to the farm, leading to a delay in time to feed. Feed restriction induces physiological and functional modification in the gut that may impair the intestinal barrier function, and has a profound impact on gut microbiota profile, mainly by increasing the abundance of *Lactobacillus* and other Firmicutes while decreasing members of *Turicibacteraceae* and *Enterobacteriaceae* (Metzler-Zebeli et al. 2019). The entire fasting period of 48–72 h has a detrimental effect on gut microbiota development and thus, on gut health and growth performance of chicks (Willemsen et al. 2010).

The strict hygiene conditions in the hatchery lead to an early low microbial diversity in the GIT that makes chicks susceptible to transient colonization by pathogens and to different infections with long-term detrimental effects (Wilkinson et al. 2020). Colonization of gut mainly depends on environmental sources such as bedding material, feed, and human contact, among other factors. Furthermore, modern production system demands higher feed efficiency to complete short productive cycles in a short period of time, which may result in metabolic disorders and in a delay in immune system development (Rubio 2019). In this context, early modulation of gut microbiota through novel proceedings such as feeding embryos and recently hatched birds is one of the novel methods to improve avian productivity (Jha et al. 2019). *In-ovo* technology implementation at early age modulates the microbiome, gene expression and affects the development and maturation of the immune system with long-term effects in birds (Siwek et al. 2018; Rubio 2019). Prebiotics and symbiotics are generally administered at day 12 to stimulate egg microbiota while probiotics are delivered at 17/18 days of incubation to trigger competitive exclusion and inhibit colonization of gut by pathogens. These two time points have been called *in-ovo* stimulation and *in-ovo* feeding, respectively, each with different strategies, biological mechanisms, and technical tools (Siwek et al. 2018). Both strategies may help to overcome the stress resulting from the hatching window and post-hatching fasting. More detailed description of the effects of pre and probiotics *in-ovo* administration has been recently reviewed (Roto et al. 2016; Siwek et al. 2018). Early nutrition programs that include the inoculation with prebiotics, probiotics, and vaccines immediately after hatching and specific pre-starter diets can also improve gut health and growth performance of chickens. Direct inoculation within the egg or on its eggshell with prebiotics, probiotics, and even with cecal contents from other birds has been studied as a strategy to direct early colonization of the GIT with microorganisms associated with a better gut health and productive performance from the moment of hatching (Simon et al. 2016; Baldwin et al. 2018; Rubio 2019; Richards-Rios et al. 2020). These procedures give the opportunity to reshape the microbiota toward the desired one that protects against infections and inflammatory responses triggered by pathogens (Varmuzova et al. 2016; Baldwin et al. 2018; Jha et al. 2019; Wilkinson et al. 2020).

10.2.2 Litter Microbiota and Litter Management Practices

The litter on which birds are raised is composed of wood shavings and other vegetal materials as well as by feed, water, and chicken excreta that is mixed and composted with the bedding materials. Broiler chickens are raised almost exclusively on litter system, while layers are initially raised on litter before transfer to cage systems. Young birds ingest litter particles along with feed and consequently the initial assembly of GIT microbiota incorporates microorganisms from these environmental sources. The type of litter material used has a significant influence in the development gut microbiota of birds and can also alter GIT morphology and physiology (Torok et al. 2009; Wang et al. 2018). A recent meta-analysis found that certain bedding materials such as straw or rice husks are significantly associated with worse productive performance in broilers (Toledo et al. 2019). There is still little information available about the environmental drivers of litter microbiota variation. Moisture content has a pronounced effect on its composition. Wet litter areas within the poultry farms, underneath drinkers, contain higher bacterial diversity compared to dry litter areas (Dumas et al. 2011; Oakley et al. 2013). Part of the bacterial species that compose litter microbiota are involved in the degradation of wood and cycling of nitrogen and sulfur (Lu et al. 2003). Dust levels, air humidity and ammonia levels inside the barn are influenced by litter quality and litter humidity, and in turn these factors depend on the type of litter material, the type of drinkers, water spillage, and diet composition, as well as health status (EFSA Panel on Animal Health and Welfare 2010; de Jong et al. 2012). Therefore, the selection of poultry litter materials as well as the farm management practices that may alter its physicochemical and biological properties is important for the establishment of gut microbiota in poultry farms, and such decisions are in turn influenced by economic and environmental factors (Waziri and Kaltungo 2017).

The litter is usually not cleaned out during the growing of the broiler. In the European Union, the litter is completely removed after each flock, and the house is cleaned, disinfected, and replaced with new litter. In contrast, in other countries including Argentina, Brazil, and the USA it is more common for the litter to be renewed only once or twice a year. The reuse of poultry litter is a widespread practice to reduce production costs and for environmental sustainability purposes. Some producers reuse litter using techniques like windrowing or clay addition to compost the used litter between flocks and reduce the pathogens load, so going even longer between total litter clean-outs for their broiler houses. It is known that reusing litter has a marked effect on GIT microbiota development, and thus can impact in host nutritional and intestinal health status. Although the population structure of intestinal and litter microbiota is clearly different, several studies have found correlations in their composition and diversity, which can be attributed to the continuous exchange that exists between these microbial communities (Danzeisen et al. 2015; Mancabelli et al. 2016; Díaz Carrasco 2018). As a general concept, fresh poultry litter contains more bacteria of environmental origin, while reused litter harbors more bacterial species from intestinal origin (Cressman et al. 2010). Halotolerant/alkaliphilic bacteria tend to increase in litter while certain butyrate-producing and *Lactobacillus*

species are augmented in the gut of young chicks when a litter-reusing regime is followed (Wang et al. 2016). There is a tendency to think that reusing poultry litter implies a higher risk associated with carryover of pathogens between flocks. Although this seems theoretically reasonable, many studies demonstrate the opposite. Several detailed studies show that the reuse of treated broiler litter is a safe practice and contrary to expectations, it substantially decreases the bacterial load of zoonotic and animal pathogens as *Salmonella* (Lu et al. 2003; Roll et al. 2011; Bucher et al. 2020). Chickens reared on reused litter tended to have a lower abundance of generic *Clostridium perfringens* compared with those reared on fresh litter (Wei et al. 2013). In the same way, the levels of *Campylobacter* and *Escherichia coli* in litter and cecal contents of broiler chickens were not influenced by litter reuse during commercial farming across six rearing cycles (Chinivasagam et al. 2016).

In the last years, the housing types of laying hens have been expanded and many producers have moved from conventional cages to deep litter, perchery and free-range systems (Thiele and Pottgüter 2008; Janczak and Riber 2015). All poultry systems provide a substrate from which birds pick and ingest bedding materials allowing the exchange of microbiota between birds and litter (Kers et al. 2018). There are few studies investigating the role of litter in intensive layers production systems as a source of gut microbiota. A recent study demonstrated a clear preference for feeding and foraging on substrate without excreta in laying hens (Von Waldburg-Zeil et al. 2019). The chemical and microbiological characteristics of layer hens' litter are different from that of broilers, with lower load of total coliform and *Staphylococcus* species (Omeira et al. 2006). Litter availability is of great importance to laying hens due to its impact on the hens' natural behavior and on the reduction of anxiety, fear, and damaging behaviors (De Haas et al. 2014; Campbell et al. 2016).

10.2.2.1 Composting and Amendment of Poultry Litter

With the rapid expansion of aviculture over the last decades, concerns regarding management and disposal of poultry waste have raised. Ammonia and greenhouse gas emissions produced by poultry litter have a negative impact on the environment (Naseem and King 2018). Additionally, organic manure decomposition within poultry houses produces biogases such as amides, amines, mercaptans, sulfides, and disulfides that may irritate and disrupt the respiratory tract epithelial lining of animals and men leading to high degree of susceptibility to respiratory tract infections, and therefore the ventilation is a key factor to avoid moisture and ammonia concentration within poultry houses and to maintain the health and well-being of the birds (Waziri and Kaltungo 2017). Reused litter has been shown to harbor higher richness and diversity of fungi than fresh litter, which correlated with air fungal contamination (Viegas et al. 2012). Mycotoxins are known to increase the risk and severity of infectious bursal disease and can lead to poor performance of the flocks (Waziri and Kaltungo 2017). The rules of litter management and amendments are few and usually depend on decisions made by farm operators based on unclear criteria. There are different types of litter treatments to reduce ammonia

volatilization which also contribute to improvements in bird performance, welfare, pathogen loads, fertilizer value of spent litter, and reduced costs associated with purchasing new bedding materials (Cockerill et al. 2020). Litter amendments practices include application of acidifiers, alkaline materials, absorbers, inhibitors, microbial and enzymatic treatments and even dietary manipulations (Waziri and Kaltungo 2017; Cockerill et al. 2020). Among these agents, the use of acidifiers has been shown to reproducibly reduce moisture, ammonia levels, and pathogenic bacteria in the litter as well as in the GIT of poultry reared on acidifier-amended litter (Garrido et al. 2004; de Toledo et al. 2020).

As mentioned above, the fate of the poultry litter can vary depending on socio-economic and environmental factors. When litter is reused in consecutive flocks or sold as organic fertilizer, a composting process is performed between flocks, which involve windrowing and heating of the litter to achieve aerobic degradation of organic matter, and reduction of moisture, ammonia, odor, and microbial load in the litter. The process usually takes 4 to 6 weeks to reach a stabilized material, resulting in a reduction of 40–80% in litter volume and weight (Kelleher et al. 2002; Waziri and Kaltungo 2017). One disadvantage of this practice is the loss of nitrogen and other nutrients during composting, which reduces its value as organic fertilizer (Chen and Jiang 2014). Another important issue with this practice is the environmental impact of ammonia and nitrous oxide emissions generated from the windrowed litter (Ro et al. 2017). In some countries, farmers use more sophisticated technology to deal with litter disposal such as anaerobic digestion and direct combustion plants which produce biofuels, heat, and energy used to power the farms themselves, and therefore making the whole system more sustainable and environmentally friendly (Beausang et al. 2020).

According to insights and perspectives recently gathered from industry stakeholders, it is believed that reusing litter will become even more frequent in the future to reduce aviculture costs and ease pressures on both the supply of new bedding materials and disposal of poultry litter (Cockerill et al. 2020). Since intensive poultry farming keeps growing as a source of animal protein worldwide, it is logical to think that other possible destinations for the poultry litter such as its use as crop fertilizer or as a feed supplement for fish and ruminants will also grow in the midterm (Bolan et al. 2010). Therefore, the combined use of *omics* tools in future studies will be essential to obtain detailed information regarding the effectiveness of different litter treatments in reducing chemical contaminants, toxins, antibiotic resistance genes, and potentially harmful microorganisms for the health of poultry, humans, and the environment.

10.3 Gut Microbiota in Conventional and Alternative Poultry Systems

The population structure and functional profile of the intestinal microbiota of birds raised under intensive farming conditions is shaped by a long list of factors, including host intrinsic characteristics such as genetic background, sex, age and

health status, and environmental factors mainly determined by rearing conditions and farm management decisions, such as the nutritional composition of feed and utilization of feed additives, welfare factors such as bird density and access to range, among others (Pan and Yu 2014; Kers et al. 2018; Díaz Carrasco et al. 2019). The degree to which each of these factors contributes to shape gut microbiota is still unclear, since microbiota shifts are usually associated with several interacting factors acting at the same time. Therefore, sometimes it is challenging to reliably identify what factor to attribute the observed effect. For example, when comparing animals under different rearing systems, the particularities of each system must be considered to disaggregate the data and hypothesize about the key drivers of gut microbiota variation within each system. This section will describe the characteristics that differentiate conventional and alternative broiler and laying hen rearing systems and the impact of feed additives on the intestinal microbiota of birds.

10.3.1 Influence of Rearing Systems on Gut Microbiota

10.3.1.1 Intensive Broiler Production

The birds that are used to breed the chickens that become broiler meat chickens are called parent birds/stock or broiler breeders. Young broiler breeder birds are kept in single-sex flocks in rearing barns, which are set-up like the broiler grow-out facilities as enclosed ventilated barns with litter floors. Then at 16–21 weeks of age, the birds are moved into mixed-sex groups in the laying houses or production farms. Egg production usually starts between 18 and 22 weeks of age and lasts until 60–65 weeks of age. The percentage of males in the group can range between 7% and 11% when egg production starts. Group size during the production period ranges from 3000 to 8000 birds in some countries or more than 10,000 as in Brazil or the USA. The majority of breeders laying houses have raised slatted areas (covering approx. 50–66% floor surface) which allows the manure to accumulate underneath in collection pits. The remaining floor surface is littered. Nests are positioned on the slats and can either be collective nests with an automated egg collection belt or individual nests. Cage housing of broiler breeders is less common. In these animals, feed restriction is practiced because if broiler breeders were fed standard diets, they would grow too rapidly and become too heavy to maintain good health before reaching the age of sexual maturity. This would have detrimental effects on their health, fertility, and welfare. However, feed restriction causes welfare problems associated with hunger (e.g., redirected pecking) and increased aggression around feeding time, as well as susceptibility to diseases.

Globally, over 70% of broiler chickens are raised in quite similar indoor intensive farming systems (Steinfeld et al. 2006). Nowadays, in several developed countries a growing proportion of commercial chickens are reared in less intensive, higher welfare systems. Most of the chicken produced for consumption in the world today comes from flocks of conventionally bred broiler chickens that grow to market weight in about 35–48 days. The efficiency in use of natural resources can be considered more sustainably than animal production some decades ago. Compared

to 25 years ago, today's chickens now require much less feed to grow to similar levels. In 1985, at 35 days of age a 1.40-kg broiler required 3.22 kg of feed but 25 years later a 2.44-kg broiler was produced on 3.66 kg of feed (Siegel 2014). Slower-growing chickens or "Heritage breeds" are chickens that do not convert feed to muscle as quickly and take almost twice as long to reach market weight, typically about 81 days. These breeds require more feed, fuel, water, and land to sustain their growth. As such, these products are typically much more expensive than their counterparts, limiting their expansion at worldwide level.

Broilers used in intensive systems are from strains that have been bred to be very fast growing to gain weight quickly. Keeping broiler production indoors, without any access to outside areas can help with pest control. In temperate countries, broiler sheds are closed, climate-controlled (e.g., fan-ventilated), and have artificial lighting (Wageningen UR Livestock Research 2010). In warmer areas, the barns are more open with curtain sides so that the chickens are exposed to daylight and natural ventilation but have no outside access (Pym and Alders 2012). For example, in Europe the standard industry broiler barn is window-less, but in some countries, windows are required to allow natural daylight (de Jong et al. 2012). In Sweden, windows to let in daylight are obligatory. Most industry standard barns are generally open spaces with feeding and drinking lines. Broilers are reared on a littered floor often composed of straw, wood shavings, hulls, peat, or paper, to absorb the chickens' manure. Feed is always available and involves a pelleted diet high in energy and protein, usually delivered via an automated feeding system.

Broiler chicks are placed in these rearing/grow-out barns are typically kept in large, mixed-sex flocks. These flocks can consist of 10,000 or 20,000 birds, or more, in a single house (ISUST & USDA Poultry Industry Manual 2013). Broilers stay at the same barn until they reach slaughter age. In some countries or companies, when birds get close to final slaughter weights, flocks are often thinned. This involves the removal of a fraction of the flock (usually the female birds that are lighter) for slaughter, to allow the remaining birds more room to grow on to a greater weight. The birds remaining in the house are likely to be stressed because of the thinning process, making them more susceptible to infections and dysbiosis (de Jong et al. 2012).

The gut microbiota of young broilers suffers a rapid increase of microbial richness and diversity as the birds grow, until eventually reaches a mature state. This process takes place in broilers around 3 weeks of life, but the succession patterns may vary depending on a combination of internal (host) and external factors (enviroment) which have been extensively reviewed (Kers et al. 2018; Díaz Carrasco et al. 2019). For example, early colonization of broilers GIT involves members of *Enterobacteriaceae* family which are gradually succeeded by different *Firmicutes* groups mainly belonging to the *Clostridiales* order (Ballou et al. 2016). However, the patterns of species succession vary among chicken breeds, since Cobb and Hubbard chickens are colonized by *Bifidobacterium*, *Enterococcaceae* and *Clostridiaceae* in early life, and have lower proportion of *Enterobacteriaceae* compared to Ross chickens (Richards et al. 2019). As discussed in Sect. 10.2.2, early colonization of chicken GIT can have a lasting impact on gut microbiota

diversity and functionality, and colonization by bifidobacteria and other lactic acid bacteria is particularly important due to their important role in the competitive exclusion of intestinal pathogens in poultry (Yadav and Jha 2019).

Fiber content in poultry feed has a strong influence on gut microbiota composition, since certain bacterial species attach themselves to the insoluble polysaccharides and form colonies around fiber particles that can work as hubs for different microbial processes and interactions (Mahmood and Guo 2020). Certain diet components such as wheat bran with small particle size can increase the levels of lactate producing *Lactobacillus* and *Bifidobacterium* species in the cecum of broilers, which stimulate the growth of members of the *Lachnospiraceae* family that use lactate to produce butyrate (Vermeulen et al. 2017). In the same paper, a significant reduction in gut colonization by *Salmonella* Enteritidis was shown, highlighting the impact that composition and structure of poultry feed can have on intestinal health. It is very likely that part of the effect of litter on gut microbiota described above (Sect. 10.2.2) is linked to the fiber content of the bedding materials.

Farm management factors affecting gut microbiota of broiler chickens have been less studied than other environmental factors such as diet or the use of feed additives (Sect. 10.3.2). A recent study showed that the rearing environment of broilers alters gut microbiota composition and functionality in a stronger way than dietary intervention (Kers et al. 2019). An exception is outdoor access in broilers under alternative rearing systems, which is discussed in Sect. 10.3.1.3.

10.3.1.2 Laying Hen Husbandry

The housing systems of laying hens are usually classified in cage and non-caged systems (Table 10.1). The cage systems can be further divided in conventional cages, furnished or enriched cages, and colony nests. The second category includes barn (floor) management systems, aviaries, and outdoor systems. Laying hens systems have a different genetic background than broilers, as well as physiology, lifespan, housing systems, and dietary requirements; thus, their gut microbiota composition is also different. Though broilers and layers share a core microbiota, there are differences in the microbial richness and relative abundance of specific microorganisms related to the characteristics of each genetic line (Khan et al. 2020). The microbiota of layer hens is usually more complex than that of broilers, and over their whole life four different stages of development have been described (Videnska et al. 2014a, b). At an early age, the microbiota is dominated by *Proteobacteria* which is slowly replaced by *Firmicutes*. Then, the phylum *Bacteroidetes* increase at expenses of *Firmicutes* which correlates with higher body weight and egg production (Videnska et al. 2014b). Finally, the microbiota of older hens is stabilized and mostly composed by an equal proportion of *Bacteroidetes* and *Firmicutes* (Videnska et al. 2014b). The differences between the microbiota composition of young and older layers could be explained, at least in part, by the management conditions of each farm and the physiological changes as the birds mature and enter the laying period (Ngunjiri et al. 2019).

There is limited literature related to the impact of environmental conditions on the development of gut microbiota in laying hens. A recent study showed that hens in

Table 10.1 Housing systems in laying hen husbandry

	Cage system			Non-caged system		
	Conventional cage	Furnished or enriched cage	Colony nest	Barn/floor management	Aviary	Outdoor system
Birds density	High	High	Medium	Medium	Low	Low
Outdoor access	No	No	No	No	Yes/ No	Yes
Use of perches/ nest	No/No	V/Yes	V/Yes	V/Yes	V/Yes	V/Yes
Natural behavior	No	V	V	Yes	Yes	Yes
Stress/ anxiety	High	High	High	Low	Low	Low
Automation	Yes	Yes	Yes	V	V	No

V variable

cage-free housing systems have higher bacterial richness in the gut microbiota and lower susceptibility to avian pathogenic *E. coli* during the early laying stage compared to hens under conventional cage systems (Van Goor et al. 2020). Hens with outdoor access showed higher proportion of *Bacteroidetes* and higher abundance of microbial species involved in amino acid and glycan metabolism in the cecum compared to caged birds (Xu et al. 2016). The house climate is a major environmental factor in the welfare and productivity of chickens. Room temperatures around 20–25 °C are considered optimal for laying hens. Chickens can adapt to lower temperatures, but high ambient temperature is a well-known stressor in poultry production whose effects mostly depend on the intensity and duration of the stimulus. Heat stress reduces feed intake and nutrient absorption, leads to immunosuppression and intestinal dysfunction, and alters the community structure of GIT microbiota, increasing the risk of gut colonization by pathogens and the mortality rate of laying hens (Zhu et al. 2019; Xing et al. 2019). The performance of laying hens is also compromised by heat stress showing decreased production and lower egg quality due to feed intake restriction, an altered absorption of nutrient and impairment of endocrine system (Sugiharto et al. 2017). Although the authors described dissimilar results of the relative abundance of *Bacteroidetes* and *Firmicutes*, which may depend on the different sample type, both studies suggested that heat stress-induced restriction of feed intake is the major driver of the microbiota changes.

Diet is one of the most important drivers of gut microbiota composition and metabolism; both macro and micronutrients have significant effects on the microbiome (Biesalski 2016). In poultry industry, the main sources of protein and energy are soybeans, corn and wheat because of their low cost/benefit ratio. However, the presence of non-starch polysaccharides in wheat and barley predisposes to

necrotic enteritis due to the proliferation of *C. perfringens* while corn and soy-based diets can also modify the intestinal microbiome and augment the risk of infectious diseases (Pan and Yu 2014). Moreover, the rearing conditions (free-range, cage, etc.) and thus the different feeding patterns have a profound influence on the microbiota of hens, which in turn affect the intestinal health (Cui et al. 2017). In line with these concepts, a recent study found that laying hens reared in different environments and fed with soybean or cottonseed meal exhibited little differences in the cecal microbiota and egg production due to the dietary protein sources (Hubert et al. 2019). Although there is no evidence about the effect of fat and laying hens' microbiota, it has been reported that the gut microbiota of broiler chickens was modified depending on the fat sources (Pan and Yu 2014).

Behavior abnormalities are common in layer hens and have a negative impact on birds' performance and welfare (Hartcher and Jones 2017). Gut microbiota has been shown to influence host behavior and physiology and could thus affect the welfare of laying hens. Recent studies have shown differences between the gut microbiota of high and low feather-pecking adult hens under the same housing conditions. The authors described an increased relative abundance of bacteria within the order *Clostridiales* and a reduced relative abundance of the genus *Lactobacillus* in high feather-pecking birds compared to low feather-pecking birds (Birkl et al. 2018; van der Eijk et al. 2019). Both groups of bacteria are involved in metabolic pathways that produce detrimental (e.g., phenol derivatives) or beneficial (e.g., indole derivatives) metabolites which may modulate the responses of the immune and central nervous systems and, potentially, affect bird's health.

Further studies are needed to determine if the changes in gut microbiota composition are causal or consequential to behavioral and physiological characteristics, which could aid in the development of tailored interventions to enhance welfare and gut health of laying hens.

10.3.1.3 Higher Welfare (Alternative) Poultry Systems

In recent years, alternative poultry productive systems are increasing around the world, mostly prompted by consumer demand for what is considered a less intensive and more welfare-friendly management practices, which is coupled with an increased popularity of locally produced food products (Gifford and Bernard 2011). High welfare systems include different and very diverse productive settings. In a broad sense, pasture-raised or *free-range* poultry productive systems can be defined as a production system where birds are totally or partially raised outdoors using some sort of small, moveable, and ventilated pen arrangement (Fanatico 2006; Shi et al. 2019). In the EU, a growing proportion of commercial broilers are reared in alternative systems, while in Brazil and the USA, less than 1% of chickens are raised as with access to range. The rearing environment can also be augmented, for example with indoor enrichment and/or with an outdoor area. Chickens use a range more if other amenities are available like presence of cover in the form of trees, bushes or hedges or with artificial shelters (European Commission Regulation 2008). Additionally, some free-range/pasture-based productive settings can be defined as organic farms, but this designation is based on specific requirements

including depending on the country, age of slaughter, growing density, access to open areas, total continuous confinement indoors is prohibited, feed must be certified organic, cannot contain any animal-by products, antibiotics, GMO derived products, or synthetic preservatives.

Despite the perception that naturally-raised poultry are produced in small scale productive units and have low impact, as the market for these products grows, further considerations on factors such as environmental impact and food safety concerns should be considered (Luján-Rhenals et al. 2016; O'Bryan et al. 2017). Changes in soil levels of nitrogen and phosphorus as well as antimicrobial runoff are some of the major environmental consequences of these productive systems account (O'Bryan et al. 2017), and while management practices such as pasture rotation can be used to avoid over grazing and buildup of excess nutrients, this practice may increase food safety problems since the access to new environments and contact with other animals potentially can increase the incidence of foodborne pathogens within pasture-raised flocks (Siemon et al. 2007; Park et al. 2013). Some foodborne pathogens that have been associated with free-range birds either in pre-harvest production or from retail birds include *Campylobacter*, *Listeria*, and *Salmonella* (Esteban et al. 2008; Gonçalves-Tenório et al. 2018; Golden and Mishra 2020). However, making general and uniform recommendations represent a serious challenge, mostly due to the diverse range of management approaches, food safety, and sustainability problems can be somewhat unpredictable.

As with laying hens, there is limited knowledge about the structure and dynamics of gut microbiota of poultry raised under free-range conditions and how interactions with such diverse rearing environment contribute to productive outcomes. Most of the existing studies directly compare gut microbiotas from conventional (industrial) poultry and free-range birds without considering that free-range production usually is based on slow-growing breeds with different genetic background and longer productive cycles where slaughter is normally after than 12 weeks, while in commercial fast-growing genetic lines slaughter age is around 5–7 weeks (Lewis et al. 1997), and these differences are also related with differences in intestinal development (Lumpkins et al. 2010; Mignon-Grasteau et al. 2015). An exception is the study performed by Cui et al. (2017), where the authors characterize the intestinal microbiota of caged and free-range hens and found that depending on rearing conditions and age laying hens had different microbiota patterns, with higher values of evenness and richness for the gut microbiota of the hens under free-range conditions (Cui et al. 2017; Van Goor et al. 2020). In this study, hens from the same breed and origin were used, but as the study is based on PCR-DGGE fingerprints, it was not possible to assess taxon representation and abundance.

Although works describing poultry gut microbiota show variable results, it is possible to identify a core microbiota that is shared across diverse poultry breeds and productive systems, allowing to make some comparisons between conventional and alternative systems (Hubert et al. 2019; Ocejo et al. 2019). In general, available works describe that free-range birds have a more diverse gut microbiota than conventionally raised birds, and those differences are related with the presence and abundance of particular microbial taxa (Hubert et al. 2019; Ocejo et al. 2019). For

example, it is well known that as young chicks grow, their gut microbiota goes through a series of temporal successions and becomes increasingly diverse and complex (Van Der Wielen et al. 2002). Ocejo et al. (2019) showed that conventional broiler and free-range chickens have similar patterns of microbial diversity and taxa succession associated with age, where certain species are replaced by others as chickens grow. Based on their results the authors describe microbiota maturation stages for the whole chicken lifespan which clearly showed the evolution from an early immature stage to a mature microbiota that differed between breeds. Although gut microbiota from free-range poultry is characterized by higher diversity and longer productive cycles, some authors propose that birds raised under free-range/pasture may develop a stable and mature gut microbiota earlier than conventionally-grown broilers. For example, while in conventionally-grown broilers, mature microbiota have been described at different ages ranging from 3 to 6 weeks (Oakley et al. 2014; Ballou et al. 2016; Park et al. 2017), Rothrock et al. (2019) describe that in pasture-based flocks core gut microbiota is established on the first week with minimal changes during rearing period (16 weeks). Exposition to diverse environmental sources of bacteria may contribute to the fast maturation of the gut microbiota (Wang et al. 2016; Kers et al. 2018; Shi et al. 2019; Ocejo et al. 2019).

Cage-free and free-range housing typically offers more space per individual bird as well as access to the outdoors and allows birds to forage with some impact on the gut microbial activities and function. In fact, after outdoor access, when grass was introduced in the diet, notable changes were observed in free-range birds after 12 days of outdoor grazing, also new classes of bacteria were described (*Fusobacteria* and *Lentisphaerae*) (Ocejo et al. 2019). Given the diverse nature of pastures with potential differences in forages as well as exposure to a wide range of environmental conditions, it would not be surprising that the birds' gastrointestinal microbial populations might also reflect this diversity (Torok et al. 2009; Kers et al. 2018) and the gut microbiota structure shows patterns which differ given the differences in the pasture or the soil with which the birds interact. Contact with other animal species also can contribute to higher microbiota diversity and the presence of particular microbial taxa. In the mentioned work (Rothrock et al. 2019), broilers were raised on pastures exposed to other animal species and the authors found that broilers shared ~75% of the OTUs with other bird species (layers, guinea hens), while only shared ~25% of the OTUs with mammal fecal microbiomes (sheep, pigs). Although this situation seems to have a negative impact on biosecurity (Jacob et al. 2008; Pohjola et al. 2016) and on the prevalence of foodborne pathogens (Esteban et al. 2008), however available works suggest that rearing broilers concomitantly with other mammal species do not significantly increase pathogens prevalence, potentially due to the rapid establishment of a mature broiler gut microbiome (Rothrock et al. 2019).

Access to outdoor environment favors bird natural behavior and contributes to some of the gut microbiota patterns described for free-range poultry. Foraging exposes birds to new sources of microbial taxa and provides diverse nutrients for the established gut microbiota. Foraging and other behaviors appear to be essential for alternative poultry broiler and egg-laying production, probably some of the

benefits of using slower-growing breeds for alternative productive systems will be related to behavior patterns which are more suited for actively foraging in comparison with conventional, fast-growing breeds (Sossidou et al. 2015). In conventional poultry, coprophagy may contribute to a reduced diversity and other microbiota patterns observed in these systems, while the increased space and diversity of substrate in cage-free or free-range environments may support the natural fecal-avoidance behavior and in turn contribute to the higher microbial diversity represented in their gastrointestinal tract (Von Waldburg-Zeil et al. 2019).

Although further studies are needed to fully understand the role environmental contributions to shape poultry gut microbiota, reported results suggest that the outdoor access and contact with soil and natural vegetation are all potentially crucial in increasing gut microbiota diversity. If greater diversity modulates immune and metabolic performance (Díaz Carrasco et al. 2019), these data have implications for managing gut health in cage-free and organic production systems. Comprehensive knowledge of how these external factors interact with host factors will contribute to define states of health and disease. Also, understanding this interaction should contribute to determining the extent to which diets can be used to modulate gut microbiota, and whether microbiota differences elicited by the rearing environment will contribute to promote efficient and sustainable production.

10.3.2 Impact of Feed Additives on Gut Microbiota

The initial development of gut microbiota is a highly dynamic process, making the immature microbiota susceptible to interventions that can have lasting effects on immune development and host energy harvest (Ballou et al. 2016). To modulate symbiotic interactions between gut microbes and host several feed additives are used to promote the development of stable and beneficial microbiota (Stanley et al. 2013; Adedokun and Olojede 2019). Although the benefits of control/modulation of gut bacteria by AGPs (Dibner and Richards 2005; Niewold 2007; Costa et al. 2017) and other feed additives such as probiotics are largely known (Gao et al. 2017), the recent extension in the use of next generation sequencing increases the number of works describing how poultry gut microbiota is largely influenced by several kinds of feed additives.

Antimicrobials growth promoters (AGPs) have been included in animal feed for nearly 70 years to improve growth performance and feed efficiency (Jukes et al. 1956; Dibner and Richards 2005; Castanon 2007). Despite the final results may depend on several factors, it was described that in-feed antimicrobials can increase chickens body weight gain up to 8% and decrease the feed conversion ratio up to 5% (Butaye et al. 2003). During that period several mechanisms were proposed to explain how antimicrobial promotes growth, but recent research suggests that they act mainly through modulation of gastrointestinal microbiota (Brüssow 2015; Angelakis 2017). Results from these studies are highly variable and while some authors describe increasing diversity and richness after use of AGPs (Pedroso et al. 2006; Gong et al. 2008; Lin et al. 2013), most of the existing works describe a

reduction in both parameters compared with non-antimicrobial controls or birds treated with other feed additives (Lu et al. 2008; Díaz Carrasco et al. 2018). Despite differences in the results associated with the global structure of the gut microbiota, changes in the relative abundance of certain taxa were described in each case. Among the observed changes increasing levels of *Escherichia* and other *Proteobacteria* coupled with a decrease in *Lactobacillus* and other *Firmicutes* are some of the most commonly described changes (Díaz Carrasco et al. 2018).

The use of antimicrobials in laying hens is lower compared to other productions, especially during the laying phase. Antibiotics disrupt the intestinal microbiota composition and therefore impair gut homeostasis and health. The administration of a single and repeated doses of tetracycline or streptomycin induced changes on fecal microbiota of 15-week and 46-week-old hens within 48 h after treatment (Videnska et al. 2013). The microbiota of younger hens was quite complex and consisted mainly of representatives of the order Clostridiales while those of older hens were dominated by *Lactobacillus*. Even when flock age-related differences were observed, the genera *Enterococcus* (upper gastrointestinal tract) and *Escherichia* (lower gastrointestinal tract) increased in response to antibiotics therapy in both groups. After 2 weeks, the microbiota composition of treated hens became similar to those of non-treated layers. Although the authors suggested that stomach microorganisms could be related to the restoration of gut microbiota (Videnska et al. 2013), currently, there is not enough evidence to support that hypothesis. In another study, the prevalence of antibiotic resistance genes and the composition of fecal microbiota of broilers and laying hens not treated with antibiotics were analyzed by real-time PCR and 16s rRNA gene pyrosequencing, respectively (Videnska et al. 2014a). The prevalence of antibiotic resistance genes was low in both lines which could be related to the absence of recent therapy in the flocks and the fast mechanism of restoration of microbiota to "normal" levels. Other authors described that the administration of a broad-spectrum antibiotic cocktail to 1-day-old birds induced a considerable reduction in the number of cultivable bacteria in feces and the modification of fecal microbiota composition despite similar diversity and richness found between groups (Simon et al. 2016). The microbiota profile of treated birds consisted of a majority of *Proteobacteria*, mainly *E. coli*, and a lower abundance of *Firmicutes*, such as *Lactobacillus*. Like the studies mentioned above, the changes in the microbiota were transient and the gut microbiota had recovered 2 weeks after antibiotic withdrawal. However, antibiotic-induced dysbiosis in early life may have long-term effects on adaptive immunity in birds (Simon et al. 2016) and metabolism (Gadde et al. 2018).

Due to the constant interaction between microbiota and host immune system, antimicrobial induced changes in microbiota composition may affect host immunity and make the host more susceptible to pathogens (Kumar et al. 2018), increasing the need for more antimicrobial to control infectious diseases and sustain productive performance. For example, necrotic enteritis is associated with perturbations in microbiota composition, which maybe produced either by unbalanced diets or environmental factors, for this reason AGPs are used to control these alterations. However, the microbiota imbalance caused by the AGPs itself (Díaz Carrasco et al.

2018) coupled with the increased frequency of resistant *C. perfringens* strains (Redondo et al. 2015) would lead to alter gut microbiota that support pathogen proliferation and increase infection susceptibility (Stanley et al. 2012b; Antonissen et al. 2016; Moore 2016). Worldwide increased concerns on the emergence and spread of antimicrobial resistant microorganisms raise questions about the production animal industries' dependency on antimicrobials leading to the ban of use of growth-promoting antibiotics (AGPs) and a general reassessment of their use for livestock. Thus, current research is focused on alternatives to antibiotics for sustainable food animal production (Huyghebaert et al. 2011; Polycarpo et al. 2017; Al-Khalaifah 2018; Lillehoj et al. 2018). Unlike consequences of feeding antibiotics on gut microbiota, alternative nutritional strategies aim to stimulate beneficial components of the gastrointestinal microbiota in chickens (Stanley et al. 2014).

Considering that some of the bacterial species members of the normal poultry gut microbiota are associated with positive productive outcomes (Xi et al. 2019), selective enrichment of these bacterial groups may contribute to the implementation of rational growth-promoting strategies. In this context, probiotics and prebiotics are well-known health promoting additives and in the last years are being intensively explored to help reduce the dependency on antimicrobials in production (Borda-Molina et al. 2018). Members of the bacterial genus *Lactobacillus*, *Ruminococcus*, and *Clostridium* clusters IV and XIVa are associated with enhanced bird performance (Patterson and Burkholder 2003; Eeckhaut et al. 2011), and strategies oriented to increase their proportions are suggested to improve productive efficiency and health (Torok et al. 2011a; Stanley et al. 2016; Fasina et al. 2016). Probiotics are viable bacteria that provide health benefits after ingestion, including enhancing the function of the intestinal barrier of the host. The main mechanisms involved in described effects of probiotic bacteria or derived products are diverse and depend on multiple mode of action, including microbiota homeostasis, reduction of pathogens by competitive exclusion and production of bacteriocins, higher immune response, and improvement of gut barrier function (Yang et al. 2009; Gaggìa et al. 2010). Prebiotics are indigestible but fermentable feed additives that suppress pathogen loads while maintaining productivity by directly feeding beneficial microorganism within the microbiota (Pourabedin et al. 2014; Micciche et al. 2018). After fermenting these dietary substances, bacteria with probiotic potential obtain their survival energy for growth or production of inhibitory metabolites like bacteriocins and lactic acid (Gibson et al. 2004). For example, while the inclusion of fructooligosaccharides (FOS) favors groups of bifidobacteria, lactulose in poultry diets increases *Lactobacillus* counts (Cho et al. 2014; Calik and Ergün 2015). Additionally, prebiotics can selectively influence gut microbiota (Flint et al. 2012) by binding directly to cells, like mannooligosaccharides (MOS), which binds to some bacterial pathogens and contribute to excretion with digesta flow (Kim et al. 2011; Huyghebaert et al. 2011). It is important to consider that main beneficial effects of prebiotics are related to the increase of beneficial bacteria, therefore these products share many of the probiotics modes of action and when provided in combination show synergistic effect on the gut health (Mookiah et al. 2014).

Plant tissues are rich in a wide variety of secondary metabolites and some of them in the right concentration and presentation can be incorporated into animal feed to enhance productivity (Mueller-Harvey 2006; Windisch et al. 2008; Surai 2014). Among the naturally occurring plant compounds those with the greatest potential to replace AGPs are essential oils, tannins, and saponins. Phytochemicals contribute to modulate gut microbiota by a combination of inhibitory and prebiotic effect against different groups of bacterial populations as described for tannins (Viveros et al. 2011; Díaz Carrasco et al. 2018). These bioactive compounds have antimicrobial effect against several pathogens including Gram-negative and Gram-positive bacteria and parasites, preventing adhesion, colonization, and proliferation in the gut of broilers (Franz et al. 2010; Redondo et al. 2014; Díaz Carrasco et al. 2016). Also, chickens fed tannin-rich products showed increased diversity in cecum microbiota with higher proportions of potential tannins-degrading bacteria and *Lactobacillus* and *Enterococcus* (Viveros et al. 2011; Díaz Carrasco et al. 2018). Additional considerations for polyphenols and probably other phytochemicals include modifications by digestive or microbe enzymes which may improve bioavailability and health effects (Molino et al. 2018). Moreover, phytochemicals also present several biological properties that have made them attractive for use as growth promoters in animal production, including antioxidant, anti-stress, nutritional, physiological, and immunological effects.

A variety of feed additives are normally used for enhancing growth and feed efficiency (Kogut 2019; Yadav and Jha 2019). Exogenous enzymes are included to complement the activity of endogenous enzymes or to counter the anti-nutritional factors present in conventional and unconventional components of poultry diet (Melo-Duràn et al. 2019). Commonly used exogenous enzymes in poultry diets are β-glucanase, xylanase, amylase, α-galactosidase, protease, lipase, and phytase (Kiarie et al. 2013; Bedford 2018). As fed components are degraded or modified in the intestine by enzymes diverse substrates are available for gut microbes (Kiarie et al. 2013). Besides the catalytic and indirect effect proposed for exogenous enzymes, recently a direct and new effect on gut microbiota was described for xylanase (Bedford 2018). The degradation of xylan by this enzyme is not producing a prebiotic per se but produces a signaling molecule which stimulates bacterial species that could produce other xylanases. Microbiota modulation by enzymes is multifactorial in action due to its role in the partitioning of nutrients and helps in the growth of specific microbiota by producing nutrients for them. Also, accelerating digestibility avoids that undigested feed reaches ceca and changes microbiota, allowing undesired microorganisms, like *C. perfringens*, to overgrowth.

The use of feed additives to manipulate gut microbiota in order to achieve an optimal gut function/microbiota for better growth and improved health of poultry is a promising, but not a new concept for the poultry industry. Growing antimicrobial restrictions highlights the need to conduct new research to develop cost-effective feeding programs while reducing antimicrobial dependency among livestock production. Also, a better understanding of the chicken microbiome, including diverse aspects such as gut ecology, the effect of feed supplements, microbiota plasticity, and the host–microbiota interactions will provide an attractive platform to develop

future rational strategies to prevent pathogen colonization or improve intestinal barrier function in order to achieve a more efficient and sustainable poultry production.

10.4 Conclusions

Modern poultry rearing systems generate an artificial environment with highly variable sources of microorganisms, which directly influence the assembly of the gut microbiota, and through this in turn may alter the vulnerability to GIT colonization by pathogens and bird productivity. Other stress and welfare factors inside poultry houses can alter the feeding and social behavior of the birds. The use of feed additive programs, early interventions, and management practices tailored to the particularities of each poultry system may help to guide intestinal microbiota development toward a desired one in order to overcome some of these issues. To achieve this, it is necessary to unravel the colonization and filtering mechanisms that govern the assembly of GIT communities under different poultry productive setting through the combined use of *omics* tools with zootechnical and physiological measurements either under experimental or commercial trials.

References

Adedokun S, Olojede O (2019) Optimizing gastrointestinal integrity in poultry: the role of nutrients and feed additives. Front Vet Sci 5:348. https://doi.org/10.3389/fvets.2018.00348

Al-Khalaifah H (2018) Benefits of probiotics and/or prebiotics for antibiotic-reduced poultry. Poult Sci 97:3807–3815. https://doi.org/10.3382/ps/pey160

Angelakis E (2017) Weight gain by gut microbiota manipulation in productive animals. Microb Pathog 106:162–170. https://doi.org/10.1016/j.micpath.2016.11.002

Antonissen G, Eeckhaut V, Van Driessche K et al (2016) Microbial shifts associated with necrotic enteritis. Avian Pathol 45:308–312. https://doi.org/10.1080/03079457.2016.1152625

Atarashi K, Tanoue T, Oshima K et al (2013) Treg induction by a rationally selected mixture of clostridia strains from the human microbiota. Nature 500:232–236. https://doi.org/10.1038/nature12331

Bae Y, Koo B, Lee S et al (2017) Bacterial diversity and its relationship to growth performance of broilers. Korean J Vet Res 57:159–167. https://doi.org/10.14405/kjvr.2017.57.3.159

Baldwin S, Hughes RJ, Van TTH et al (2018) At-hatch administration of probiotic to chickens can introduce beneficial changes in gut microbiota. PLoS One 13:e0194825. https://doi.org/10.1371/journal.pone.0194825

Ballou AL, Ali RA, Mendoza MA et al (2016) Development of the Chick microbiome: how early exposure influences future microbial diversity. Front Vet Sci 3:1–12. https://doi.org/10.3389/fvets.2016.00002

Beausang C, McDonnell K, Murphy F (2020) Anaerobic digestion of poultry litter – a consequential life cycle assessment. Sci Total Environ 735:139494. https://doi.org/10.1016/j.scitotenv.2020.139494

Bedford MR (2018) The evolution and application of enzymes in the animal feed industry: the role of data interpretation. Br Poult Sci 59:486–493. https://doi.org/10.1080/00071668.2018.1484074

Biesalski HK (2016) Nutrition meets the microbiome: micronutrients and the microbiota. Ann N Y Acad Sci 1372:53–64. https://doi.org/10.1111/nyas.13145

Birkl P, Bharwani A, Kjaer JB et al (2018) Differences in cecal microbiome of selected high and low feather-pecking laying hens. Poult Sci 97:3009–3014. https://doi.org/10.3382/ps/pey167

Bolan NS, Szogi AA, Chuasavathi T et al (2010) Uses and management of poultry litter. Worlds Poult Sci J 66:673–698. https://doi.org/10.1017/S0043933910000656

Bolnick DI, Snowberg LK, Caporaso JG et al (2014) Major histocompatibility complex class IIb polymorphism influences gut microbiota composition and diversity. Mol Ecol 23:4831–4845. https://doi.org/10.1111/mec.12846

Borda-Molina D, Seifert J, Camarinha-Silva A (2018) Current perspectives of the chicken gastrointestinal tract and its microbiome. Comput Struct Biotechnol J 16:131–139. https://doi.org/10.1016/J.CSBJ.2018.03.002

Breytenbach JH (2005) Guidelines for effective vaccination of broilers. http://www.positiveaction.info/pdfs/articles/pp13.7p7.pdf

Brüssow H (2015) Growth promotion and gut microbiota: insights from antibiotic use. Environ Microbiol 17:2216–2227. https://doi.org/10.1111/1462-2920.12786

Bucher MG, Zwirzitz B, Oladeinde A et al (2020) Reused poultry litter microbiome with competitive exclusion potential against Salmonella Heidelberg. J Environ Qual 49:869–881. https://doi.org/10.1002/jeq2.20081

Butaye P, Devriese LA, Haesebrouck F (2003) Antimicrobial growth promoters used in animal feed: effects of less well known antibiotics on gram-positive bacteria. Clin Microbiol Rev 16:175–188. https://doi.org/10.1128/cmr.16.2.175-188.2003

Calik A, Ergün A (2015) Effect of lactulose supplementation on growth performance, intestinal histomorphology, cecal microbial population, and short-chain fatty acid composition of broiler chickens. Poult Sci 94:2173–2182. https://doi.org/10.3382/ps/pev182

Campbell DLM, Makagon MM, Swanson JC, Siegford JM (2016) Litter use by laying hens in a commercial aviary: dust bathing and piling. Poult Sci 95:164–175. https://doi.org/10.3382/ps/pev183

Castanon JIR (2007) History of the use of antibiotic as growth promoters in European poultry feeds. Poult Sci 86:2466–2471. https://doi.org/10.3382/ps.2007-00249

Chen Z, Jiang X (2014) Microbiological safety of chicken litter or chicken litter-based organic fertilizers: a review. Agriculture 4:1–29. https://doi.org/10.3390/agriculture4010001

Cheng HY, Ning MX, Chen DK, Ma WT (2019) Interactions between the gut microbiota and the host innate immune response against pathogens. Front Immunol 10:607. https://doi.org/10.3389/fimmu.2019.00607

Chinivasagam HN, Estella W, Rodrigues H et al (2016) On-farm campylobacter and Escherichia coli in commercial broiler chickens: re-used bedding does not influence campylobacter emergence and levels across sequential farming cycles. Poult Sci 95:1105–1115. https://doi.org/10.3382/ps/pew003

Cho JH, Kim HJ, Kim IH (2014) Effects of phytogenic feed additive on growth performance, digestibility, blood metabolites, intestinal microbiota, meat color and relative organ weight after oral challenge with Clostridium perfringens in broilers. Livest Sci 160:82–88. https://doi.org/10.1016/j.livsci.2013.11.006

Cockerill SA, Gerber PF, Walkden-Brown SW, Dunlop MW (2020) Suitability of litter amendments for the Australian chicken meat industry. Anim Prod Sci 60:1469. https://doi.org/10.1071/AN19587

Cook MI, Beissinger SR, Toranzos GA, Arendt WJ (2005) Incubation reduces microbial growth on eggshells and the opportunity for trans-shell infection. Ecol Lett 8:532–537. https://doi.org/10.1111/j.1461-0248.2005.00748.x

Costa MC, Bessegatto JA, Alfieri AA et al (2017) Different antibiotic growth promoters induce specific changes in the cecal microbiota membership of broiler chicken. PLoS One 12:e0171642. https://doi.org/10.1371/journal.pone.0171642

Cressman MD, Yu Z, Nelson MC et al (2010) Interrelations between the microbiotas in the litter and in the intestines of commercial broiler chickens. Appl Environ Microbiol 76:6572–6582. https://doi.org/10.1128/AEM.00180-10

Cui Y, Wang Q, Liu S et al (2017) Age-related variations in intestinal microflora of free-range and caged hens. Front Microbiol 8:1310. https://doi.org/10.3389/fmicb.2017.01310

Danzeisen JL, Clayton JB, Huang H et al (2015) Temporal relationships exist between cecum, ileum, and litter bacterial microbiomes in a commercial Turkey flock, and subtherapeutic penicillin treatment impacts ileum bacterial community establishment. Front Vet Sci 2:56. https://doi.org/10.3389/fvets.2015.00056

De Haas EN, Bolhuis JE, Kemp B et al (2014) Parents and early life environment affect behavioral development of laying hen chickens. PLoS One 9:e90577. https://doi.org/10.1371/journal.pone.0090577

De Jong I, Berg C, Butterworth A, Estevéz I (2012) Scientific report updating the EFSA opinions on the welfare of broilers and broiler breeders. Wiley-Blackwell, New York. https://doi.org/10.2903/sp.efsa.2012.en-295

de Toledo TDS, Roll AAP, Rutz F et al (2020) An assessment of the impacts of litter treatments on the litter quality and broiler performance: a systematic review and metaanalysis. PLoS One 15:e0232853. https://doi.org/10.1371/journal.pone.0232853

Díaz Carrasco JM (2018) Seasonality as a driver of cecal and litter microbiota variation in commercial broiler chickens. Symposium on Gut Health in Production of Food Animals, St. Louis, p 13

Díaz Carrasco JM, Redondo LM, Redondo EA et al (2016) Use of plant extracts as an effective manner to control Clostridium perfringens induced necrotic enteritis in poultry. Biomed Res Int 2016:1–15. https://doi.org/10.1155/2016/3278359

Díaz Carrasco JM, Redondo EA, Pin Viso ND et al (2018) Tannins and bacitracin differentially modulate gut microbiota of broiler chickens. Biomed Res Int 2018:1879168. https://doi.org/10.1155/2018/1879168

Díaz Carrasco JM, Casanova NA, Fernández Miyakawa ME et al (2019) Microbiota, gut health and chicken productivity: what is the connection? Microorganisms 7:1–15. https://doi.org/10.3390/microorganisms7100374

Dibner JJ, Richards JD (2005) Antibiotic growth promoters in agriculture: history and mode of action. Poult Sci 84:634–643. https://doi.org/10.1093/ps/84.4.634

Ding J, Dai R, Yang L et al (2017) Inheritance and establishment of gut microbiota in chickens. Front Microbiol 8:1967. https://doi.org/10.3389/fmicb.2017.01967

Dominguez-Bello MG, Costello EK, Contreras M et al (2010) Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. Proc Natl Acad Sci U S A 107:11971–11975. https://doi.org/10.1073/pnas.1002601107

Donaldson EE, Stanley D, Hughes RJ, Moore RJ (2017) The time-course of broiler intestinal microbiota development after administration of cecal contents to incubating eggs. PeerJ 5:e3587. https://doi.org/10.7717/peerj.3587

Druyan S (2010) The effects of genetic line (broilers vs. layers) on embryo development. Poult Sci 89:1457–1467. https://doi.org/10.3382/ps.2009-00304

Dumas MD, Polson SW, Ritter D et al (2011) Impacts of poultry house environment on poultry litter bacterial community composition. PLoS One 6:e24785. https://doi.org/10.1371/journal.pone.0024785

Edgar J, Held S, Jones C, Troisi C (2016) Influences of maternal care on chicken welfare. Animals 6:2. https://doi.org/10.3390/ani6010002

Eeckhaut V, Van Immerseel F, Croubels S et al (2011) Butyrate production in phylogenetically diverse Firmicutes isolated from the chicken caecum. Microb Biotechnol 4:503–512. https://doi.org/10.1111/j.1751-7915.2010.00244.x

EFSA Panel on Animal Health and Welfare (2010) Scientific opinion on the influence of genetic parameters on the welfare and the resistance to stress of commercial broilers. EFSA J 8:1–82. https://doi.org/10.2903/j.efsa.2010.1666

Esteban JI, Oporto B, Aduriz G et al (2008) A survey of food-borne pathogens in free-range poultry farms. Int J Food Microbiol 123:177–182. https://doi.org/10.1016/j.ijfoodmicro.2007.12.012
European Commission Regulation (2008) Commission Regulation (EC) No 543/2008
Fanatico AC (2006) Alternative poultry production systems and outdoor access. In: ATTRA - National Sustainable Agriculture Information Service. http://www.fao.org/ag/againfo/themes/animal-welfare/news-detail/es/c/42603
Fasina YO, Newman MM, Stough JM, Liles MR (2016) Effect of Clostridium perfringens infection and antibiotic administration on microbiota in the small intestine of broiler chickens. Poult Sci 95:247–260. https://doi.org/10.3382/ps/pev329
Flint HJ, Scott KP, Louis P, Duncan SH (2012) The role of the gut microbiota in nutrition and health. Nat Rev Gastroenterol Hepatol 9:577–589. https://doi.org/10.1038/nrgastro.2012.156
Fouhse JM, Zijlstra RT, Willing BP (2016) The role of gut microbiota in the health and disease of pigs. Anim Front 6:30–36. https://doi.org/10.2527/af.2016-0031
Franz C, Baser KHC, Windisch W (2010) Essential oils and aromatic plants in animal feeding - a European perspective. A review. Flavour Fragr J 25:327–340. https://doi.org/10.1002/ffj.1967
Funkhouser LJ, Bordenstein SR (2013) Mom knows best: the universality of maternal microbial transmission. PLoS Biol 11:e1001631. https://doi.org/10.1371/journal.pbio.1001631
Gadde UD, Oh S, Lillehoj HS, Lillehoj EP (2018) Antibiotic growth promoters virginiamycin and bacitracin methylene disalicylate alter the chicken intestinal metabolome. Sci Rep 8:3592. https://doi.org/10.1038/s41598-018-22004-6
Gaggìa F, Mattarelli P, Biavati B (2010) Probiotics and prebiotics in animal feeding for safe food production. Int J Food Microbiol 141. https://doi.org/10.1016/j.ijfoodmicro.2010.02.031
Gantois I, Ducatelle R, Pasmans F et al (2009) Mechanisms of egg contamination by Salmonella enteritidis: review article. FEMS Microbiol Rev 33:718–738. https://doi.org/10.1111/j.1574-6976.2008.00161.x
Gao P, Ma C, Sun Z et al (2017) Feed-additive probiotics accelerate yet antibiotics delay intestinal microbiota maturation in broiler chicken. Microbiome 5:91. https://doi.org/10.1186/s40168-017-0315-1
Garrido MN, Skjervheim M, Oppegaard H, Sørum H (2004) Acidified litter benefits the intestinal flora balance of broiler chickens. Appl Environ Microbiol 70:5208–5213. https://doi.org/10.1128/AEM.70.9.5208-5213.2004
Gibson GR, Probert HM, Van Loo J et al (2004) Dietary modulation of the human colonic microbiota: updating the concept of prebiotics. Nutr Res Rev 17:259–275. https://doi.org/10.1079/nrr200479
Gifford K, Bernard JC (2011) The effect of information on consumers' willingness to pay for natural and organic chicken. Int J Consum Stud 35:282–289. https://doi.org/10.1111/j.1470-6431.2010.00929.x
Glendinning L, McLachlan G, Vervelde L (2017) Age-related differences in the respiratory microbiota of chickens. PLoS One 12:e0188455. https://doi.org/10.1371/journal.pone.0188455
Golden CE, Mishra A (2020) Prevalence of salmonella and campylobacter spp. in alternative and conventionally produced chicken in the United States: a systematic review and meta-analysis. J Food Prot 83:1181–1197. https://doi.org/10.4315/JFP-19-538
Gonçalves-Tenório A, Nunes SB, Rodrigues V et al (2018) Prevalence of pathogens in poultry meat: a meta-analysis of European published surveys. Foods 7:69. https://doi.org/10.3390/foods7050069
Gong J, Yu H, Liu T et al (2008) Effects of zinc bacitracin, bird age and access to range on bacterial microbiota in the ileum and caeca of broiler chickens. J Appl Microbiol 104:1372–1382. https://doi.org/10.1111/j.1365-2672.2007.03699.x
Haiser HJ, Turnbaugh PJ (2012) Is it time for a metagenomic basis of therapeutics. Science 336:1253–1255. https://doi.org/10.1126/science.1224396
Hartcher KM, Jones B (2017) The welfare of layer hens in cage and cage-free housing systems. Worlds Poult Sci J 73:767–781. https://doi.org/10.1017/S0043933917000812

Hildebrandt MA, Hoffmann C, Sherrill-Mix SA et al (2009) High-fat diet determines the composition of the murine gut microbiome independently of obesity. Gastroenterology 137:1716–1724. https://doi.org/10.1053/j.gastro.2009.08.042

Hubert SM, Al-Ajeeli M, Bailey CA, Athrey G (2019) The role of housing environment and dietary protein source on the gut microbiota of chicken. Animals 9:1085. https://doi.org/10.3390/ani9121085

Huyghebaert G, Ducatelle R, Van Immerseel F (2011) An update on alternatives to antimicrobial growth promoters for broilers. Vet J 187:182–188. https://doi.org/10.1016/j.tvjl.2010.03.003

Inatomi T (2016) Laying performance, immunity and digestive health of layer chickens fed diets containing a combination of three probiotics. Sci Postprint. 1:e00058. https://doi.org/10.14340/spp.2016.03a0001

ISUST & USDA Poultry Industry Manual (2013) FAD PReP poultry industry manual. The Foreign Animal Disease Preparedness and Response Plan (FAD PReP)/National Animal Health Emergency Management System (NAHEMS) guidelines

Jacob JP, Griggs JP, Bender JB (2008) Characterization of small-scale antibiotic-free broiler production in Minnesota. J Appl Poult Res 17:412–420. https://doi.org/10.3382/japr.2007-00057

Janczak AM, Riber AB (2015) Review of rearing-related factors affecting the welfare of laying hens. Poult Sci 94:1454–1469. https://doi.org/10.3382/ps/pev123

Janke O, Tzschentke B, Boerjan M (2004) Comparative investigations of heat production and body temperature in embryos of modern chicken breeds. In: Avian and Poultry Biology Reviews. Science Reviews, pp 191–196

Jha R, Singh AK, Yadav S et al (2019) Early nutrition programming (in ovo and post-hatch feeding) as a strategy to modulate gut health of poultry. Front Vet Sci 6:82. https://doi.org/10.3389/fvets.2019.00082

Johnson TJ, Youmans BP, Noll S et al (2018) A consistent and predictable commercial broiler chicken bacterial microbiota in antibiotic-free production displays strong correlations with performance. Appl Environ Microbiol 84:e00362. https://doi.org/10.1128/AEM.00362-18

Jukes HG, Hill DC, Branion HD (1956) Effect of feeding antibiotics on the intestinal tract of the Chick. Poult Sci 35:716–723. https://doi.org/10.3382/ps.0350716

Kelleher BP, Leahy JJ, Henihan AM et al (2002) Advances in poultry litter disposal technology - a review. Bioresour Technol 83:27–36. https://doi.org/10.1016/S0960-8524(01)00133-X

Kers JG, Velkers FC, Fischer EAJ et al (2018) Host and environmental factors affecting the intestinal microbiota in chickens. Front Microbiol 9:235. https://doi.org/10.3389/fmicb.2018.00235

Kers JG, Velkers FC, Fischer EAJ et al (2019) Take care of the environment: housing conditions affect the interplay of nutritional interventions and intestinal microbiota in broiler chickens. Anim Microbiome 1:10. https://doi.org/10.1186/s42523-019-0009-z

Khan S, Moore RJ, Stanley D, Chousalkar KK (2020) The gut microbiota of laying hens and its manipulation with prebiotics and probiotics to enhance gut health and food safety. Appl Environ Microbiol 86:e00600–e00620. https://doi.org/10.1128/AEM.00600-20

Kiarie E, Romero LF, Nyachoti CM (2013) The role of added feed enzymes in promoting gut health in swine and poultry. Nutr Res Rev 26:71–88. https://doi.org/10.1017/S0954422413000048

Kim GB, Seo YM, Kim CH, Paik IK (2011) Effect of dietary prebiotic supplementation on the performance, intestinal microflora, and immune response of broilers. Poult Sci 90:75–82. https://doi.org/10.3382/ps.2010-00732

Kogut MH (2019) The effect of microbiome modulation on the intestinal health of poultry. Anim Feed Sci Technol 250:32–40. https://doi.org/10.1016/j.anifeedsci.2018.10.008

Kogut MH, Arsenault RJ (2016) Editorial: gut health: the new paradigm in food animal production. Front Vet Sci 3:71. https://doi.org/10.3389/fvets.2016.00071

Kubasova T, Kollarcikova M, Crhanova M et al (2019) Contact with adult hen affects development of caecal microbiota in newly hatched chicks. PLoS One 14:e0212446. https://doi.org/10.1371/journal.pone.0212446

Kumar S, Chen C, Indugu N et al (2018) Effect of antibiotic withdrawal in feed on chicken gut microbial dynamics, immunity, growth performance and prevalence of foodborne pathogens. PLoS One 13:e0192450. https://doi.org/10.1371/journal.pone.0192450

Lee S, La T-M, Lee H-J et al (2019) Characterization of microbial communities in the chicken oviduct and the origin of chicken embryo gut microbiota. Sci Rep 9:6838. https://doi.org/10.1038/s41598-019-43280-w

Lei K, Li YL, Yu DY et al (2013) Influence of dietary inclusion of Bacillus licheniformis on laying performance, egg quality, antioxidant enzyme activities, and intestinal barrier function of laying hens. Poult Sci 92:2389–2395. https://doi.org/10.3382/ps.2012-02686

Lewis PD, Perry GC, Farmer LJ, Patterson RLS (1997) Responses of two genotypes of chicken to the diets and stocking densities typical of UK and "Label Rouge" production systems: I. Performance, behaviour and carcass composition. Meat Sci 45:501–516. https://doi.org/10.1016/S0309-1740(96)00084-8

Lillehoj H, Liu Y, Calsamiglia S et al (2018) Phytochemicals as antibiotic alternatives to promote growth and enhance host health. Vet Res 49:76. https://doi.org/10.1186/s13567-018-0562-6

Lin J, Hunkapiller AA, Layton AC et al (2013) Response of intestinal microbiota to antibiotic growth promoters in chickens. Foodborne Pathog Dis 10:331–337. https://doi.org/10.1089/fpd.2012.1348

Lorenz EN (1963) Deterministic nonperiodic flow. J Atmos Sci 20:130–141. https://doi.org/10.1175/1520-0469(1963)020<0130:dnf>2.0.co;2

Lu J, Sanchez S, Hofacre C et al (2003) Evaluation of broiler litter with reference to the microbial composition as assessed by using 16S rRNA and functional gene markers. Appl Environ Microbiol 69:901–908. https://doi.org/10.1128/AEM.69.2.901-908.2003

Lu J, Hofacre C, Smith F, Lee MD (2008) Effects of feed additives on the development on the ileal bacterial community of the broiler chicken. Animal 2:669–676. https://doi.org/10.1017/S1751731108001894

Luján-Rhenals D, Morawicki R, Van Loo EJ, Ricke S (2016) Energy and water use in poultry processing. In: Achieving sustainable production of poultry meat, volume 1: safety, quality and sustainability. Thomas D. Burleigh, Cambridge, UK, pp 389–409

Lumpkins BS, Batal AB, Lee MD (2010) Evaluation of the bacterial community and intestinal development of different genetic lines of chickens. Poult Sci 89:1614–1621. https://doi.org/10.3382/ps.2010-00747

Mahmood T, Guo Y (2020) Dietary fiber and chicken microbiome interaction: where will it lead to? Anim Nutr 6:1–8. https://doi.org/10.1016/j.aninu.2019.11.004

Maki JJ, Bobeck EA, Sylte MJ, Looft T (2020) Eggshell and environmental bacteria contribute to the intestinal microbiota of growing chickens. J Anim Sci Biotechnol 11:60. https://doi.org/10.1186/s40104-020-00459-w

Mancabelli L, Ferrario C, Milani C et al (2016) Insights into the biodiversity of the gut microbiota of broiler chickens. Environ Microbiol 18:4727–4738. https://doi.org/10.1111/1462-2920.13363

Melo-Duràn D, Solà-Oriol D, Villagomez-Estrada S, Pérez JF (2019) Enzymes as an alternative to antibiotics: an overview. In: The value of fibre. Wageningen Academic Publishers, New York, pp 351–3710

Metzler-Zebeli BU, Siegerstetter S-C, Magowan E et al (2019) Feed restriction modifies intestinal microbiota-host mucosal networking in chickens divergent in residual feed intake. mSystems 4:e00261-18. https://doi.org/10.1128/mSystems.00261-18

Micciche AC, Foley SL, Pavlidis HO et al (2018) A review of prebiotics against salmonella in poultry: current and future potential for microbiome research applications. Front Vet Sci 5:191. https://doi.org/10.3389/fvets.2018.00191

Mignon-Grasteau S, Narcy A, Rideau N et al (2015) Impact of selection for digestive efficiency on microbiota composition in the chicken. PLoS One. https://doi.org/10.1371/journal.pone.0135488

Mitchell MA, Kettlewell PJ (2009) Transport of chicks, pullets and spent hens. In: Welfare of the laying hen. CABI, New York, pp 361–374

Molino S, Fernández-Miyakawa M, Giovando S, Rufián-Henares JÁ (2018) Study of antioxidant capacity and metabolization of quebracho and chestnut tannins through in vitro gastrointestinal digestion-fermentation. J Funct Foods 49:188–195. https://doi.org/10.1016/j.jff.2018.07.056

Mookiah S, Sieo CC, Ramasamy K et al (2014) Effects of dietary prebiotics, probiotic and synbiotics on performance, caecal bacterial populations and caecal fermentation concentrations of broiler chickens. J Sci Food Agric 94:341–348. https://doi.org/10.1002/jsfa.6365

Moore RJ (2016) Necrotic enteritis predisposing factors in broiler chickens. Avian Pathol 45:1–22. https://doi.org/10.1080/03079457.2016.1150587

Mueller-Harvey I (2006) Unravelling the conundrum of tannins in animal nutrition and health. J Sci Food Agric 86:2010–2037. https://doi.org/10.1002/jsfa.2577

Naseem S, King AJ (2018) Ammonia production in poultry houses can affect health of humans, birds, and the environment—techniques for its reduction during poultry production. Environ Sci Pollut Res 25:15269–15293. https://doi.org/10.1007/s11356-018-2018-y

Ngunjiri JM, Taylor KJM, Abundo MC et al (2019) Farm stage, bird age, and body site dominantly affect the quantity, taxonomic composition, and dynamics of respiratory and gut microbiota of commercial layer chickens. Appl Environ Microbiol 85:e03137–e03118. https://doi.org/10.1128/AEM.03137-18

Nicholson JK, Holmes E, Kinross J et al (2012) Host-gut microbiota metabolic interactions. Science 336:1262–1267. https://doi.org/10.1126/science.1223813

Niewold TA (2007) The nonantibiotic anti-inflammatory effect of antimicrobial growth promoters, the real mode of action? A hypothesis. Poult Sci 86:605–609. https://doi.org/10.1093/ps/86.4.605

O'Bryan CA, Crandall P, Jaroni D et al (2017) Assessment of nitrogen and phosphorus loads present in environments impacted by alternative poultry processing operations utilized in pasture-raised poultry production. Renew Agric Food Syst 32:33–42. https://doi.org/10.1017/S1742170515000514

O'Dwyer JP, Kembel SW, Green JL (2012) Phylogenetic diversity theory sheds light on the structure of microbial communities. PLoS Comput Biol 8:e1002832. https://doi.org/10.1371/journal.pcbi.1002832

Oakley BB, Morales CA, Line J et al (2013) The poultry-associated microbiome: network analysis and farm-to-fork characterizations. PLoS One 8:e57190. https://doi.org/10.1371/journal.pone.0057190

Oakley BB, Buhr RJ, Ritz CW et al (2014) Successional changes in the chicken cecal microbiome during 42 days of growth are independent of organic acid feed additives. BMC Vet Res 10:282. https://doi.org/10.1186/s12917-014-0282-8

Ocejo M, Oporto B, Hurtado A (2019) 16S rRNA amplicon sequencing characterization of caecal microbiome composition of broilers and free-range slow-growing chickens throughout their productive lifespan. Sci Rep 9:2506. https://doi.org/10.1038/s41598-019-39323-x

Omeira N, Barbour EK, Nehme PA et al (2006) Microbiological and chemical properties of litter from different chicken types and production systems. Sci Total Environ 367:156–162. https://doi.org/10.1016/j.scitotenv.2006.02.019

Pan D, Yu Z (2014) Intestinal microbiome of poultry and its interaction with host and diet. Gut Microbes 5:108–119. https://doi.org/10.4161/gmic.26945

Pantoja-Feliciano IG, Clemente JC, Costello EK et al (2013) Biphasic assembly of the murine intestinal microbiota during early development. ISME J 7:1112–1115. https://doi.org/10.1038/ismej.2013.15

Park SH, Hanning I, Perrota A et al (2013) Modifying the gastrointestinal ecology in alternatively raised poultry and the potential for molecular and metabolomic assessment. Poult Sci 92:546–561. https://doi.org/10.3382/ps.2012-02734

Park SH, Lee SI, Kim SA et al (2017) Comparison of antibiotic supplementation versus a yeast-based prebiotic on the cecal microbiome of commercial broilers. PLoS One 12:e0182805. https://doi.org/10.1371/journal.pone.0182805

Patterson JA, Burkholder KM (2003) Application of prebiotics and probiotics in poultry production. Poult Sci 82:627–631. https://doi.org/10.1093/ps/82.4.627

Pedroso AA, Menten JFM, Lambais MR et al (2006) Intestinal bacterial community and growth performance of chickens fed diets containing antibiotics. Poult Sci 85:747–752. https://doi.org/10.1093/ps/85.4.747

Pohjola L, Nykäsenoja S, Kivistö R et al (2016) Zoonotic public health hazards in backyard chickens. Zoonoses Public Health 63:420–430. https://doi.org/10.1111/zph.12247

Polycarpo G, Cruz-Polycarpo V, Andretta I et al (2017) Meta-analytic study of organic acids as an alternative performance-enhancing feed additive to antibiotics for broiler chickens. Poult Sci 96:3645–3653. https://doi.org/10.3382/ps/pex178

Pourabedin M, Xu Z, Baurhoo B et al (2014) Effects of mannan oligosaccharide and virginiamycin on the cecal microbial community and intestinal morphology of chickens raised under suboptimal conditions. Can J Microbiol 60:255–266. https://doi.org/10.1139/cjm-2013-0899

Pym RAE, Alders RG (2012) Introduction to village and backyard poultry production. In: Alternative systems for poultry: health, welfare and productivity. CABI Publishing, New York, pp 97–109

Redondo LM, Chacana P, a, Dominguez J.E., Fernandez Miyakawa M.E. (2014) Perspectives in the use of tannins as alternative to antimicrobial growth promoter factors in poultry. Front Microbiol 5:118. https://doi.org/10.3389/fmicb.2014.00118

Redondo LM, Dominguez JE, Rabinovitz BC et al (2015) Hydrolyzable and condensed tannins resistance in Clostridium perfringens. Anaerobe 34:139–145. https://doi.org/10.1016/j.anaerobe.2015.05.010

Richards P, Fothergill J, Bernardeau M, Wigley P (2019) Development of the caecal microbiota in three broiler breeds. Front Vet Sci 6:201. https://doi.org/10.3389/fvets.2019.00201

Richards-Rios P, Leeming G, Fothergill J et al (2020) Topical application of adult cecal contents to eggs transplants spore-forming microbiota but not other members of the microbiota to chicks. Appl Environ Microbiol 86:e02387–e02319. https://doi.org/10.1128/AEM.02387-19

Ro KS, Moore PA, Szogi AA, Millner PD (2017) Ammonia and nitrous oxide emissions from broiler houses with downtime windrowed litter. J Environ Qual 46:498–504. https://doi.org/10.2134/jeq2016.09.0368

Roll VFB, Dai Prá MA, Roll AP (2011) Research on salmonella in broiler litter reused for up to 14 consecutive flocks. Poult Sci 90:2257–2262. https://doi.org/10.3382/ps.2011-01583

Rothrock MJ, Locatelli A, Feye KM et al (2019) A Microbiomic analysis of a pasture-raised broiler flock elucidates foodborne pathogen ecology along the farm-to-fork continuum. Front Vet Sci 6:260. https://doi.org/10.3389/fvets.2019.00260

Roto SM, Kwon YM, Ricke SC (2016) Applications of in ovo technique for the optimal development of the gastrointestinal tract and the potential influence on the establishment of its microbiome in poultry. Front. Vet. Sci. 3:63. https://doi.org/10.3389/fvets.2016.00063

Rubio LA (2019) Possibilities of early life programming in broiler chickens via intestinal microbiota modulation. Poult Sci 98:695–706. https://doi.org/10.3382/ps/pey416

Schmidt CJ, Persia ME, Feierstein E et al (2009) Comparison of a modern broiler line and a heritage line unselected since the 1950s. Poult Sci 88:2610–2619. https://doi.org/10.3382/ps.2009-00055

Shawkey MD, Firestone MK, Brodie EL, Beissinger SR (2009) Avian incubation inhibits growth and diversification of bacterial assemblages on eggs. PLoS One 4:e4522. https://doi.org/10.1371/journal.pone.0004522

Shi Z, Rothrock MJ, Ricke SC (2019) Applications of microbiome analyses in alternative poultry broiler production systems. Front Vet Sci 6:157. https://doi.org/10.3389/fvets.2019.00157

Siegel PB (2014) Evolution of the modern broiler and feed efficiency. Annu Rev Anim Biosci 2:375–385. https://doi.org/10.1146/annurev-animal-022513-114132

Siemon CE, Bahnson PB, Gebreyes WA (2007) Comparative investigation of prevalence and antimicrobial resistance of Salmonella between pasture and conventionally reared poultry. Avian Dis 51:112–117. https://doi.org/10.1637/0005-2086(2007)051[0112:CIOPAA]2.0.CO;2

Simon K, Verwoolde MB, Zhang J et al (2016) Long-term effects of early life microbiota disturbance on adaptive immunity in laying hens. Poult Sci 95:1543–1554. https://doi.org/10.3382/ps/pew088

Singh KM, Shah TM, Reddy B et al (2014) Taxonomic and gene-centric metagenomics of the fecal microbiome of low and high feed conversion ratio (FCR) broilers. J Appl Genet 55:145–154. https://doi.org/10.1007/s13353-013-0179-4

Siwek M, Slawinska A, Stadnicka K et al (2018) Prebiotics and synbiotics - in ovo delivery for improved lifespan condition in chicken. BMC Vet Res 14:402. https://doi.org/10.1186/s12917-018-1738-z

Smith CCR, Snowberg LK, Gregory CJ et al (2015) Dietary input of microbes and host genetic variation shape among-population differences in stickleback gut microbiota. ISME J 9:2515–2526. https://doi.org/10.1038/ismej.2015.64

Smith NW, Shorten PR, Altermann E et al (2019) The classification and evolution of bacterial cross-feeding. Front Ecol Evol 7:153. https://doi.org/10.3389/fevo.2019.00153

Sossidou EN, Dal BA, Castellini C, Grashorn MA (2015) Effects of pasture management on poultry welfare and meat quality in organic poultry production systems. Worlds Poult Sci J 71:375–384. https://doi.org/10.1017/S0043933915000379

Stanley D, Denman SE, Hughes RJ et al (2012a) Intestinal microbiota associated with differential feed conversion efficiency in chickens. Appl Microbiol Biotechnol 96:1361–1369. https://doi.org/10.1007/s00253-011-3847-5

Stanley D, Keyburn AL, Denman SE, Moore RJ (2012b) Changes in the caecal microflora of chickens following Clostridium perfringens challenge to induce necrotic enteritis. Vet Microbiol 159:155–162. https://doi.org/10.1016/j.vetmic.2012.03.032

Stanley D, Geier MS, Hughes RJ et al (2013) Highly variable microbiota development in the chicken gastrointestinal tract. PLoS One 8:e84290. https://doi.org/10.1371/journal.pone.0084290

Stanley D, Hughes RJ, Moore RJ (2014) Microbiota of the chicken gastrointestinal tract: influence on health, productivity and disease. Appl Microbiol Biotechnol 98:4301–4310. https://doi.org/10.1007/s00253-014-5646-2

Stanley D, Hughes RJ, Geier MS, Moore RJ (2016) Bacteria within the gastrointestinal tract microbiota correlated with improved growth and feed conversion: challenges presented for the identification of performance enhancing probiotic bacteria. Front Microbiol 7:187. https://doi.org/10.3389/fmicb.2016.00187

Steinfeld H, Gerber P, Wassenaar T et al (2006) Livestock's long shadow. FAO of the UN, Rome

Sugiharto S, Yudiarti T, Isroli I et al (2017) Dietary supplementation of probiotics in poultry exposed to heat stress - a review. Ann Anim Sci 17:591–604. https://doi.org/10.1515/aoas-2016-0062

Surai PF (2014) Polyphenol compounds in the chicken/animal diet: from the past to the future. J Anim Physiol Anim Nutr (Berl) 98:19–31. https://doi.org/10.1111/jpn.12070

Sylte MJ, Chandra LC, Looft T (2017) Evaluation of disinfectants and antiseptics to eliminate bacteria from the surface of Turkey eggs and hatch gnotobiotic poults. Poult Sci 96:2412–2420. https://doi.org/10.3382/ps/pex022

Tallentire CW, Leinonen I, Kyriazakis I (2016) Breeding for efficiency in the broiler chicken: a review. Agron Sustain Dev 36:1–16. https://doi.org/10.1007/s13593-016-0398-2

Thiele H-H, Pottgüter R (2008) Management recommendations for laying hens in deep litter, Perchery and free range systems. Lohmann-information.de 43:53–63

Toledo TDSD, Pich CS, Roll AAP et al (2019) The effect of litter materials on broiler performance: a systematic review and meta-analysis. Br Poult Sci 60:605–616. https://doi.org/10.1080/00071668.2019.1639143

Torok VA, Hughes RJ, Ophel-Keller K et al (2009) Influence of different litter materials on cecal microbiota colonization in broiler chickens. Poult Sci 88:2474–2481. https://doi.org/10.3382/ps.2008-00381

Torok VA, Allison GE, Percy NJ et al (2011a) Influence of antimicrobial feed additives on broiler commensal posthatch gut microbiota development and performance. Appl Environ Microbiol 77:3380–3390. https://doi.org/10.1128/AEM.02300-10

Torok VA, Hughes RJ, Mikkelsen LL et al (2011b) Identification and characterization of potential performance-related gut microbiotas in broiler chickens across various feeding trials. Appl Environ Microbiol 77:5868–5878. https://doi.org/10.1128/AEM.00165-11

Trudeau S, Thibodeau A, Côté J-C et al (2020) Contribution of the broiler breeders' fecal microbiota to the establishment of the eggshell microbiota. Front Microbiol 11:666. https://doi.org/10.3389/fmicb.2020.00666

Van der Eijk JAJ, de Vries H, Kjaer JB et al (2019) Differences in gut microbiota composition of laying hen lines divergently selected on feather pecking. Poult Sci 98:7009–7021. https://doi.org/10.3382/ps/pez336

Van Der Wielen PWJJ, Keuzenkamp DA, Lipman LJA et al (2002) Spatial and temporal variation of the intestinal bacterial community in commercially raised broiler chickens during growth. Microb Ecol 44:286–293. https://doi.org/10.1007/s00248-002-2015-y

Van Goor A, Redweik GAJ, Stromberg ZR et al (2020) Microbiome and biological blood marker changes in hens at different laying stages in conventional and cage free housings. Poult Sci 99:2362–2374. https://doi.org/10.1016/j.psj.2020.01.011

Van Veelen HPJ, Salles JF, Tieleman BI (2018) Microbiome assembly of avian eggshells and their potential as transgenerational carriers of maternal microbiota. ISME J 12:1375–1388. https://doi.org/10.1038/s41396-018-0067-3

Varmuzova K, Kubasova T, Davidova-Gerzova L et al (2016) Composition of gut microbiota influences resistance of newly hatched chickens to salmonella enteritidis infection. Front Microbiol 7:957. https://doi.org/10.3389/fmicb.2016.00957

Vermeulen K, Verspreet J, Courtin CM et al (2017) Reduced particle size wheat bran is butyrogenic and lowers salmonella colonization, when added to poultry feed. Vet Microbiol 198:64–71. https://doi.org/10.1016/j.vetmic.2016.12.009

Videnska P, Faldynova M, Juricova H et al (2013) Chicken faecal microbiota and disturbances induced by single or repeated therapy with tetracycline and streptomycin. BMC Vet Res 9:30. https://doi.org/10.1186/1746-6148-9-30

Videnska P, Rahman MM, Faldynova M et al (2014a) Characterization of egg laying hen and broiler fecal microbiota in poultry farms in Croatia, Czech Republic, Hungary and Slovenia. PLoS One 9:e110076. https://doi.org/10.1371/journal.pone.0110076

Videnska P, Sedlar K, Lukac M et al (2014b) Succession and replacement of bacterial populations in the caecum of egg laying hens over their whole life. PLoS One 9:e115142. https://doi.org/10.1371/journal.pone.0115142

Viegas C, Carolino E, Malta-Vacas J et al (2012) Fungal contamination of poultry litter: a public health problem. J Toxicol Environ Health A 75:1341–1350

Viveros A, Chamorro S, Pizarro M et al (2011) Effects of dietary polyphenol-rich grape products on intestinal microflora and gut morphology in broiler chicks. Poult Sci 90:566–578. https://doi.org/10.3382/ps.2010-00889

Von Waldburg-Zeil CG, Van Staaveren N, Harlander-Matauschek A (2019) Do laying hens eat and forage in excreta from other hens? Animal 13:367–373. https://doi.org/10.1017/S1751731118001143

Wageningen UR Livestock Research (2010) Animal welfare risk assessment guidelines on housing and management. Wiley-Blackwell, New York

Wang L, Lilburn M, Yu Z (2016) Intestinal microbiota of broiler chickens as affected by litter management regimens. Front Microbiol 7:593. https://doi.org/10.3389/fmicb.2016.00593

Wang S, Chen L, He M et al (2018) Different rearing conditions alter gut microbiota composition and host physiology in Shaoxing ducks. Sci Rep 8:7387. https://doi.org/10.1038/s41598-018-25760-7

Waziri MI, Kaltungo BY (2017) Poultry litter selection, management and utilization in the tropics. In: Poultry science. IntechOpen, pp 191–209

Wei S, Gutek A, Lilburn M, Yu Z (2013) Abundance of pathogens in the gut and litter of broiler chickens as affected by bacitracin and litter management. Vet Microbiol 166:595–601. https://doi.org/10.1016/j.vetmic.2013.06.006

Wilkinson N, Hughes RJ, Bajagai YS et al (2020) Reduced environmental bacterial load during early development and gut colonisation has detrimental health consequences in Japanese quail. Heliyon 6:e03213. https://doi.org/10.1016/j.heliyon.2020.e03213

Willemsen H, Debonne M, Swennen Q et al (2010) Delay in feed access and spread of hatch: importance of early nutrition. Worlds Poult Sci J 66:177–188. https://doi.org/10.1017/S0043933910000243

Windisch W, Schedle K, Plitzner C, Kroismayr A (2008) Use of phytogenic products as feed additives for swine and poultry. J Anim Sci 86:E140–E148. https://doi.org/10.2527/jas.2007-0459

Wong S, Rawls JF (2012) Intestinal microbiota composition in fishes is influenced by host ecology and environment. Mol Ecol 21:3100–3102. https://doi.org/10.1111/j.1365-294X.2012.05646.x

Xi Y, Shuling N, Kunyuan T et al (2019) Characteristics of the intestinal flora of specific pathogen free chickens with age. Microb Pathog 132:325–334. https://doi.org/10.1016/j.micpath.2019.05.014

Xing S, Wang X, Diao H et al (2019) Changes in the cecal microbiota of laying hens during heat stress is mainly associated with reduced feed intake. Poult Sci 98:5257–5264. https://doi.org/10.3382/ps/pez440

Xu Y, Yang H, Zhang L et al (2016) High-throughput sequencing technology to reveal the composition and function of cecal microbiota in Dagu chicken. BMC Microbiol 16:1–9. https://doi.org/10.1186/s12866-016-0877-2

Yadav S, Jha R (2019) Strategies to modulate the intestinal microbiota and their effects on nutrient utilization, performance, and health of poultry. J Anim Sci Biotechnol 10:2. https://doi.org/10.1186/s40104-018-0310-9

Yan W, Sun C, Yuan J, Yang N (2017) Gut metagenomic analysis reveals prominent roles of lactobacillus and cecal microbiota in chicken feed efficiency. Sci Rep 7:45308. https://doi.org/10.1038/srep45308

Yang Y, Iji PA, Choct M (2009) Dietary modulation of gut microflora in broiler chickens: a review of the role of six kinds of alternatives to in-feed antibiotics. Worlds Poult Sci J 65:97–114. https://doi.org/10.1017/S0043933909000087

Yeoman CJ, Chia N, Jeraldo P et al (2012) The microbiome of the chicken gastrointestinal tract. Anim Health Res Rev 13:89–99. https://doi.org/10.1017/S1466252312000138

Zheng D, Liwinski T, Elinav E (2020) Interaction between microbiota and immunity in health and disease. Cell Res 30:492–506. https://doi.org/10.1038/s41422-020-0332-7

Zhu L, Liao R, Wu N et al (2019) Heat stress mediates changes in fecal microbiome and functional pathways of laying hens. Appl Microbiol Biotechnol 103:461–472. https://doi.org/10.1007/s00253-018-9465-8

Role of Early Life Intestinal Microbiota in Modulating Immunity in Broiler Chickens

11

Denise R. Rodrigues

Abstract

The successional changes in the neonatal intestinal microbiota occur concomitantly with the development, expansion, and education of the mucosal immune system. The early gut colonization with undesirable bacteria may dysregulate the host immunological and physiological mechanisms to restore homeostasis later in life. In contrast, early life exposure to probiotics may lead to beneficial intestinal colonization of the avian microbiome favoring the fitting development of immune functions. Therefore, early microbial communities' modulation toward beneficial bacterial colonization holds a great promise for improving health and productivity in poultry. This review illustrates the symbiotic relationship between the intestinal pioneer microbiota and the immune system in poultry. It has focused on describing how early exposure to commensal microorganisms can shape the gut microbiome potentially impacting the lifelong immune functions. Lastly, it has highlighted how prenatal probiotic applications can translate effective interventions to modulate immune competence and influence health in broiler chickens.

Keywords

Dysbiosis · Immune system · Poultry · Prenatal · Probiotics

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11.1 Introduction

In recent years, the attention of researchers has been focused on understanding the linkage between microbiota and immune functions. Such an intimate relationship creates mechanisms for mutual benefits to both microbes and host (Chow et al. 2010). At homeostasis, this mutualistic partnership enables the maintenance of microbial tolerance in the intestinal ecosystem; in turn, the establishment of commensal communities contributes to the development, maturation, and function of immune system (Bar-Shira et al. 2003; Kelly et al. 2007; Brisbin et al. 2008; Chung et al. 2012). Nevertheless, the cooperative arrangements between microbiota and host mucosal immunity are constantly threatened during the bird's early life. Environmental insults like improper pioneer colonization in the intestinal tract can disturb the equilibrium of the neonatal microbiome, favor pathogen overgrowth, and lead to infection-induced dysbiosis and high mortality in newly hatched chicks (Matulova et al. 2013; Juricova et al. 2013; Kogut 2019; Rodrigues et al. 2020a). Considering the limited exposure of chicks to maternal origin bacteria, an immediate strategy to promote a beneficial postnatal microbial settlement in the intestinal tract is, therefore, more meaningful in avian species than other farm animals (Kabir 2009).

In this context, accumulating evidence has indicated that prenatal life corresponds to a preferred phase for applying nutritional-based interventions to modulate the metabolic and immunologic profiles (Gensollen et al. 2016; Rubio 2019; Kogut 2019; Rodrigues et al. 2020b). Several studies discussed in this review demonstrate that the early exposure of probiotics to chicks alters the intestinal microbiota with positive effects on the immune fitness. The interest in early life programming comes at a time when poor feed conversion and low resistance to pathogens continue to challenge the poultry industry.

This review illustrates the symbiotic relationship between the intestinal pioneer microbiota and the avian immune system. It has focused on describing how early exposure to beneficial microorganisms can shape the neonatal microbiome and influence the development and function of the immune system. Lastly, it has highlighted how prenatal probiotic applications can translate effective interventions to modulate immune competence in broiler chickens, with the purpose of motivating further research to identify novel approaches to increase poultry productivity and health.

11.2 The Microbiota and the Developing Immune System

11.2.1 The Early Life Microbial Colonization

Colonization and establishment of newborn mammalian microbiota likely begin in utero and expand rapidly after birth (Gensollen et al. 2016). Opposing mammals, the assembly of the poultry microbiota starts at hatch, and factors mainly related to the environment have been shown to influence the initial colonization of the neonatal microbiome. In nature, newly hatched chicks are exposed to maternal microbiota on

the egg's surface and in the immediate nest environment (Stanley et al. 2013). However, in a commercial setting, eggs are usually washed and fumigated to remove bacterial contamination; therefore, a maternal core microbiota might not be completely transferred to the hatching chicks.

Such a circumstance, the hatchery microbiota represents a critical source of microorganisms for the neonatal chicks and serves as the basis from which the intestinal microbial communities will settle at a later age (Stanley et al. 2013; Ballou et al. 2016; Kubasova et al. 2019). Some reports have shown that the neonatal chick's microbiota composition is primarily Proteobacteria, derived from opportunistic environmental communities (Ballou et al. 2016; Donaldson et al. 2017; Rodrigues et al. 2020d; Wilson et al. 2020). In the event of pathogenic contamination in the hatcher, the sterile chick's gastrointestinal tract (GIT) represents an empty ecological niche for multiplication followed by prolonged colonization of pathogens (Crhanova et al. 2011). Thus, the microbiota abundance and structure in an adult may be, in part, a reflection of exposure history to microorganisms and environmental modulators through early life (Gensollen et al. 2016). Given the importance of host–microbiome mutualistic symbiosis to the chick's initial development, as well as implications for long-term outcomes, mechanisms to promote a reliable horizontal transmission of microbial symbionts have been increasingly investigated.

11.2.2 The Developing Immune System

The successional changes in the gut microbiome occur concomitantly with the development, expansion, and education of the mucosal immune system (Chow et al. 2010; Gensollen et al. 2016; Zheng et al. 2020). The success of this relationship is clearly demonstrated by the capacity of the mucosal immune system to maintain tolerance to innocuous stimuli (Zheng et al. 2020). Germ-free mouse models have been essential to reveal the strong influence of intestinal microbial communities on the development of proper immune functions. Comparative studies of germ-free and colonized mice have indicated that the lack of intestinal microbiota has caused extensive deficits in the gut-associated lymphoid tissues (GALT) with smaller Peyer's patches, fewer intraepithelial lymphocytes, impaired secretion of antimicrobial peptides and IgA, along with other immunological deficiencies (Round and Mazmanian 2009; Chung et al. 2012). Another important host–microbe mutualism finding discovered from these studies was that host immune maturation may be dependent on specific commensal microbes (Chung et al. 2012; Hedblom et al. 2018).

From that perspective, recent studies have shown that experimentally induced changes in the pioneer microbiota of monogastric livestock species can affect the immune programming and lead to differences in the maintenance of immunologic and microbial homeostasis upon exposure to external factors (Schokker et al. 2015b, 2017). For chickens, the early life intervention with an antibiotic may perturb the initial microbial colonization and negatively affect the expression of genes involved

in immune processes, including antigen processing and presentation, natural killer cell-mediated cytotoxicity, and hematopoietic cell lineage (Schokker et al. 2017).

The mucosa-associated lymphoid tissue (MALT) of birds is based on lymphoid cells distributed in the lamina propria mucosae and submucosa of intestinal and respiratory tracts. As most poultry commercial species do not have other peripheral encapsulated lymph nodes, the GALT serves as the major secondary lymphoid organ (Bar-Shira et al. 2003). The avian GALT is composed by diffuse lymphoid tissue in the esophagus, rectum, proventriculus, and the wall of the proctodeum, along with the pharyngeal tonsil, oesophageal tonsil, pyloric tonsil, Peyer's patches, Meckel's diverticulum, two cecal tonsils, and the bursa of Fabricius (Casteleyn et al. 2010).

Widely denoted, the innate immune system is the first line of defense against antigens and includes all aspects of immune defense mechanisms encoded in the mature functional forms by the host's germ-line genes (Akira et al. 2006; Chaplin 2010). The innate immune system recognizes microorganisms via a limited number of germline-encoded pattern-recognition receptors (PRRs), which are expressed broadly on many cells (Akira et al. 2006; Chaplin 2010).

Unlike the innate immune response, the acquired immune system is composed of lymphoid cells (B and T cells) and is characterized by specificity generated by a mechanism known as gene rearrangement (Akira et al. 2006; Kelly et al. 2007). The adaptive response expresses itself temporally after the innate response in the host defense (Chaplin 2010).

The adaptive immune functions of newly hatched chicks develop only toward the end of the first-week post-hatch (Barshira and Friedman 2006). Therefore, the maternal antibodies and innate immune system are the main apparatus for dealing with any early pathogenic assault. As part of innate immune mechanism, the mucus covers the GIT mucosal surface and limits exposure of epithelial cells to the microbiome. Additionally, mucus facilitates the formation of sIgA-mediated immune defense. A significant component of intestinal mucus is secretory mucin 2 (MUC2), whose gene expression is dramatically increased on hatching day in chickens and ducks, followed by steady expression levels. The MUC2 expression pattern is consistent with the reported kinetics of bacterial colonization in the gut (Zhang et al. 2015).

Maternal milk is an important primary source of IgA (and IgM) in mammals, which transfers the mother's ability to exclude non-pathogenic luminal bacteria to her offspring (Kelly et al. 2007). In chickens, although no maternal antibodies are obtained from milk as in mammals, it is reported that certain amounts of IgA (approximately 0.3 mg) are transported from the egg albumen to the chick's intestine before hatch (Bar-Shira et al. 2003; Barshira and Friedman 2006). In fact, Zhang et al. (2015) have demonstrated that GIT IgA was lowly expressed during the first-week post-hatch. The highest IgA cecal expression was earlier than in the ileum, partly due to the faster and more abundant bacterial colonization in the hindgut.

Furthermore, the innate regulatory mechanisms seem to be critical for microbial-immune surveillance. Many innate immune mechanisms accountable for providing differential responsiveness to commensal and pathogenic bacteria are based on their ability to recognize the microbial-associated molecular patterns (MAMPs) found

ubiquitously on both pathogenic and symbiotic bacteria (Kelly et al. 2007; Chow et al. 2010). Functional expression of antimicrobial peptides (AMPs) and Toll-like receptors (TLRs) such as TLR2, TLR15, and TLR21 during the chick embryonic development indicated that innate mechanisms can be expanded at this stage (Barshira and Friedman 2006; Meade et al. 2009). Further, the expression of beta-defensins (AvBD2, AvBD4, AvBD6, and AvBD7) and cathelicidin genes has been shown to be evident on day nine of embryonic development, suggesting that the induction of AMPs is more likely to represent a preparatory mechanism to protect the newly hatched chick, rather than a response to *in ovo* infection (Meade et al. 2009).

Although significant innate functions are developed at hatch, some components of innate system are expanded as a result of microbial exposure and sampling (Barshira and Friedman 2006; Kelly et al. 2007; Brisbin et al. 2008). Both aspects associated with innate preparedness in the developing chick were thoroughly studied by Barshira and Friedman (2006). Barshira and Friedman reported an increased transcription of beta-defensin gene in the intestine of chicks at hatch, suggesting that those proteins maturation is independent of bacterial colonization. On the other hand, innate pro-inflammatory cytokine and chemokine gene expression was established in the chick's intestine only after hatch. Noteworthy, the expression of pro-inflammatory cytokine was enhanced in the large gut, which is the leading bacterial colonization site in poultry. It, therefore, follows that the cross-talk between the early life microbiota and immune cells plays a pivotal role in shaping the maturation and function of the immune system (Chow et al. 2010; Crhanova et al. 2011; Schokker et al. 2015a; Rodrigues et al. 2020b). However, when the equilibrium between the host immune system and microbiota is disrupted, inflammation and disease can be triggered.

11.2.3 Immune Plasticity in Response to the Microbiota

A proper immune response has the purpose of protecting the host from pathogenic insults and other environmental challenges without harming self-tissues. The immune system's ability to prevent damage to its tissues is characterized by self-tolerance (Wells 2011). The immune system has evolved to induce immunological tolerance to commensal microbiota while resisting pathogens by activating strong pro-inflammatory reactions (Kelly et al. 2007). This back and forth between tolerance and immune activation denotes plasticity mechanisms within the host and microbial dynamics, which are necessary for avoiding a dysbiosis condition (Chow et al. 2010).

Targeted research on the interaction between microbiota and host immune system has found pieces of evidence of specific microbes regulating anti-inflammatory regulatory T cells (T_{reg}) or pro-inflammatory T helper 17 cells (T_H17) in mice (Mazmanian et al. 2005; Arpaia et al. 2013; Furusawa et al. 2013). For instance, Atarashi et al. (2011) demonstrated that the induction of colonic T_{reg} was dependent on commensal microorganisms' colonization. Establishing a defined mix of

Clostridium strains in the mouse gut affected numbers of Foxp3+ T_{reg} cells and their function. Furthermore, *Clostridium*'s oral inoculation during early life resulted in resistance to colitis and systemic immunoglobulin E responses in adult mice (Atarashi et al. 2011).

Based on the concept of mutualism, Mazmanian et al. (2005) proposed a novel class of molecules referred to as "symbiosis factors," which are present on symbiotic bacteria and have been proved to mediate host immune-system response through specific cellular and molecular interactions. In particular, this research revealed that *Bacillus fragilis*, a ubiquitous constituent of the mammalian lower GIT microbiota, acquires a bacterial polysaccharide that controls the development of CD4 T cells in mice.

Additionally, it has been postulated that the bacterial metabolites can also mediate the communication between the commensal microbes and immune cells. In support of this idea, Furusawa et al. (2013) showed that luminal concentrations of short-chain fatty acids (SCFAs) positively correlate to the number of T_{reg} cells in the colon of mice. The authors suggested that among the SCFAs, butyrate produced by butyrate-producing microbes induced functional colonic T_{reg} cells, specifically among CD4 T cell subsets. Also, a report by Arpaia et al. (2013) has demonstrated that butyrate, produced by commensal microorganisms during starch fermentation, facilitated the extrathymic generation of T_{reg} cells, directly affecting the balance between pro- and anti-inflammatory mechanisms.

Similarly, work by Ivanov et al. (2009b) demonstrated that T_H17 cells were induced in the small intestinal lamina propria of mice in response to specific components of the commensal microbiota. The emergence of intestinal T_H17 cells occurred only after colonization with specific pathogen-free microbiota, and their differentiation was inhibited by treating mice with selective antibiotics. The T_H17 subset represents an arm of the adaptive immune system that works to clear specific types of pathogens that were not effectively neutralized by T_H1 or T_H2 immune responses (Korn et al. 2009). A hallmark of this T_H cell subset was the production of IL-17A, a pro-inflammatory cytokine that plays an important role in several diseases (Zhao et al. 2014). Research in chicken models has reported the role of IL-17 in mediating host defense against Marek's disease, *Cryptosporidium baileyi,* and *Eimeria* species (Zhang et al. 2013; Zhao et al. 2014; Welch 2015).

Subsequent investigation has found that colonization of the small intestine of mice with a single commensal microbe, a segmented filamentous bacterium (SFB), was sufficient to induce growth of CD4+ T_H cells in the lamina propria (Ivanov et al. 2009a). The T_H17 cells modification following intestinal colonization with SFB, or reduction of microbial communities mediated by antibiotics, indicates plasticity in T cell responses to microbiota changes (Chow et al. 2010). Segmented filamentous bacterium, a Gram-positive spore-forming commensal bacterium, are a microorganism that has been recently subjected to intense research as an intestinal colonizer related to enhanced mucosal immunity in humans and livestock. The primary site for SFB in chickens, designated recently as *Candidatus Savagella,* is the mucosa of lower ileum (Liao et al. 2012; Rodrigues et al. 2020d). This bacterium has been allied with inducing maturation of all immune system components. On one side,

SFB may mediate stimulation of T_H17 cell responses, boost pro-inflammatory cytokine and IgA production. On the other side, the immune-enhancing SFB may trigger inflammation-like responses (Ivanov et al. 2009a; Chung et al. 2012; Rodrigues et al. 2020c). Additionally, SFB have been positively related to body weight gain in commercial turkeys (Danzeisen et al. 2013) and chickens (Johnson et al. 2018).

Colonization by SFB is considered to be time-dependent in broilers, presumably because they are related to immune system maturation, which occurs with the aging host (Liao et al. 2012). Our recent research has shown that the highest population of SFB in the ileum occurred in 10 days-old broilers (Rodrigues et al. 2020a, b). At the same age, the mass spectrometry-based ileal proteome and Spearman's coefficient analysis revealed a strong positive correlation between the SFB population and several inflammation-related proteins; Integrin subunit alpha 1(ITGA-1) and Myosin light chain 9 (MYL9). Notably, it has been speculated that the high SFB population may have driven a predicted ileal pro-inflammatory status without tissue damage. It is worth mentioning that a transient physiological inflammatory response in the gut may be associated with the development and maturation of the mucosal-associated lymphatic tissue (Chow et al. 2010; Crhanova et al. 2011; Kogut et al. 2018). At present, it is unclear what distinguishes an inflammatory transcriptional profile elicited by pathogens or commensals as SFB.

Likewise, it is becoming accepted that Lactobacillales can interact with immune cells or epithelial cells lining the mucosa to modulate specific immune functions system. The genus *Lactobacillus* is an autochthonous resident in chickens. These Gram-positive, non-sporulating, and facultative anaerobe species are found in low numbers within the distal intestine and are predominant in the proximal GIT of poultry. Lactobacilli have been shown to elicit innate and adaptive immune responses in the host by binding to PRR expressed on immune cells and many other tissues, including the intestinal epithelium (Wells 2011). However, the capacity of different Lactobacilli species to stimulate the PRR signaling varies considerably. Moreover, there is encouraging evidence based on in vivo studies that specific Lactobacilli-based probiotics can modulate host barrier functions, defensin production, and inflammatory pathways (Wells 2011).

11.3 Implications of Improper Microbial Colonization

The cross-talk between microbial communities and immune cells has been positively associated with establishing immune competence (Crhanova et al. 2011; Schokker et al. 2017; Rodrigues et al. 2020c). On account of this fact, intestinal dysbiosis during the neonatal chick phase may have short and long-term consequences on immune responses (Simon et al. 2016; Schokker et al. 2017). Work conducted by Schokker and co-authors (2017) reported that 24 h of antibiotic therapy on newly hatched chicks displayed a notable increase in jejunal microbial diversity and altered the microbial composition by decreasing the relative abundance of the *Lactobacillaceae* population at 14 days post-antibiotic treatment. These temporal

changes in the early microbiota caused by antibiotic usage significantly impacted the cell-mediated immune development in the chicks.

Current evidence has been proposed that exposure to a large variety of environmental microbial communities and beneficial bacterial colonization during the neonatal period may lead to a less diverse microbiome, composed of microorganisms with proven health-promoting properties (Mulder et al. 2009; Schokker et al. 2017; Rodrigues et al. 2020a). Although lower diversity is widely associated with microbiota disturbance, it has become apparent that not all drops in microbial diversity features are responsive to pathogen colonization (Reese and Dunn 2018). In fact, a rise in diversity through the early life colonizing gut system can generate more chaos and is assumed to be detrimental for immune development (Schokker et al. 2017).

Perturbation of the pioneer microbial colonization with antibiotic exposure affects the early immune programming and has been shown to boost negative antibody response in hens after antibiotic treatment cessation (Simon et al. 2016). Antibiotic therapy can be used as a model for inducing dysbiosis of intestinal microbial communities in different livestock species (Schokker et al. 2015b, 2017). In a commercial setting, early application of antibiotics can be utilized followed by delayed feeding procedures in hatcheries or acquired infections (Simon et al. 2016). Furthermore, colonization by pathogenic bacteria may actively employ mechanisms that disrupt the functional, morphological, and immunological processes in the chicken gut. During the hatch and post-hatching period, newly hatched chicks are exposed to stressors, derived from practices used in modern broiler production, such as transportation and processing at hatcheries, which may threaten the developing immune system and thus predispose chicks to intestinal colonization by pathogens (Rubio 2019).

Early intestinal colonization by *Salmonella enterica* serotype Enteritidis in chicks has been demonstrated to delay the jejunum's morphological processes, thereby interrupting the spatial-temporal development of the immune system. While genes involved in the natural killer cell-mediated cytotoxicity pathways were overexpressed earlier in infected birds, the number for CD8+ cells, TCRαβ, and TCRγδ cells were lower at later ages, suggesting that the early dysbiosis onset by *Salmonella* colonization may influence the immune maturation and competence in broiler chickens (Schokker et al. 2010).

Another example of early disturbance by enteric microbiota that triggered a dysregulated immune response in chicks was shown in our recent study (Wilson et al. 2020). It was revealed that the neonatal non-pathogenic *Enterobacteriaceae* colonization promoted intestinal proteomic changes accompanied by inflammation in chicks (Wilson et al. 2020). The complexity of intestinal microbiota caused by early colonization of *Enterobacteriaceae* strains may have dysregulated the host immunological and physiological mechanisms to restore homeostasis later in life (Rodrigues et al. 2020b). Further evidence provided by the microbiome and mass spectrometry-based proteome analysis demonstrated that although the *Enterobacteriaceae* population had declined over 3 days of age, its early colonization led to a later inhibition of functions linked to immune cell migration. This

inhibition seemed to promote immunosuppression and induced long-term inflammation in the intestine of 10-day-old broilers.

It is crucial to bear in mind that chickens can respond to natural cecal colonization by an increased expression of IL-8 and IL-17 in the first week of life, indicating physiological inflammation and maturation of the gut immune system (Crhanova et al. 2011). Physiological inflammation can be characterized by a controlled inflammatory response and is dependent on the balance of the innate immune response which mediates host defense and tolerance in the gut (Kogut et al. 2018). In general, the inflammatory response may be beneficial for the host as an acute and transient mechanism to mediate inciting agents' clearance in the GIT. Alternatively, failure to control inflammation could inflict chronic and severe tissue damage (Xiao 2017). Considering that a pathogenic exposure is likely to occur prior or during the chick's placement on the farms, the early stimulation of beneficial intestinal microbiota is warranted, as there may be critical effects in modulating health in broilers.

11.4 Role of Probiotics in the Early Life Programming

The chicken GIT is arranged by a miscellaneous bacterial community in which each bacterium is adapted to a unique ecological niche (Shang et al. 2018). In the first weeks of age, the luminal and mucosa-associated microbial colonization is characterized by robust shifts in composition, and a progressive stabilization to an adult-like community structure may happen after 10–14 days of age in broilers (Awad et al. 2016; Rodrigues et al. 2020a, c). The most diverse GIT section in chickens is the ceca, which harbors up to 10^{10} microbes, predominantly composed by the Proteobacteria, Bacteroides, and Firmicutes (Stanley et al. 2013; Oakley and Kogut 2016; Rodrigues et al. 2020a). The poultry GIT microbiota undergoes a period of heavy changes during the first days of life. Even though the pioneer intestinal bacterial settlers are generally transients, they may drive the course of microbial community composition and diversity over time.

Concomitant with this process, the immune system's maturation starts during first week of life in broilers (Crhanova et al. 2011). Although epigenetic mechanisms can significantly determine the immune functionality, the initial microbial exposure has been identified to carry out a significant role in promoting the development of the immune response (Crhanova et al. 2011; Chung et al. 2012). Therefore, early microbiota modulation toward beneficial bacterial colonization holds a great promise for inducing health and better productivity in broilers.

It is increasingly evident that early manipulation of microbiota by probiotics provides a valuable tool to favor beneficial intestinal colonization and influence systemic and gut-associated immune responses. Probiotics were defined by the World Health Organization (WHO) as live microorganisms, which, when administered in adequate amounts, confer health benefits on the host. Synbiotic, which contains both probiotic and prebiotic components, has also drawn recent attention due to its potential for modifying the gut microbiota and its metabolites

(Markowiak 2017; Rodrigues et al. 2020c, d). Prebiotics are food components, usual carbohydrates of various molecular structures that are not digestible by the host, that can be selectively fermented by potentially beneficial bacteria (Markowiak 2017). Since prebiotics are used mostly as a selective medium for probiotic growth, alterations in the intestinal microbial community may occur at the level of individual strains and species (Markowiak 2017). Regardless of the fact that several bacterial species and yeasts from *Bacillus, Lactobacillus, Enterococcus, Bifidobacterium, Pediococcus*, and *Saccharomyces* genera have been described as probiotic for broiler chickens, the probiotic features are more specific to the selected strain rather than the genus of origin.

In addition to the effects on microbial dynamics toward beneficial bacterial growth, early exposure to microbial preparations has been acknowledged as an approach to reduce pathogen colonization and induce GIT development by stimulating growth of the villus surface area. Other probiotic action mechanisms include maturation of the immune system, improvement of gut barrier function, and the presence of highly competitive microbial communities (Ballou et al. 2016; Pedroso et al. 2016; Varmuzova et al. 2016; Kogut 2019). While not all probiotic mechanisms of action have been totally elucidated, some of the factors claimed to be responsible for probiotic's efficiency include the GIT microbial viability, capability to reproduce itself in the host, production of essential metabolites, ability to adhere and colonize the epithelial cells. Interestingly, the positive outcomes from probiotics are not dependent on their GIT colonization. Lactobacilli or Clostridiales may affect the development of the intestinal tract in chicks by merely passing through it (Kubasova et al. 2019).

Beyond probiotics, active plant derivatives, and other feed additives have been used as a strategy to enable a favorable intestinal colonization in chickens as a means of improving health status (Markowiak 2017). However, rather than discuss all nutritional interventions described in the literature with effects on modulating the neonatal GIT microbial communities, this review provides a comprehensive account of probiotic approaches exploited to manipulate the early intestinal microbiota and the immune programming in broiler chickens.

11.4.1 Probiotic Modulation of Early Life Microbiota

The emergence of 16S rRNA sequencing and metagenomics pipelines has promoted deep insights into the intestinal microbiota taxonomic profile in developing chicks. Indeed, research from many laboratories has allowed a better characterization of the microbial communities' temporal succession throughout the different growth phases of broiler chickens. Although pioneer intestinal colonization has a significant influence in shaping the postnatal microbial acquisition and maturation, researchers agreed unanimously that age of the bird is a significant driver of microbiome composition and functionality in commercial broilers (Ballou et al. 2016; Oakley and Kogut 2016; Awad et al. 2016; Rodrigues et al. 2020d). Thereby, as has been proposed by Kogut (2019), there are some windows of opportunity during the broiler

life-span, including the neonatal phase, which can successfully manipulate the microbiome toward beneficial bacterial growth. Since the maternal microbiota is not an influential provider of bacterial species for commercial chicks, early life probiotic usage is recognized as a strategic tool to support a rapid and initial GIT colonization with known beneficial bacteria (Donaldson et al. 2017; Ding et al. 2017; Kogut 2019; Lee et al. 2019).

Probiotics may exert the greatest impact in poultry when applied in the perinatal period (the last few days before hatch and the first few days after hatch). Supporting this claim, some reports have shown that probiotic application by *in ovo* technique, spraying hatching egg, neonatal oral administration, and feed supplementation throughout the first weeks of age can be very effective for modulating the microbiota in chickens (Pedroso et al. 2016; Graham et al. 2018; Wilson et al. 2020).

Recently, the emergence of *in ovo* techniques made it possible to manipulate the intestinal bacteria colonization before chicks have even been hatched or exposed to farm environments (Pedroso et al. 2016; Rubio 2019; Rodrigues et al. 2020d; Wilson et al. 2020). Our previous work addressed *in ovo* technique as an experimental model to study how early intestinal colonization can shape the development and persistence of microbiome in chicks (Rodrigues et al. 2020a; Wilson et al. 2020). In those studies, it was shown that different bacterial isolates provided *in ovo,* resulted in distinct microbiome profiles on the day of the hatch (DOH) and by 10 days of age. Notably, inoculation of a lactic acid bacteria (LAB) based probiotic resulted in increased Lactobacilli populations at DOH, which may have influenced the establishment of butyrate-producing bacteria and SFB in young broilers.

The *in ovo* administration of probiotics has also been associated with reducing intestinal pathogen adhesion in broilers. Previous work has demonstrated that chicks from eggs inoculated *in ovo* with a competitive exclusion probiotic product reduced the GIT abundance of *Enterobacteriaceae* (Pedroso et al. 2016). Early acquisition of unfavorable microorganisms is alarming as it may occupy crucial ecological niches and resist colonization by beneficial microbes (Pedroso et al. 2016; Rodrigues et al. 2020a).

Other hatchery options to deliver probiotics for neonatal chicks are via coarse spray and oral gavage (including droplet, gel, and inoculations). Both methods have shown positive outcomes in programming the early GIT microbiota composition to favor beneficial consortia in chicks. Work conducted by Graham et al. (2018) reported multiple spray applications in hatching cabinets containing LAB yielded lower Gram-negative bacterial counts in the intestinal tracts of probiotic-treated chicks on DOH, and that this significant reduction persisted for 24 h post-hatch. In agreement, Richards-Rios et al. (2019) found that eggs sprayed with dilute adult cecal content during incubation provide a mechanism to transfer desirable intestinal microbes to chicks with earlier colonization by *Ruminococcaceae* and SFB. Similarly, applying a coarse spray containing probiotics to the newly hatched chicks seemed to reduce *Salmonella* GIT colonization 5 days post-challenge (Wolfenden et al. 2007). To reach this technique's efficacy, the aforementioned authors highlighted the importance of maximizing the chicks' s preening activity by adding

a green food coloring to the probiotic solution and increasing the photo intensity post spraying.

Research on turkeys has disclosed that tailored probiotics administered continually through a gel carrier to the poults may induce positive effects on the gut fungal community and commensal bacterial microbiota with an enhanced population of SFB in the ileum (Ward et al. 2019). While inoculating one-day-old chicks with a single probiotic dose may also offer a great opportunity to influence the microbial structure and individual taxa as reducing *Shigella/Escherichia* populations in the chicken's GIT (Baldwin et al. 2018).

Although there are some innovative commercial products in the market designed to introduce probiotic solutions for neonatal chicks, few studies are approaching the new delivery routes for probiotics during early life in chickens. Moreover, in-feed is still the most common method for delivering probiotics in poultry production (Olnood et al. 2015). Taking into account the fact that the timing of probiotic application may influence the beneficial outcomes, research by Nakphaichit et al. (2011) investigated probiotic feed supplementation during the first-week post-hatch and its impact on gut microbial composition along with bacterial diversity in broilers throughout 6 weeks of age. The authors found that early probiotic administration did not alter the microbiota richness at 21 days but may have influenced the higher microbial diversity by 42 days. At 42 days, there was an enrichment of Lactobacilli and Actinobacteria, while colonization of Proteobacteria was suppressed in the gut. Together, these studies demonstrate that early probiotic application may shape the intestinal pioneer colonization, promoting settlement of beneficial microbial communities in poultry.

11.4.2 Link Between Prenatal Probiotics and Immune Responses

Early GIT colonization by commensals can positively influence the immune system development, contributing to the host survival and fitness (Bar-Shira et al. 2003; Madej and Bednarczyk 2016). As microbiome research has spread in poultry production, there was an opportunity to learn more about how nutritional interventions could shift the microbiota toward beneficial colonization in the GIT. Although the relationship between microbiota and the immune system is not yet fully elucidated, a substantial body of evidence has claimed that probiotics are a promising nutritional tool to manipulate the avian microbiome, potentially impacting the lifelong immune functions.

The *in ovo* experimental model has provided a complementary approach for characterizing how the early exposure to microbes affects the chick's neonatal microbiota and immune function development. Our recent investigation compared the *in ovo* injection of a LAB probiotic or *Enterobacteriaceae* cultures to evaluate the mucosal innate immune response in 10-days-old chicks by analyzing differentially expressed proteins in the GIT. Based on immune-related proteins' expression, the predicted biological functions of probiotic-treated chicks were associated with activation and movement of immune cells. At the same time, the *Enterobacteriaceae*

exposed birds presented a downregulation of processes related to immune development. Those findings highlighted that proper immune function was dependent on specific GIT microbiota profiles, in which early life exposure to probiotics has led to beneficial colonization of the neonatal microbiome favoring the fitting development of immune functions. In contrast, neonatal colonization of *Enterobacteriaceae* strains may have led to an imbalance of microbial communities dysregulating the local immune features (Rodrigues et al. 2020c).

Another study consolidated evidence demonstrating that prebiotics, particularly synbiotics, delivered *in ovo* may assist GALT expansion by influencing the composition and number of adaptive immune cells in growing chicks (Madej and Bednarczyk 2016). In similar work, prebiotics and live microbials administrated *in ovo* during early development of chicks stimulated the formation of germinal centers in the spleens of 21- and 35-day-old chickens, indicating intensified B-cell proliferation in secondary lymphatic organs (Madej et al. 2015).

A further meaningful innate immunostimulatory effect of probiotics may be involved in heterophil function in poultry. Farnell et al. (2006) administered selected probiotic bacteria orally to day-of-hatch chicks and isolated heterophils 24 h post-treatment to screen heterophil activity by oxidative burst and degranulation assays. Results found an enhanced heterophil function in treated chicks suggesting that early probiotic application may stimulate innate immunity. The next major probiotic effect on immune status is related to the stimulation of natural antibodies. Accordingly, a study by Haghighi and co-authors unraveled a potential role of early intestinal microbial manipulation by probiotics. Probiotic administration enhanced the serum and natural antibodies against several foreign antigens in treated chicks, which might be important for reducing intestinal pathogen occupation (Haghighi et al. 2006).

Concordantly, the early continuous inoculation of host-tailored probiotic treatment has been associated with immunostimulation mechanisms at the gut level involving T_H17 and IL-17 in turkeys (Ward et al. 2019). This may be due, in part, as explained by the authors, to the high population of SFB found in the ileum, which can induce T_H17 cells accumulation and enhance mucosal barrier protection. The importance of early beneficial microbial colonization to host immunity has led Redweik et al. (2020) to investigate the impact of day-old chicks inoculated with microbial spores on the immunometabolic processes in young birds. The findings included the upregulation of several immune pathways associated with innate (Toll-like receptor, JAK-STAT) and adaptive (T/B cell receptor, T_H17 differentiation) responses in treated birds. Additionally, SFB were detected in ceca and ileum, indicating that SFB-based treatment can stimulate innate and adaptive immune responses and potentially protect chickens from enteric pathogens.

11.5 Future Perspectives

Manipulation of the neonatal microbiome by probiotics is an emerging interest area due to its evidenced influence in modulating a favorable microbiota establishment. Although the culture-independent genomic techniques and bioinformatic approaches

have widened the view on the association between early exposure of probiotics and commensal GIT microbiota colonization, there are many other factors such as genetics, diet, age, and environment, all of which can play a role in microbial settlement. Regardless of the administration method, early exposure of probiotics has repeatedly shown positive responses in programming the immune functions in poultry. Still, much remains to be elucidated beyond the present correlative connections between microbiota-mediated probiotic supplementation and long-standing health consequences to broiler chickens. A better understanding of how the early life microbiota integrates with the innate and adaptive immune system, along with advanced research focused on identifying probiotic immunologic activities, will provide meaningful tools to enhance tolerance to early environmental exposures and prevent the expansion of dysbiosis-mediated diseases in poultry.

References

Akira S, Uematsu S, Takeuchi O (2006) Pathogen recognition and innate immunity. Cell 124(4):783–801. https://doi.org/10.1016/j.cell.2006.02.015

Arpaia N, Campbell C, Fan X, Dikiy S, van der Veeken J, deRoos P, Liu H, Cross JR, Pfeffer K, Coffer PJ, Rudensky AY (2013) Metabolites produced by commensal bacteria promote peripheral regulatory T-cell generation. Nature 504(7480):451–455. https://doi.org/10.1038/nature12726

Atarashi K, Tanoue T, Shima T, Imaoka A, Kuwahara T, Momose Y, Cheng G, Yamasaki S, Saito T, Ohba Y, Taniguchi T, Takeda K, Hori S, Ivanov II, Umesaki Y, Itoh K, Honda K (2011) Induction of colonic regulatory T cells by indigenous clostridium species. Science 331(6015):337–341. https://doi.org/10.1126/science.1198469

Awad WA, Mann E, Dzieciol M, Hess C, Schmitz-Esser S, Wagner M, Hess M (2016) Age-related differences in the luminal and mucosa-associated gut microbiome of broiler chickens and shifts associated with Campylobacter jejuni infection. Front Cell Infect Microbiol 6:154. https://doi.org/10.3389/fcimb.2016.00154

Baldwin S, Hughes RJ, Hao Van TT, Moore RJ, Stanley D. (2018) At-hatch administration of probiotic to chickens can introduce beneficial changes in gut microbiota. PLoS One 13(3): e0194825. https://doi.org/10.1371/journal.pone.0194825

Ballou AL, Ali RA, Mendoza MA, Ellis JC, Hassan HM, Croom WJ, Koci MD (2016) Development of the chick microbiome: how early exposure influences future microbial diversity. Front Vet Sci. 3:2. https://doi.org/10.3389/fvets.2016.00002

Barshira E, Friedman A (2006) Development and adaptations of innate immunity in the gastrointestinal tract of the newly hatched chick. Dev Comp Immunol 30(10):930–941. https://doi.org/10.1016/j.dci.2005.12.002

Bar-Shira E, Sklan D, Friedman A (2003) Establishment of immune competence in the avian GALT during the immediate post-hatch period. Dev Comp Immunol 27(2):147–157. https://doi.org/10.1016/S0145-305X(02)00076-9

Brisbin JT, Gong J, Sharif S (2008) Interactions between commensal bacteria and the gut-associated immune system of the chicken. Anim Health Res Rev 9(1):101–110. https://doi.org/10.1017/S146625230800145X

Casteleyn C, Doom M, Lambrechts E, Van den Broeck W, Simoens P, Cornillie P (2010) Locations of gut-associated lymphoid tissue in the 3-month-old chicken: a review. Avian Pathol 39(3):143–150. https://doi.org/10.1080/03079451003786105

Chaplin DD (2010) Overview of the immune response. J Allergy Clin Immunol 125(2):S3–S23. https://doi.org/10.1016/j.jaci.2009.12.980

Chow J, Lee SM, Shen Y, Khosravi A, Mazmanian SK (2010) Host–bacterial symbiosis in health and disease. In: Advances in immunology. Elsevier, New York, pp 243–274

Chung H, Pamp SJ, Hill JA, Surana NK, Edelman SM, Troy EB, Reading NC, Villablanca EJ, Wang S, Mora JR, Umesaki Y, Mathis D, Benoist C, Relman DA, Kasper DL (2012) Gut immune maturation depends on colonization with a host-specific microbiota. Cell 149(7):1578–1593. https://doi.org/10.1016/j.cell.2012.04.037

Crhanova M, Hradecka H, Faldynova M, Matulova M, Havlickova H, Sisak F, Rychlik I (2011) Immune response of chicken gut to natural colonization by gut microflora and to Salmonella enterica serovar Enteritidis infection. Infect Immun 79(7):2755–2763. https://doi.org/10.1128/IAI.01375-10

Danzeisen JL, Calvert AJ, Noll SL, McComb B, Sherwood JS, Logue CM, Johnson TJ (2013) Succession of the Turkey gastrointestinal bacterial microbiome related to weight gain. PeerJ 1: e237. https://doi.org/10.7717/peerj.237

Ding J, Dai R, Yang L, He C, Xu K, Liu S, Zhao W, Xiao L, Luo L, Zhang Y, Meng H (2017) Inheritance and establishment of gut microbiota in chickens. Front Microbiol 8:1967. https://doi.org/10.3389/fmicb.2017.01967

Donaldson EE, Stanley D, Hughes RJ, Moore RJ (2017) The time-course of broiler intestinal microbiota development after administration of cecal contents to incubating eggs. PeerJ 5: e3587. https://doi.org/10.7717/peerj.3587

Farnell MB, Donoghue AM, de los Santos FS, Blore PJ, Hargis BM, Tellez G, Donoghue DJ (2006) Upregulation of oxidative burst and degranulation in chicken heterophils stimulated with probiotic bacteria. Poult Sci 85(11):1900–1906. https://doi.org/10.1093/ps/85.11.1900

Furusawa Y, Obata Y, Fukuda S, Endo TA, Nakato G, Takahashi D, Nakanishi Y, Uetake C, Kato K, Kato T, Takahashi M, Fukuda NN, Murakami S, Miyauchi E, Hino S, Atarashi K, Onawa S, Fujimura Y, Lockett T, Clarke JM, Topping DL, Tomita M, Hori S, Ohara O, Morita T, Koseki H, Kikuchi J, Honda K, Hase K, Ohno H (2013) Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells. Nature 504(7480):446–450. https://doi.org/10.1038/nature12721

Gensollen T, Iyer SS, Kasper DL, Blumberg RS (2016) How colonization by microbiota in early life shapes the immune system. Science 352(6285):539–544. https://doi.org/10.1126/science.aad9378

Graham LE, Teague KD, Latorre JD, Yang Y, Baxter MFA, Mahaffey BD, Hernandez-Velasco X, Bielke LR, Hargis BM, Tellez G (2018) Use of probiotics as an alternative to formaldehyde fumigation in commercial broiler chicken hatch cabinets. J Appl Poult Res 27(3):371–379. https://doi.org/10.3382/japr/pfy008

Haghighi HR, Gong J, Gyles CL, Hayes MA, Zhou H, Sanei B, Chambers JR, Sharif S (2006) Probiotics stimulate production of natural antibodies in chickens. Clin Vaccine Immunol 13(9):975–980. https://doi.org/10.1128/CVI.00161-06

Hedblom GA, Reiland HA, Sylte MJ, Johnson TJ, Baumler DJ (2018) Segmented filamentous bacteria – metabolism meets immunity. Front Microbiol 9:1991. https://doi.org/10.3389/fmicb.2018.01991

Ivanov II, Atarashi K, Manel N, Brodie EL, Shima T, Karaoz U, Wei D, Goldfarb KC, Santee CA, Lynch SV, Tanoue T, Imaoka A, Itoh K, Takeda K, Umesaki Y, Honda K, Littman DR (2009a) Induction of intestinal Th17 cells by segmented filamentous bacteria. Cell 139(3):485–498. https://doi.org/10.1016/j.cell.2009.09.033

Ivanov II, de Frutos RL, Manel N, Yoshinaga K, Rifkin B, Sartor RB, Finlay BB, Littman DR (2009b) Specific microbiota direct the differentiation of Th17 cells in the mucosa of the small intestine. Cell Host Microbe 4(4):337–349

Johnson TJ, Youmans BP, Noll S, Cardona C, Evans NP, Karnezos TP, Ngunjiri JM, Abundo MC, Lee C-W (2018) A consistent and predictable commercial broiler chicken bacterial microbiota in antibiotic-free production displays strong correlations with performance. Appl Environ Microbiol 84(12):e00362–e00318. https://doi.org/10.1128/AEM.00362-18

Juricova H, Videnska P, Lukac M, Faldynova M, Babak V, Havlickova H, Sisak F, Rychlik I (2013) Influence of Salmonella enterica Serovar Enteritidis infection on the development of the cecum microbiota in newly hatched chicks. Appl Environ Microbiol 79(2):745–747. https://doi.org/10.1128/AEM.02628-12

Kabir SML (2009) The role of probiotics in the poultry industry. Int J Mol Sci 10(8):3531–3546. https://doi.org/10.3390/ijms10083531

Kelly D, King T, Aminov R (2007) Importance of microbial colonization of the gut in early life to the development of immunity. Mutat Res Mol Mech Mutagen 622(1–2):58–69. https://doi.org/10.1016/j.mrfmmm.2007.03.011

Kogut MH (2019) The effect of microbiome modulation on the intestinal health of poultry. Anim Feed Sci Technol 250:32–40. https://doi.org/10.1016/j.anifeedsci.2018.10.008

Kogut MH, Genovese KJ, Swaggerty CL, He H, Broom L (2018) Inflammatory phenotypes in the intestine of poultry: not all inflammation is created equal. Poult Sci 97(7):2339–2346. https://doi.org/10.3382/ps/pey087

Korn T, Bettelli E, Oukka M, Kuchroo VK (2009) IL-17 and Th17 cells. Annu Rev Immunol 27:485–517

Kubasova T, Kollarcikova M, Crhanova M, Karasova D, Cejkova D, Sebkova A, Matiasovicova J, Faldynova M, Pokorna A, Cizek A, Rychlik I (2019) Contact with adult hen affects development of caecal microbiota in newly hatched chicks. PLoS One 14(3):e0212446. https://doi.org/10.1371/journal.pone.0212446

Lee S, La T-M, Lee H-J, Choi I-S, Song C-S, Park S-Y, Lee J-B, Lee S-W (2019) Characterization of microbial communities in the chicken oviduct and the origin of chicken embryo gut microbiota. Sci Rep 9(1):6838. https://doi.org/10.1038/s41598-019-43280-w

Liao N, Yin Y, Sun G, Xiang C, Liu D, Yu HD, Wang X (2012) Colonization and distribution of segmented filamentous bacteria (SFB) in chicken gastrointestinal tract and their relationship with host immunity. FEMS Microbiol Ecol 81(2):395–406. https://doi.org/10.1111/j.1574-6941.2012.01362.x

Madej JP, Bednarczyk M (2016) Effect of in ovo- delivered prebiotics and synbiotics on the morphology and specific immune cell composition in the gut-associated lymphoid tissue. Poult Sci 95(1):19–29. https://doi.org/10.3382/ps/pev291

Madej JP, Stefaniak T, Bednarczyk M (2015) Effect of in ovo-delivered prebiotics and synbiotics on lymphoid-organs' morphology in chickens. Poult Sci 94(6):1209–1219. https://doi.org/10.3382/ps/pev076

Markowiak Ś (2017) Effects of probiotics, prebiotics, and synbiotics on human health. Nutrients 9(9):1021. https://doi.org/10.3390/nu9091021

Matulova M, Varmuzova K, Sisak F, Havlickova H, Babak V, Stejskal K, Zdrahal Z, Rychlik I (2013) Chicken innate immune response to oral infection with Salmonella enterica serovar Enteritidis. Vet Res 44(1):37. https://doi.org/10.1186/1297-9716-44-37

Mazmanian S, Liu C, Tzianabos A, Kasper D (2005) An immunomodulatory molecule of symbiotic bacteria directs maturation of the host immune system. Cell 122(1):107–118

Meade KG, Higgs R, Lloyd AT, Giles S, O'Farrelly C (2009) Differential antimicrobial peptide gene expression patterns during early chicken embryological development. Dev Comp Immunol 33(4):516–524. https://doi.org/10.1016/j.dci.2008.10.003

Mulder IE, Schmidt B, Stokes CR, Lewis M, Bailey M, Aminov RI, Prosser JI, Gill BP, Pluske JR, Mayer C-D, Musk CC, Kelly D (2009) Environmentally-acquired bacteria influence microbial diversity and natural innate immune responses at gut surfaces. BMC Biol 7(1):79. https://doi.org/10.1186/1741-7007-7-79

Nakphaichit M, Thanomwongwattana S, Phraephaisarn C, Sakamoto N, Keawsompong S, Nakayama J, Nitisinprasert S (2011) The effect of including Lactobacillus reuteri KUB-AC5 during post-hatch feeding on the growth and ileum microbiota of broiler chickens. Poult Sci 90(12):2753–2765. https://doi.org/10.3382/ps.2011-01637

Oakley BB, Kogut MH (2016) Spatial and temporal changes in the broiler chicken cecal and fecal microbiomes and correlations of bacterial taxa with cytokine gene expression. Front Vet Sci 3: 11. https://doi.org/10.3389/fvets.2016.00011

Olnood CG, Beski SSM, Iji PA, Choct M (2015) Delivery routes for probiotics: effects on broiler performance, intestinal morphology and gut microflora. Anim Nutr 1(3):192–202. https://doi.org/10.1016/j.aninu.2015.07.002

Pedroso AA, Batal AB, Lee MD (2016) Effect of in ovo administration of an adult-derived microbiota on establishment of the intestinal microbiome in chickens. Am J Vet Res 77(5):514–526. https://doi.org/10.2460/ajvr.77.5.514

Redweik GAJ, Kogut MH, Arsenault RJ, Mellata M (2020) Oral treatment with ileal spores triggers immunometabolic shifts in chicken gut. Front Vet Sci. 7:629. https://doi.org/10.3389/fvets.2020.00629

Reese AT, Dunn RR (2018) Drivers of microbiome biodiversity: a review of general rules, feces, and ignorance. mBio 9(4):e01294-18. https://doi.org/10.1128/mBio.01294-18

Richards-Rios P, Leeming G, Fothergill J, Bernardeau M, Wigley P (2019) Topical application of adult cecal contents to eggs transplants spore-forming microbiota but not other members of the microbiota to chicks. Appl Environ Microbiol. 86(5):e02387-19. https://doi.org/10.1128/AEM.02387-19

Rodrigues DR, Briggs W, Duff A, Chasser K, Murugesan R, Pender C, Ramirez S, Valenzuela L, Bielke L (2020a) Cecal microbiome composition and metabolic function in probiotic treated broilers. PLoS One 15(6):e0225921. https://doi.org/10.1371/journal.pone.0225921

Rodrigues DR, Briggs W, Duff A, Chasser K, Murugesan R, Pender C, Ramirez S, Valenzuela L, Bielke LR (2020b) Comparative effectiveness of probiotic-based formulations on cecal microbiota modulation in broilers. PLoS One 15(5):e0225871. https://doi.org/10.1371/journal.pone.0225871

Rodrigues DR, Wilson KM, Trombetta M, Briggs WN, Duff AF, Chasser KM, Bottje WG, Bielke L (2020c) A proteomic view of the cross-talk between early intestinal microbiota and poultry immune system. Front Physiol 11:20. https://doi.org/10.3389/fphys.2020.00020

Rodrigues DR, Winson E, Wilson KM, Briggs WN, Duff AF, Chasser KM, Bielke LR (2020d) Intestinal pioneer colonizers as drivers of ileal microbial composition and diversity of broiler chickens. Front Microbiol 10:2858. https://doi.org/10.3389/fmicb.2019.02858

Round J, Mazmanian J (2009) The gut microbiota shapes intestinal immune responses during health and disease. Nat Rev Immunol 9:313–323. https://doi.org/10.1038/nri2515

Rubio LA (2019) Possibilities of early life programming in broiler chickens via intestinal microbiota modulation. Poult Sci 98(2):695–706. https://doi.org/10.3382/ps/pey416

Schokker D, Peters T, Hoekman A, Rebel J (2010) Effects of Salmonella on spatial-temporal processes of jejunal development in chickens. Dev Comp Immunol 34(10):1090–1100

Schokker D, Veninga G, Vastenhouw SA, Bossers A, de Bree FM, Kaal-Lansbergen LMTE, Rebel JMJ, Smits MA (2015a) Early life microbial colonization of the gut and intestinal development differ between genetically divergent broiler lines. BMC Genomics 16(1):418. https://doi.org/10.1186/s12864-015-1646-6

Schokker D, Zhang J, Vastenhouw SA, Heilig HGHJ, Smidt H, Rebel JMJ, Smits MA (2015b) Long-lasting effects of early-life antibiotic treatment and routine animal handling on gut microbiota composition and immune system in pigs. PLoS One 10(2):e0116523. https://doi.org/10.1371/journal.pone.0116523

Schokker D, Jansman AJM, Veninga G, de Bruin N, Vastenhouw SA, de Bree FM, Bossers A, Rebel JMJ, Smits MA (2017) Perturbation of microbiota in one-day old broiler chickens with antibiotic for 24 hours negatively affects intestinal immune development. BMC Genomics 18(1):241. https://doi.org/10.1186/s12864-017-3625-6

Shang Y, Kumar S, Oakley B, Kim WK (2018) Chicken gut microbiota: importance and detection technology. Front Vet Sci. 5:254. https://doi.org/10.3389/fvets.2018.00254

Simon K, Verwoolde MB, Zhang J, Smidt H, de Vries RG, Kemp B, Lammers A (2016) Long-term effects of early life microbiota disturbance on adaptive immunity in laying hens. Poult Sci 95(7):1543–1554. https://doi.org/10.3382/ps/pew088

Stanley D, Geier MS, Hughes RJ, Denman SE, Moore RJ (2013) Highly variable microbiota development in the chicken gastrointestinal tract. PLoS One 8(12):e84290. https://doi.org/10.1371/journal.pone.0084290

Varmuzova K, Kubasova T, Davidova-Gerzova L, Sisak F, Havlickova H, Sebkova A, Faldynova M, Rychlik I (2016) Composition of gut microbiota influences resistance of newly hatched chickens to Salmonella Enteritidis infection. Front Microbiol 7:957. https://doi.org/10.3389/fmicb.2016.00957

Ward TL, Weber BP, Mendoza KM, Danzeisen JL, Llop K, Lang K, Clayton JB, Grace E, Brannon J, Radovic I, Beauclaire M, Heisel TJ, Knights D, Cardona C, Kogut M, Johnson C, Noll SL, Arsenault R, Reed KM, Johnson TJ (2019) Antibiotics and host-tailored probiotics similarly modulate effects on the developing avian microbiome, mycobiome, and host gene expression. mBio 10(5):e02171-19. https://doi.org/10.1128/mBio.02171-19

Welch LM (2015) Th17 immune responses in the chicken. Era. http://hdl.handle.net/1842/15909

Wells JM (2011) Immunomodulatory mechanisms of lactobacilli. Microb Cell Factories 10(Suppl 1):S17. https://doi.org/10.1186/1475-2859-10-S1-S17

Wilson KM, Rodrigues DR, Briggs WN, Duff AF, Chasser KM, Bottje WG, Bielke LR (2020) Impact of in ovo administered pioneer colonizers on intestinal proteome on day of hatch. Poult Sci 99(3):1254–1266. https://doi.org/10.1016/j.psj.2019.10.017

Wolfenden AD, Pixley CM, Higgins JP, Higgins SE, Hargis BM, Tellez G, Vicente JL, Torres-Rodriguez A (2007) Evaluation of spray application of a Lactobacillus-based probiotic on Salmonella enteritidis colonization in broiler chickens. Int J Poult Sci 6(7):493–496. https://doi.org/10.3923/ijps.2007.493.496

Xiao TS (2017) Innate immunity and inflammation. Cell Mol Immunol 14(1):1–3. https://doi.org/10.1038/cmi.2016.45

Zhang RL, Song M, Yanxin HU (2013) Eimeria tenella: interleukin 17 contributes to host immunopathology in the gut during experimental infection. Exp Parasitol 133(2):120–130

Zhang Q, Eicher SD, Applegate TJ (2015) Development of intestinal mucin 2, IgA, and polymeric Ig receptor expressions in broiler chickens and Pekin ducks. Poult Sci 94(2):172–180. https://doi.org/10.3382/ps/peu064

Zhao G-H, Cheng W-Y, Wang W, Jia Y-Q, Fang Y-Q, Du S-Z, Yu S-K (2014) The expression dynamics of IL-17 and Th17 response relative cytokines in the trachea and spleen of chickens after infection with Cryptosporidium baileyi. Parasit Vectors 7(1):212. https://doi.org/10.1186/1756-3305-7-212

Zheng D, Liwinski T, Elinav E (2020) Interaction between microbiota and immunity in health and disease. Cell Res 30(6):492–506. https://doi.org/10.1038/s41422-020-0332-7

Adaptive Poultry Gut Capacity to Resist Oxidative Stress

12

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Abstract

The effective control of oxidative stress is a prerequisite for poultry health and sustainable production. Diet supplies animals with antioxidant components such as vitamins (e.g., C and E), trace elements (e.g., Se and Cu) and phytochemicals (e.g., flavonoids and polyphenols) that directly support the oxidative defense. The gut is at the forefront line of contact with challenge stressors of dietary and environmental origin (e.g., xenobiotics, pathogens, heat). It is therefore important that stressors get effectively dealt with at the gut level. In this respect, the endogenous cellular signaling pathways related to the innate detoxifying and antioxidant defense system can be critical.

The aim of this work was to review current knowledge on signaling pathways, and the relevant gene battery components that, when activated induce a cellular cytoprotective response mediated by enzymes having detoxifying, antioxidant and anti-inflammatory functions. Subsequently, case studies assessing relevant cytoprotective responses upon physiological non-challenge and challenge experimental conditions were analyzed. Measuring the adaptive capacity for cryoprotection through the signaling pathways addressed in this work could be a valuable analytical tool in research and development protocols for bioactive compounds with gut and overall health-protective properties.

Keywords

Xenobiotics · Detoxification · Oxidative stress · AHRs · Nrf2 · Phytochemicals

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12.1 Introduction

Poultry in intensive farming operations may be exposed to an array of stressor challenges of endogenous (e.g., metabolic) and exogenous—environmental (e.g., temperature, diet and xenobiotics) origin. Whenever the cellular concentration of pro-oxidant reactive oxygen species (ROS) over exceeds the bird's antioxidant capacity, a dysregulation of the intracellular redox status could occur (Stefanson and Bakovic 2014). Such a condition, if not promptly and adequately controlled, will lead to oxidative stress. The latter will, in turn, result in cellular protein oxidation and lipid peroxidation, DNA damage and inflammation with detrimental effects for poultry performance, health and product quality (Sahin et al. 2013; Lee et al. 2017, Da Silva et al. 2018; Bortoli et al. 2018; Carvalho et al. 2019).

Poultry may resist oxidative stress via direct and indirect mechanisms. The direct mechanism involves the immediate engagement of dietary non-enzymatic antioxidant compounds such as vitamins, phytochemicals, trace minerals (e.g., copper, zinc, selenium), carotenoids and cofactors such as folic and uric acids (Allen and Tressini 2000; Lee et al. 2017). The indirect mechanism involves the inducible gene expression of cytoprotective enzymes with detoxifying, antioxidant and anti-inflammatory functions (Köhle and Bock 2006; Wullaert et al. 2011; Huang et al. 2015).

In particular, the inducible cellular cytoprotection is mediated by two signaling pathways referred to as AHR and Nrf2 from the transcription factors that activate them, namely the aryl hydrocarbon receptor (AHR) and the nuclear factor-erythroid-derived 2-like 2 (Nrf2), respectively (Köhle and Bock 2006). The AHR pathway is responsible for the detoxification of xenobiotic compounds such as dioxins, mycotoxins, phytochemicals and bacterial pathogens. Transcription factors AHRs are responsible for the regulation of target genes related to detoxification and elimination of xenobiotics (Larigot et al. 2018). They exist as a multiprotein complex in the cytoplasm and bind xenobiotic AHR ligands entering the cell and subsequently translocate to the nucleus and heterodimerize with AHR Nuclear Translocator—ARNT (Bortoli et al. 2018). Then, AHR/ARNT recognizes the xenobiotic-responsive elements (XREs) region of target genes known as Phase I xenobiotic-metabolizing enzymes (XMEs) and regulates their expression and downstream xenobiotic detoxification (Guo et al. 2020).

The Nrf2 pathway is known as the master regulator of cell defense (Stefanson and Bakovic 2014), as it is one of the most important regulators of the antioxidant response and inflammation (Vomund et al. 2017). Physiologically Nrf2 is in the cytoplasm and bound with its inhibitor Kelch-like ECH-associated protein-1 (Keap1). Upon activation from ROS and electrophilic insults, Nrf2 separates from Keap1, translocates to the nucleus, dimerizes with small musculoaponeurotic fibrosarcoma protein (sMAF), and binds at antioxidant response element (ARE) DNA regions of its target genes. This binding results in the transcription of Phase II antioxidant and cytoprotective genes (Muhammad et al. 2017).

Both signaling pathways are essential for cellular protection. In the following sections, the pathway components and their functions will be reviewed. In addition, relevant research studying how various challenge stressors and dietary components

may affect their activation, downstream events and potential synergies in the gastrointestinal tract of poultry will be assessed.

12.2 AHR Signaling Pathway and Regulation of Xenobiotic Response Element (XRE) Genes

Xenobiotics such as mycotoxins and plant natural components (phytochemicals), bacterial pathogens, and other various exogenous ligands can be processed in the cell in four different ways. They could be eliminated, retained in tissues, chemically transformed and metabolized by enzymes (Croom 2012). Generally, xenobiotics activate the aryl hydrocarbon receptor (AHR) signaling pathway. In particular, AHR is a transcription factor of the bHLH/PAS (basic helix-loop-helix/Per-Arnt-Sim) family expressed in animal tissues that promote the metabolism of xenobiotics (Köhle and Bock 2006). Two types of AHRs, AHR1 and AHR2, have been identified in avian species (Yasui et al. 2007). AHR1 is the only type of AHR gene expressed in mammals, while AHR1 and AHR2 are expressed in fish and avian species (Antos et al. 2015). Furthermore, in birds, AHR1 is expressed at higher levels than AHR2 (Yasui et al. 2007).

AHRs structure in an inactive state is a multiprotein complex consisting of the heat shock protein 90 (Hsp90), hepatitis B virus X-associated protein (XAP2) and protein p23 (Wang et al. 2020). Under the induced state, AHR ligands such as mycotoxins, phytochemicals and bacterial pathogens bind to AHRs and get transferred to the nucleus. After the binding with AHR nuclear translocator (ARNT), they formulate an active heterodimer that adjusts the expression of genes by attaching to xenobiotic-responsive elements (XRE) (Lee et al. 2018). In particular, the AHR-ARNT complex binds to XRE and regulates the expression of xenobiotic-metabolizing enzymes (XME) such as quinone oxidoreductase 1 (NQO1), glutathione transferase A2 (GSTA2), and cytochrome P450 (CYP) enzymes (CYP1A1, CYP1A2, CYP1B1). Cytochrome P450 enzymes are involved in Phase I metabolism, and their enzyme activity participates in the oxidative metabolism and elimination of many xenobiotics (Köhle and Bock 2006).

According to their functions, AHRs have been characterized as a multifunctional molecular switch that enhances xenobiotic metabolism and additionally cell proliferation and differentiation (Bock and Köhle 2006). Moreover, nuclear factor [erythroid-derived 2]-like 2 (Nrf2) is a downstream target of AHRs. In the gastrointestinal tract, it has been reported that AHR–Nrf2 interaction promotes detoxification by synergistically activating, Phase I and II xenobiotic-metabolizing enzymes (XMEs) (Köhle and Bock 2006). Furthemore, according to cited literature xenobiotic biotransformation occurs in three phases (Vrzal et al. 2004; Patel and Sen 2013). Firstly, in Phase I, the expression of CYP enzymes is induced by the entry of xenobiotics which are transcriptionally controlled by nuclear receptors such as PXR (pregnane X receptor), CAR (constitutive androstane receptor), and AHRs (Vrzal et al. 2004; Croom 2012). Secondly, in Phase II of xenobiotic metabolism, the conjugated products of Phase I are transformed by Phase II enzymes such as GSTs

and UGTs to small hydrophilic substances such as glutathione, sulfate, cysteine, or acetate, producing more hydrophilic products than those which could be easily excreted (Vrzal et al. 2004). Finally, Phase III is considered as the process of the export of xenobiotic biotransformation products from the cell. The membrane proteins and enzymes which are responsible for this transfer are called Phase III enzymes. A Phase III example is the multidrug-resistant protein (MDR1) which is a P-glycoprotein known for the regulation of the trans-epithelial leakage of xenobiotics (Ito and Alcorn 2003), and the topic Phase III enzymes will not be dealt here any further.

12.2.1 Phase I Enzymes

Phase I enzymes participate in oxidative metabolism via their synergies with Phase II enzymes (Croom 2012). One of the most important groups of Phase I enzymes is the family of cytochrome P450 type 1 - CYP1s (Bock and Köhle 2006). CYP1s are mainly located in the liver and distributed throughout the body, playing key roles in the xenobiotic metabolism (Antos et al. 2015; Lee et al. 2018). Phase I metabolism takes place in the liver, intestine, lungs, kidneys, and plasma, and it is regulated by translocation of AHRs and pregnane X receptor (PXR) (Lee et al. 2018). The CYP1s family includes enzymes that are encoded by more than 5000 genes. This gene family generally includes three subfamilies: the CYP1As, CYP1Bs, and CYP1Cs (Goldstone and Stegeman 2006). These enzymes catalyze the oxidative metabolism of various organic compounds such as polycyclic, often halogenated, aromatic hydrocarbons, aromatic amines, and some endogenous substrates (Antos et al. 2015).

Due to their genetic polymorphism and environmental factors, the action of the CYP1s differs among the species (Kapelyukh et al. 2019). Mammals have two CYP1A isoforms, CYP1A1 and CYP1A2, and the CYP1B1 enzyme (Goldstone and Stegeman 2006). The CYP1A1 enzyme is the most dynamic among the CYP1s family for the metabolization of polycyclic aromatic hydrocarbons. CYP1A1 was chosen as an up-and-coming enzyme in the prevention and treatment of human diseases (Ye et al. 2019). On the other hand, CYP1A2 is a very important CYP1 enzyme in humans that can detoxify several chemical species like drugs, industrial chemicals, and environmental toxicants (Antos et al. 2015). In addition, CYP1B1 is a critical part of the oxidative metabolism of xenobiotics. It metabolizes chemical parts of retinol metabolism, melatonin, dietary plant flavonoids, and the composition of genotoxic catechol estrogens (Shah et al. 2019).

Chickens have two CYP1A isoforms CYP1A4 and CYP1A5 that are orthologous to the mammalian CYP1A1 and CYP1A2, respectively (Goldstone and Stegeman, 2006). In particular, CYP1A4 levels are monitored by ethoxyresorufin-O-deethylase and aryl hydrocarbon hydroxylase. In addition, CYP1A5 is known to participate in three reactions, arachidonic acid epoxygenation, 4-hydroxylation of tamoxifen, and uroporphyrinogen oxidation (Antos et al. 2015). Finally, in chickens, CYP1B1 is

also expressed. The CYP1B1 monooxygenase is responsible for the elimination of a large variety spectrum of xenobiotics (Goldstone and Stegeman 2006).

12.3 Nrf2 Signaling Pathway and Regulation of ARE-Responsive Genes

In 1994, Moi and his colleagues discovered a new basic leucine zipper transcription factor called nuclear factor [erythroid-derived 2]-like 2 (Nrf2), a critical redox-sensitive transcription factor that is considered as a key regulator of cellular antioxidant response and xenobiotic metabolism in all mammals (Ma 2013; Vomund et al. 2017; Dai et al. 2020).

At the physiological state, Nrf2 in the cell cytoplasm is secluded by its actin-bound inhibitor protein Keap1 (Stefanson and Bakovic 2014). Disruption of Nrf2 and Keap1 complex by oxidation of cysteine residues in Keap1 makes Nrf2 translocate into the nucleus. There it heterodimerizes with small musculoaponeurotic fibrosarcoma protein (sMaf) and co-activator proteins and binds to antioxidant response element (ARE), leading to the transcription of a cascade of cytoprotective genes (Lee et al. 2017).

The interaction between Nrf2 and ARE leads to transcriptional activation and up-regulation of several Phase II antioxidant and detoxifying enzymes such as catalase (CAT), superoxide dismutase (SOD), glutathione reductase (GSR), glutathione peroxidase (GPx), glutathione S-transferase (GST), NAD(P)H: quinone oxidoreductase 1 (NQO1), uridine 5-diphospho (UDP)-glucuronosyltransferase, and thioredoxin (TXN) (Na and Surh 2008; Muhammad et al. 2017). Phase II proteins and antioxidant enzymes are capable of preventing chronic oxidative stress, increasing toxin metabolism and preserving cellular homeostasis (Bocci and Valacchi 2015).

12.3.1 Keap1 as Nrf2 Inhibitor

Keap1 is an Nrf2 cytoskeleton binding protein related to the regulation of the Nrf2 signaling pathway. Keap1 is localized in the cytoplasm near to plasma membrane and, due to its intensively reactive thiol content (It has at least 25 reactive thiols), is a very sensitive redox sensor that deals with exogenous electrophiles and the products of lipid peroxidation (Stefanson and Bakovic 2014). In particular, Keap1 interacts with Cullin 3 and forms an E3 ubiquitin ligase complex that is responsible for Nrf2 proteasomal degradation (Lu et al. 2016; Lee et al. 2017). In addition, Cul3-E3 regulated proteasomal degradation of Nrf2 promoted by Keap1 results in inhibition of ARE activation (Stefanson and Bakovic 2014). Under physiological conditions, the complex Nrf2-Keap1 exists in the cytoplasm with a half-life of approximately 20 minutes if it is not in need, is degraded by the proteasome (Bocci and Valacchi 2015). On the other hand, under induced state, inducers react with cysteines in Keap1, causing the release of Nrf2 and its subsequent translocation to the nucleus

and activation of the expression of Phase II cytoprotective genes (Baird and Dinkova-Kostova 2011).

12.3.2 Phase II Enzymes and Proteins

Two of the most important Phase II enzymes are catalase (CAT) and superoxide dismutase (SOD). Antioxidant enzymes CAT and SOD directly react with ROS (Patlevič et al. 2016; Ighodaro and Akinloye 2018; Wang et al. 2018a). In particular, CAT catalyzes the decomposition of hydrogen peroxide (H_2O_2) to water (H_2O) and oxygen (O_2) in a breakdown reaction which prohibits the formation of hydroxyl radicals from H_2O_2 (Jung and Kwak 2010). Moreover, superoxide dismutase (SOD) catalyzes the dismutation of superoxide anion-free radical (O_2^-) into O_2 and H_2O_2 and reduces the O_2^- level that injures the cells at an excessive concentration (Younus 2018).

Another important group of Phase II enzymes is associated with glutathione (GSH). Glutathione (GSH) is a three amino acid (cysteine, glycine, and glutamic acid) peptide thiol that plays a key role in the prevention of radical-mediated injuries and, for this reason, is one of the most adequate intracellular antioxidant proteins existing in the cytoplasm (Browne and Armstrong 1998; Stefanson and Bakovic 2014). GSH is referred to as a radical scavenger that is used by the antioxidant Phase II enzymes glutathione peroxidase (GPx), glutathione reductase (GSR) and glutathione-S-transferases (GSTs) (Browne and Armstrong 1998). It is generally known that $GP_{X,}$ in association with GSR, regenerates oxidized antioxidants (Iskusnykh et al. 2013; Bacou et al. 2021). Particularly, GPx is a selenium-including antioxidant enzyme that down-regulates H_2O_2 and lipid peroxides to water and lipid alcohols. Moreover, it oxidizes glutathione to glutathione disulfide (Stefanson and Bakovic 2014). The regulation of the intracellular redox state of cells depends on low concentrations of glutathione because it contributes equivalents for a serious number of biochemical pathways. If GPx activity or GSH levels are inefficient, hydrogen peroxide and lipid peroxides are not detoxified and may be transformed to OH-radicals and lipid peroxyl radicals. In low-level oxidative stress, the glutathione system is thought to be a crucial defense (Tabet and Touyz 2007). In addition, GSR catalyzes the reduction of glutathione disulfide (GSSG) to GSH, preserving the supply of reduced glutathione and the sulfhydryl pool of cells (Couto et al. 2016; Balogh et al. 2019). Lastly, for GSH related Phase II enzymes, the GST family of enzymes catalyze the detoxication of xenobiotic compounds by GSH conjugation and protects cells against oxidative stress and several toxic molecules (Strange et al. 2001).

In cellular systems, there are many one- and two-electron reductases which are responsible for the reduction of quinones to semiquinones and hydroquinones. NAD(P)H: quinone acceptor oxidoreductases (NQOs) is a two-electron reductase family formed by NQO1 and NQO2, which can reduce endogenous and exogenous quinones to hydroquinones (Ross and Siegel 2017). Specifically, NQO1 is a highly inducible Phase II enzyme, regulated by Keap1/Nrf2/ARE pathway that promotes a

two-electron transfer to diminish quinones to hydroquinones (Dinkova-Kostova and Talalay 2010). As a result of quinones reduction, NQO1 depresses quinone levels and prevents the production of free radical oxygen intermediates (Ross and Siegel 2017). Other roles of NQO1 are related to its potential to act as a compound of cellular redox system producing antioxidant forms of ubiquinone and vitamin E and at high concentrations functions as a direct superoxide reductase (Dinkova-Kostova and Talalay 2010).

Another Phase II enzyme is heme oxygenase-1 (HO-1). HO-1 is regarded as a highly protective enzyme that directly inhibits pro-inflammatory cytokines and activates anti-inflammatory ones (Pae and Chung 2009; Puentes-Pardo et al. 2020; Campbell et al. 2021). In addition, HO-1 activates oxidative degradation of heme into free iron, carbon monoxide (CO), and bilirubin (Ahmed et al. 2017). Furthermore, bilirubin is known to act as an antioxidant, and CO functions as a nuclear factor kappa B (NF-κB), inhibitor (Lee et al. 2017). Therefore, HO-1 plays an important anti-oxidative and anti-inflammatory role in the cellular system (Chau 2015).

Furthermore, the thioredoxin (Trx) system is one of the most important cellular disulfide reducing systems, including NADPH, Trx reductase (TrxR), and Trx. Thioredoxins (Trxs) play numerous roles in the cellular system. Among other functions, Trxs act as reductases in redox control, modulate the inflammatory response, help cells to deal with oxidative stress, and regulate programmed cell death via denitrosylation (Collet and Messens 2010). Specifically, Trxs function as an electron donor for certain antioxidant enzymes like peroxiredoxins (PRDXs) and methionine sulfoxide reductases, regulate the task of transcription factors such as NF-κB, and they are associated with the adjustment of apoptosis (Muri et al. 2018).

Peroxiredoxins (PRDXs) are a family of non-seleno peroxidases that catalyze the peroxide reduction of H_2O_2, organic hydroperoxides, and peroxynitrite. They are related to the regulation of several physiological functions, like cell growth, differentiation, apoptosis, lipid metabolism as well as immune response (Nicolussi et al. 2017). PRDX1 is a considerable member of the antioxidant enzymes, and it can be quickly over-oxidized. In the nucleus, PRDX1 precisely collaborates with p53 or transcription factors such as NF-κB, and in this manner, it influences their bioactivities upon gene regulation. In the cytoplasm, it obtains anti-apoptotic potential through direct or indirect interactions with plenty of ROS-dependent (redox regulation) effectors (Ding et al. 2017a).

Finally, uridine diphosphate glucuronosyltransferase (UGT) is a xenobiotic-metabolizing enzyme that plays a key role in Phase II metabolism in the liver of birds (Kawai et al. 2019). UGT catalyzes the transformation of small lipophilic molecules, such as steroids, bilirubin, hormones, and drugs into water-soluble, excretable metabolites (Tukey and Strassburg 2000).

12.4 Interaction of AHR and Nrf2 Signaling Pathways

The synergistic function of Phase I and II enzymes against xenobiotics and oxidative stressors is regulated by the interactions of the aryl hydrocarbon receptor (AHR) and the transcription factor Nrf2 (Lee et al. 2018). In particular, Phase I enzymes metabolize xenobiotics, and Phase II enzymes interact with Phase I metabolites by catalyzing their conjugation reaction and elimination-detoxification (Croom 2012). Several studies reported that when xenobiotics enter the organism, first activate AHR and then the Nrf2 pathway (Köhle and Bock 2006).

The expression of Nrf2 is regulated, among other factors by AHRs (Shin et al. 2007). AHRs are known to have Nrf2 as a direct target gene (Bortoli et al. 2018). Two critical Phase II XMEs enzymes, which link both Nrf2 and AHR pathways, are NQO1 and GST (Fig. 12.1). NQO1 and GST have also ARE and XRE in their regulatory regions (Köhle and Bock 2006; Lee et al. 2018). Another case of AHRs and Nrf2 interaction is based on the AHR activation of CYPs that lead to the release of ROS, and in turn, ROS production activates Nrf2 (Marchand et al. 2004), as it is shown in Fig. 12.2.

12.5 Cases of AHR and Nrf2 Signaling Studies in Poultry

12.5.1 Mycotoxins

In intensive poultry, production chickens are more likely to face various stressor challenges related to dietary and environmental factors. For example, the toxicity caused by the composition of in-feed xenobiotics could lead to oxidative stress and

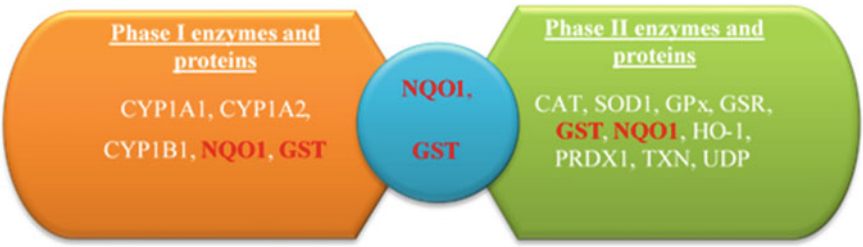

Fig. 12.1 Graphical representation of Phase I & II enzyme batteries and their interaction. Phase I enzymes battery includes: cytochrome P450 1 A1 (CYP1A1), cytochrome P450 1 A2 (CYP1A2), cytochrome P450 1 B1 (CYP1B1), quinone oxidoreductase 1 (NQO1), and glutathione transferase (GST) Phase II enzymes battery includes: catalase (CAT), superoxide dismutase 1 (SOD1), glutathione peroxidase (GPx), glutathione reductase (GSR), GST, NQO1, heme oxygenase-1 (HO-1), peroxiredoxin 1 (PRDX1), uridine 5-diphospho (UDP)-glucuronosyltransferase, and thioredoxin (TXN) and uridine 5-diphospho (UDP)-glucuronosyltransferase (UDP). NQO1 and GST possess antioxidant response element (ARE) and xenobiotic response element (XRE) in their regulatory regions and, for this reason, can be activated by both transcription factors, namely nuclear factor [erythroid-derived 2]-like 2 (Nrf2) and aryl hydrocarbon receptors (AHR)

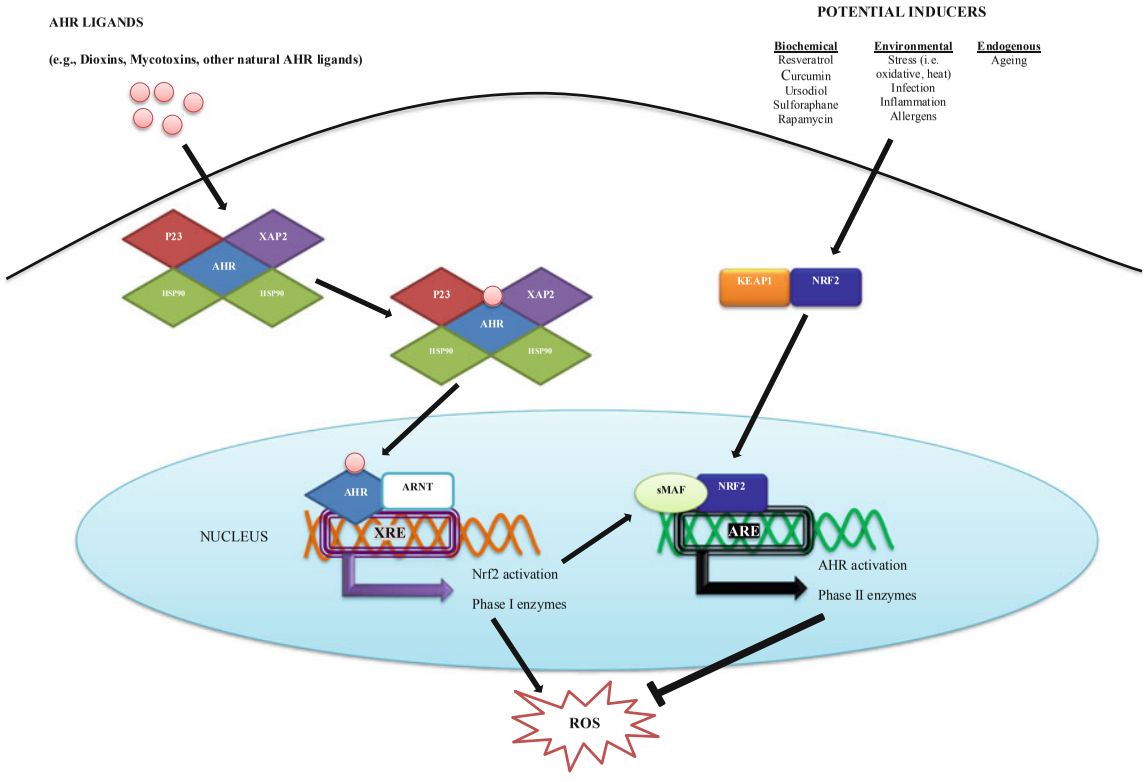

Fig. 12.2 Schematic representation of the AHR-NRF2 pathways and their interactions. The Aryl hydrocarbon receptor (AHR) forms a complex with protein p23 (P23), protein of hepatitis B virus X-associated (XAP2), heat shock protein 90 (HSP90), and bind with AHR ligands entering the cell. This multiprotein complex transfers to the nucleus to bind with AHR nuclear translocator (ARNT) to xenobiotic-responsive elements (XRE) and adjust the expression of Phase I

inflammation that in turn negatively impacts poultry health and performance. Xenobiotics such as mycotoxins and their metabolites are found as contaminants in animal feed and are highly dangerous for poultry and humans due to their high toxicity (Liu et al. 2019; Ates and Ortatatli 2021).

The topic of mycotoxin and mycotoxin metabolites' effects on the AHR signaling pathway and the transcription of its downstream genes has been addressed by only a few studies. It was shown that different types of mycotoxins and their metabolites induced AHRs and CYPs enzyme expression mainly in the chicken liver (Muhammad et al. 2017; Liu et al. 2019; Ates and Ortatatli 2021). The effects of mycotoxins on the AHR pathway in the broiler's gut are largely overlooked. Mycotoxins are known to cause intestinal toxicity by inducing oxidative stress (Marin et al. 2013). Their effects on the regulation of the Nrf2 signaling pathway and its related downstream genes have been studied more compared to AHRs in poultry (Table 12.1). Mycotoxins inhibited the antioxidant response via the decrease of Nrf2 and the expression of its downstream Phase II cytoprotective genes in the chicken liver (Liu and Wang 2016; Wang et al. 2018a, b), spleen (Rajput et al. 2019), kidneys (Li et al. 2020), and intestine (Tong et al. 2020). In organs such as the kidneys and the intestine, the effects of mycotoxins on the cellular antioxidant response might depend on their concentration and the chronic period of the exposure. Finally, there are no studies investigating the effects of mycotoxins collectively on both the AHR and Nrf2 signaling.

12.5.2 Heats Stress

It is generally known that poultry in heat stress conditions in order to maintain their homeostasis activate physiological mechanisms. Specific genes and metabolic pathways are linked to these mechanisms. Among others, the AHR pathway and its related Phase I XMEs are likely to be involved in the regulation of cellular stress caused by high thermal conditions (Guo et al. 2020). However, no studies were found to investigate heat stress effects on the AHR signaling pathway in poultry.

On the other hand, it is known that heat stress could lead to ROS production and oxidative stress (Sahin et al. 2012). The possible mechanism behind these effects is that heat stress is responsible for the de-activation of the electron transport assemblies of the mitochondrial membrane, which modulates the expression of Nrf2 (Mohammed et al. 2019). In poultry, the effects of heat stress on the Nrf2 signaling pathway have been investigated in hepatic (Sahin et al. 2012; Zhang et al.

Fig. 12.2 (continued) metabolizing enzymes like cytochrome P450 enzymes (CYP1A1, CYP1A2, and CYP1B1) which trigger the production of reactive oxygen species (ROS). Upon activation by ROS, the Nuclear factor [erythroid-derived 2]-like 2 (Nrf2) unbinds from the Keap1-Nrf2 complex and translocates to the nucleus, binds with small musculoaponeurotic fibrosarcoma protein (sMAF) and stimulates the expression of Phase II cytoprotective and antioxidant genes, which eliminate ROS. For a further description of other Phase II enzyme functions, see the text

Table 12.1 Cases of AHR and Nrf2 signaling studies in poultry addressing the topic of mycotoxins challenge

Mycotoxins	Avian species	Cells	AHR pathway	Nrf2 pathway	References
Aflatoxins	Broilers	Hepatocytes	–	↓ Nrf2, NQO1, SOD, HO-1	Liu and Wang (2016)
Aflatoxins	Broilers	Hepatocytes	↑ CYP2A6	–	Muhammad et al. (2017)
Aflatoxins	Broilers	Hepatocytes	–	↓ Nrf2, GSTs	Wang et al. (2018a, b)
T-2 toxin from fusarium genus	Chickens	Hepatocytes	↑ AHR, CYP1A5	–	Liu et al. (2019)
Aflatoxins	Broilers	Splenic	–	↓ Nrf2, HO-1, NQO1, GPx	Rajput et al. (2019)
Ochratoxin A	Chicken	Nephrons	–	↓ Nrf2, CAT, SOD, HO-1	Li et al. (2020)
Ochratoxin A	Broilers	Duodenal, Jejunal, Ileal	–	↓ Nrf2, HO-1	Tong et al. (2020)
Aflatoxins	Broilers	Hepatocytes	↑ AHR, CYP1A1, CYP2A6	–	Ates and Ortatatli (2021)

2018), fibroblasts (Wu et al. 2019) and intestinal (Song et al. 2018; Mohammed et al. 2019) cells. In all cases, the results demonstrated that heat stress decreased Nrf2 and its downstream Phase II antioxidant enzymes (Table 12.2).

12.5.3 Bacterial Pathogens

Bacterial pathogens are very critical for poultry production due to their negative effects, which lead to major economic losses and risks for overall food safety. They are responsible for immune dysregulation—inflammation, oxidative stress, contamination of poultry tissues and carcass, high mortality rates and an overall reduction in poultry productivity (Li et al. 2019; Alber et al. 2020). Pathogenic proliferation and intestinal colonization could result in disturbance of gut microbial balance and dysbiosis, cause inflammation via Toll-like receptor (TLR) signaling that could, in turn, hamper the epithelial tight junction assembly resulting in "leaky gut" with detrimental consequences beyond the intestine (Awad et al. 2017; Zhang et al. 2020; Chang et al. 2020). It has been reported that the AHR pathway and the related Phase I XMEs play a key role in bacterial infections (Zhao et al. 2019). In particular, AHR is involved in the regulation of cytokine production and the modulation of bacterial-induced inflammatory responses (Alber et al. 2020). So far, only two studies have shown that bacterial pathogens such as *Mycoplasma gallisepticum* in fibroblasts cells (Zhao et al. 2019) and *Escherichia coli* in lung cells (Alber et al. 2020) induced

Table 12.2 Cases of AHR and Nrf2 signaling studies in poultry addressing the topic of heat stress challenge

Other factors	Avian species	Cells	AHR pathway	Nrf2 pathway	References
Heat stress	Quail	Hepatocytes	–	↓ Nrf2, HO-1, ↑ HSP70	Sahin et al. (2012)
Heat stress	Broilers	Jejunal	–	↓ Nrf2, HO-1, GPx	Song et al. (2018)
Heat stress	Broilers	Hepatocytes	–	↓ Nrf2, CAT	Zhang et al. (2018)
Heat stress	Broilers	Cecal	–	↓ Nrf2, GPx	Mohammed et al. (2019)
Heat stress	Chickens	Fibroblasts cells	–	↓ Nrf2, CAT, SOD, GSTs	Wu et al. (2019)

expression of the AHR signaling pathway. In poultry production, bacterial pathogens are one of the most important causes of intestinal oxidative stress inductions, which could negatively affect gut health and productivity (Sun et al. 2020). In addition, it has been shown (Table 12.3) that bacterial pathogens and their components like the lipopolysaccharides (LPS) of Gram-ve bacteria, could cause oxidative stress via decreasing the expression levels of Nrf2 and its downstream Phase II antioxidant and cytoprotective genes (Li et al. 2019; Ding et al. 2020; Sun et al. 2020).

12.5.4 Phytochemicals

The beneficial role of phytochemicals (i.e., herbs, spices, essential oils, and bioactive components mixtures) on enhancing antioxidant response at the biochemical level has been repeatedly shown in poultry production. In particular, in broilers, different types of dietary supplemented phytochemicals have been shown to enhance plasma (Paraskeuas et al. 2017a, b), meat (Paraskeuas et al. 2016), liver (Ding et al. 2017b; Mountzouris et al. 2019) and intestinal total antioxidant status (Mountzouris et al. 2019, 2020; Griela et al. 2021). Broiler antioxidant status improvements could be positively correlated with poultry productivity (Ding et al. 2020), and all would depend on the phytochemical bioactive constituents (Polat et al. 2011) and their dietary inclusion level (Roofchaee et al. 2011).

However, the molecular mechanisms underpinning the response of poultry to oxidative stress have not been fully elucidated yet (Ding et al. 2020). Table 12.4 lists a series of studies investigating the effects of phytochemicals without or in combination with xenobiotics such as mycotoxins on the AHR signaling pathway and the expression of its downstream Phase I XMEs (Muhammad et al. 2017; Kim et al. 2019; Ates and Ortatatli 2021). It has been demonstrated that various phytochemicals decreased AHR and its related Phase I XMEs in chicken liver (Kim et al. 2019; Ates and Ortatatli 2021) spleen and cecal tonsils (Muhammad

Table 12.3 Cases of AHR and Nrf2 signaling studies in poultry addressing the topic of the pathogenic challenge

Other factors	Avian species	Cells	AHR pathway	Nrf2 pathway	References
Mycoplasma gallisepticum	Chickens	Thymus	–	↓ Nrf2, GPx, PRDX6	Li et al. (2019)
Mycoplasma gallisepticum	Chickens	Fibroblasts cells	↑ AHR, ARNT	–	Zhao et al. (2019)
Escherichia coli	Chickens	Lung cells	↑ AHR	–	Alber et al. (2020)
Escherichia coli	Chickens	Hepatocytes	–	↓ Nrf2, CAT, SOD	Ding et al. (2020)
Bacterial lipopolysaccharides	Chickens	Jejunal	–	↓ Nrf2, HO-1, NQO1, SOD	Sun et al. (2020)

et al. 2017). Contemporary intense interest in phytochemicals includes the elucidation of their potential function as AHR ligands as well as their effects on genes and metabolic pathways related to gut function and health (Gutiérrez-Vázquez and Quintana 2018).

On the other hand, most of the available studies have looked into the effects of phytochemicals on the Nrf2 signaling pathway and the expression of its downstream antioxidant and anti-inflammatory genes (Table 12.4). In particular, it has been shown that the dietary supplementation of various phytochemicals induced the expression of Nrf2 and its Phase II antioxidant genes in chicken liver (Sahin et al. 2012; Zhang et al. 2018; Wang et al. 2018a, b), spleen (Rajput et al. 2019), fibroblasts cells (Wu et al. 2019), and intestine (Mueller et al. 2012; Song et al. 2018; Mountzouris et al. 2020; Sun et al. 2020; Griela et al. 2021). Moreover, in boilers, the modulation of the Nrf2 signaling pathway was found to be dose-dependent, and in addition, the magnitude of modulation was shown to depend on the chicken intestinal site (Mountzouris et al. 2020). Enhancing the broiler adaptive capacity to resist oxidative stress via activation of Nrf2 signaling might hold the key for further protection from mycotoxins (Wang et al. 2018b; Rajput et al. 2019). So far, no study has looked into the effects of phytochemicals on AHR and Nrf2 signaling pathways in chickens. However, Singh et al. (2019) has investigated in mice intestinal epithelial cells the effects of urolithin A, a metabolite produced from berries, grapes, and walnuts, on AHR and Nrf2 pathways and their related downstream Phase I and Phase II genes. The results demonstrated that the particular phytochemical might have the potential to enhance gut barrier function by regulating AHR and Nrf2 pathways and their related downstream genes. The potential modulation of the AHR and Nrf2 pathways by dietary phytochemical is a promising way of further protecting poultry from the detrimental consequences of oxidative stress.

Table 12.4 Cases of AHR and Nrf2 signaling studies in poultry addressing the topic of dietary phytochemical inclusion

Phytochemicals	Avian species	Cells	AHR pathway	Nrf2 pathway	References
Broccoli extract, turmeric, oregano, thyme, rosemary	Broilers	Jejunal, colon, hepatocytes	–	↑ ARE genes (HO-1, TrxR1) in jejunum	Mueller et al. (2012)
Curcumin	Quails	Hepatocytes	–	↑ Nrf2, HO-1, ↓ HSP70	Sahin et al. (2012)
Curcumin	Broilers	Hepatocytes	↓ CYP2A6	–	Muhammad et al. (2017)
Artemisia annua	Broilers	Jejunal	–	↑ Nrf2, HO-1, GPx, SOD	Song et al. (2018)
Curcumin	Broilers	Hepatocytes	–	↑ Nrf2, CAT	Zhang et al. (2018)
Curcumin	Broilers	Hepatocytes	–	↑ Nrf2, GSTs	Wang et al. (2018b)
Indole	Chickens	Cecal tonsils, splenic	↓ AHR, CYP1A4, CYP1A5 (cecal tonsils)	–	Kim et al. (2019)
Grapeseed	Broilers	Splenic	–	↑ Nrf2, HO-1, NQO1, GPx	Rajput et al. (2019)
Curcumin	Chickens	Fibroblasts cells	–	↑ Nrf2, CAT, SOD, GSTs	Wu et al. (2019)
Mixture based on ginger, lemon balm, oregano, thyme	Broilers	Duodenal, Jejunal, Ileal, Cecal	–	↑ Nrf2, HO-1, NQO1, GPx duodenum = ceca>ileum	Mountzouris et al. (2020)
Quercetin	Chickens	Jejunal	–	↑ Nrf2, HO-1, NQO1, SOD	Sun et al. (2020)
Nigella sativa seeds	Broilers	Hepatocytes	↓ AHR, CYP1A1, CYP2A6	–	Ates and Ortatatli. (2021)
Blend of compounds	Broilers	Duodenal, Jejunal, Ileal, Cecal	–	↑ CAT, SOD, HO-1, NQO1, GSR, PRDX1, TXN	Griela et al. (2021)

12.6 Conclusion

This chapter has highlighted endogenous poultry adaptive mechanisms to counteract oxidative stress with an emphasis on the gut. These mechanisms involve the engagement of AHR and Nrf2 signaling pathways and their downstream Phase I XMEs and Phase II cytoprotective enzymes. The activation of these pathways increases poultry fitness to reduce the impact of stressor challenges such as xenobiotics, heat stress and pathogens on gut function and health. Nutrigenomic studies addressing the effects of dietary bioactive compounds such as phytochemicals on the activation and magnitude of poultry gut adaptive capacity are missing. Such studies are expected to provide a rationale for the mechanistic evaluation of the efficacy of promising cytoprotective applications in broiler nutrition. Further understanding of the AHR and Nrf2 pathway modulation in poultry could provide an analytical selection platform for screening various bioactive components and categorizing dietary interventions with respect to their total protective role against oxidative stress.

References

Ahmed SMU, Luo L, Namani A, Wang XJ, Tang X (2017) Nrf2 signaling pathway: pivotal roles in inflammation. Biochim Biophys Acta 1863:585–597

Alber A, Morris KM, Bryson KJ, Sutton KM, Monson MS, Chintoan-Uta C, Borowska D, Lamont SJ, Schouler C, Kaiser P, Stevens MP, Vervelde L (2020) Avian pathogenic *Escherichia coli* (APEC) strain-dependent immunomodulation of respiratory granulocytes and mononuclear phagocytes in CSF1R-reporter transgenic chickens. Front Immunol 10:30–55

Allen RG, Tressini M (2000) Oxidative stress and gene regulation. Free Radic Biol Med 28:463–499

Antos PA, Błachuta M, Hrabia A, Grzegorzewska AK, Sechman A (2015) Expression of aryl hydrocarbon receptor 1 (AHR1), AHR1 nuclear translocator 1 (ARNT1) and CYP1 family monooxygenase mRNAs and their activity in chicken ovarian follicles following in vitro exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Toxicol Lett 237:100–111

Ates MB, Ortatatli M (2021) Phase-1 bioactivation mechanisms of aflatoxin through AhR, CAR, and PXR nuclear receptors and the interactions with Nigella sativa seeds and thymoquinone in broilers. Ecotoxicol Environ Saf 208:111774

Awad WA, Hess C, Hess M (2017) Enteric pathogens and their toxin-induced disruption of the intestinal barrier through alteration of tight junctions in chickens. Toxins (Basel) 9:1–22

Bacou E, Walk C, Rider S, Litta G, Perez-Calvo E (2021) Dietary oxidative distress: a review of nutritional challenges as models for poultry, Swine and Fish. Antioxidants 10:525

Baird L, Dinkova-Kostova A (2011) The cytoprotective role of the Keap1–Nrf2 pathway. Arch Toxicol 85:241–272

Balogh K, Kövesi B, Zándoki E, Kulcsár S, Ancsin Z, Erdélyi M, Dobolyi C, Bata-Vidács I, Inotai K, Szekeres A, Mézes M, Kukolya J (2019) Effect of sterigmatocystin or aflatoxin-contaminated feed on lipid Peroxidation and glutathione redox system and expression of glutathione redox system regulatory genes in broiler chicken. Antioxidants (Basel) 8:201

Bocci V, Valacchi G (2015) Nrf2 activation as target to implement therapeutic treatments. Front Chem 3:4

Bock KW, Köhle C (2006) Ah receptor: dioxin-mediated toxic responses as hints to deregulated physiologic functions. Biochem Pharmacol 72:393–404

Bortoli S, Boutet-Robinet E, Lagadic-Gossmann D, Huc L (2018) Nrf2 and AhR in metabolic reprogramming after contaminant exposure. Curr Opin Pharmacol 8:34–41
Browne R, Armstrong D (1998) Methods in molecular biology. In: Free radical and antioxidant protocols, vol 108. Springer, New York
Campbell NK, Fitzgerald HK, Dunne A (2021) Regulation of inflammation by the antioxidant haem oxygenase 1. Nat Rev Immunol
Carvalho RH, Ida EI, Madruga MS, Shimokomaki M, Estevez M (2019) Collapse of the endogenous antioxidant enzymes in post-mortem broiler thigh muscles triggers oxidative stress and impairs water-holding capacity. J Food Sci Technol 56:1371–1379
Chang CH, Teng PY, Lee TT, Yu B (2020) Effects of multi-strain probiotic supplementation on intestinal microbiota, tight junctions, and inflammation in young broiler chickens challenged with Salmonella enterica subsp. Enterica. Asian-Australas J Anim Sci 33:1797–1808
Chau LY (2015) Heme oxygenase-1: emerging target of cancer therapy. J Biomed Sci 22:22
Collet JF, Messens J (2010) Structure, function, and mechanism of thioredoxin proteins. Antioxid Redox Signal 13:1205–1216
Couto N, Wood J, Barber J (2016) The role of glutathione reductase and related enzymes on cellular redox homoeostasis network. Free Radic Biol Med 95:27–42
Croom E (2012) Metabolism of xenobiotics of human environments. Prog Mol Biol Transl Sci 112: 31–88
Da Silva EO, Bracarense APFL, Oswald IP (2018) Mycotoxins and oxidative stress: where are we? World Mycotoxin J 11:113–134
Dai X, Yan X, Wintergerst KA, Cai L, Keller BB, Tan Y (2020) Nrf2: redox and metabolic regulator of stem cell state and function. Trends Mol Med 26:185–200
Ding C, Fan X, Wu G (2017a) Peroxiredoxin 1 - an antioxidant enzyme in cancer. J Cell Mol Med 21:193–202
Ding X, Yang CW, Yang ZB (2017b) Effects of star anise (*Illicium verum* Hook.f.), essential oil, and leavings on growth performance, serum, and liver antioxidant status of broiler chickens. J Appl Poult Res 4:459–466
Ding X, Yang C, Yang Z, Ren X, Wang P (2020) Effects of star anise (Illicium verum Hook.f) oil on the nuclear factor E2–related factor 2 signaling pathway of chickens during subclinical *Escherichia coli* challenge. Poult Sci 99:3092–3101
Dinkova-Kostova AT, Talalay P (2010) NAD(P)H:quinone acceptor oxidoreductase 1 (NQO1), a multifunctional antioxidant enzyme and exceptionally versatile cytoprotector. Arch Biochem Biophys 501:116–123
Goldstone HM, Stegeman JJ (2006) A revised evolutionary history of the CYP1A subfamily: gene duplication, gene conversion, and positive selection. J Mol Evol 62:708–717
Griela E, Paraskeuas V, Mountzouris KC (2021) Effects of diet and phytogenic inclusion on the antioxidant capacity of the broiler chicken gut. Animals 11:739
Guo K, Ge J, Zhang C, Lv M-W, Zhang Q, Talukder M, Li J-L (2020) Cadmium induced cardiac inflammation in chicken (Gallus gallus) via modulating cytochrome P450 systems and Nrf2 mediated antioxidant defense. Chemosphere 249:125858
Gutiérrez-Vázquez C, Quintana FJ (2018) Regulation of the immune response by the aryl hydrocarbon receptor. Immunity 48:19–33
Huang C, Jiao H, Song Z, Zhao J, Wang X, Lin H (2015) Heat stress impairs mitochondria functions and induces oxidative injury in broiler chickens. J Anim Sci 93:2144–2153
Ighodaro OM, Akinloye OA (2018) First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): their fundamental role in the entire antioxidant defence grid. Alex J Med 54:287–293
Iskusnykh IY, Popova TN, Agarkov AA, Pinheiro de Carvalho MA, Rjevskiy SG (2013) Expression of glutathione peroxidase and glutathione reductase and level of free radical processes under toxic hepatitis in rats. J Toxicol 2013:870628
Ito S, Alcorn J (2003) Xenobiotic transporter expression and function in the human mammary gland. Adv Drug Deliv Rev 55:653–665

Jung KA, Kwak MK (2010) The Nrf2 system as a potential target for the development of indirect antioxidants. Molecules 15:7266–7291

Kapelyukh Y, Henderson CJ, Scheer N, Rode A, Wolf CR (2019) Defining the contribution of CYP1A1 and CYP1A2 to drug metabolism using humanized CYP1A1/1A2 and Cyp1a1/Cyp1a2 knockout mice. Drug Metab Dispos 47:907–918

Kawai YK, Shinya S, Ikenaka Y, Saengtienchai A, Kondo T, Darwish WS, Nakayama SMM, Mizukawa H, Ishizuka M (2019) Characterization of function and genetic feature of UDP-glucuronosyltransferase in avian species. Comp Biochem Physiol C Toxicol Pharmacol 217:5–14

Kim WH, Lillehoj HS, Min W (2019) Indole treatment alleviates intestinal tissue damage induced by chicken coccidiosis through activation of the aryl hydrocarbon receptor. Front Immunol 10:560

Köhle C, Bock KW (2006) Activation of coupled Ah receptor and Nrf2 gene batteries by dietary phytochemicals in relation to chemoprevention. Biochem Pharmacol 72:795–805

Larigot L, Juricek L, Dairou J, Coumoul X (2018) AhR signaling pathways and regulatory functions. Biochim Open 7:1–9

Lee MT, Lin WC, Yu B, Lee TT (2017) Antioxidant capacity of phytochemicals and their potential effects on oxidative status in animals - a review. Asian-Australas J Anim Sci 30:299–308

Lee HJ, Pyo MC, Shin HS, Ryu D, Lee KW (2018) Renal toxicity through AhR, PXR, and Nrf2 signaling pathway activation of ochratoxin A-induced oxidative stress in kidney cells. Food Chem Toxicol 122:59–68

Li J, Qiao Z, Hu W, Zhang W, Shah SWA, Ishfaq M (2019) Baicalin mitigated mycoplasma gallisepticum-induced structural damage and attenuated oxidative stress and apoptosis in chicken thymus through the Nrf2/HO-1 defence pathway. Vet Res 50:1–13

Li K, Cao Z, Guo Y, Tong C, Yang S, Long M, Li P, He J (2020) Selenium yeast alleviates ochratoxin A-induced apoptosis and oxidative stress via modulation of the PI3K/AKT and Nrf2/Keap1 signaling pathways in the kidneys of chickens. Oxidative Med Cell Longev 2020:4048706

Liu Y, Wang W (2016) Aflatoxin B1 impairs mitochondrial functions, activates ROS generation, induces apoptosis and involves Nrf2 signal pathway in primary broiler hepatocytes. Anim Sci J 87:1490–1500

Liu Q, Wen J, Zhu J, Zhang T, Deng Y, Jiang J (2019) Aromatic hydrocarbon receptor regulates chicken cytochrome P450 1A5 transcription: a novel insight into T-2 toxin-induced gene expression and cytotoxicity in LMH cells. Biochem Pharmacol 168:319–329

Lu MC, Ji JA, Jiang ZY, You QD (2016) The Keap1-Nrf2-ARE pathway as a potential preventive and therapeutic target: an update. Med Res Rev 36:924–963

Ma Q (2013) Role of nrf2 in oxidative stress and toxicity. Annu Rev Pharmacol Toxicol 53:401–426

Marchand A, Barouki R, Garlatti M (2004) Regulation of NAD(P)H:Quinone oxidoreductase 1 gene expression by CYP1A1 activity. Mol Pharmacol 65:1029–1037

Marin DE, Pistol GC, Gras MA, Palade ML, Taranu I (2013) Comparative effect of ochratoxin A on inflammation and oxidative stress parameters in gut and kidney of piglets. Regul Toxicol Pharmacol 89:224–231

Mohammed AA, Jiang S, Jacobs JA, Cheng HW (2019) Effect of a synbiotic supplement on cecal microbial ecology, antioxidant status, and immune response of broiler chickens reared under heat stress. Poult Sci 98:4408–4415

Moi P, Chan K, Asunis I, Cao A, Kan YW (1994) Isolation of NF-E2-related factor 2 (Nrf2), a NF-E2-like basic leucine zipper transcriptional activator that binds to the tandem NF-E2/AP1 repeat of the beta-globin locus control region. Proc Natl Acad Sci U S A 91:9926–9930

Mountzouris KC, Paraskeuas V, Griela E, Papadomichelakis G, Fegeros K (2019) Effects of phytogenic inclusion level on broiler carcass yield, meat antioxidant capacity, availability of dietary energy, and expression of intestinal genes relevant for nutrient absorptive and cell growth–protein synthesis metabolic functions. Anim Prod Sci 60:242–253

Mountzouris KC, Paraskeuas V, Fegeros K (2020) Priming of intestinal cytoprotective genes and antioxidant capacity by dietary phytogenic inclusion in broilers. Anim Nutr 6:305–312
Mueller K, Blum NM, Kluge H, Mueller AS (2012) Influence of broccoli extract and various essential oils on performance and expression of xenobiotic- and antioxidant enzymes in broiler chickens. Br J Nutr 108:588–602
Muhammad I, Sun X, Wang H, Li W, Wang X, Cheng P, Li S, Zhang X, Hamid S (2017) Curcumin successfully inhibited the computationally identified CYP2A6 enzyme-mediated bioactivation of Aflatoxin B1 in Arbor Acres broiler. Front Pharmacol. 8:2–11
Muri J, Heer S, Matsushita M, Pohlmeier L, Tortola L, Fuhrer T, Conrad M, Zamboni N, Kisielow J, Kopf M (2018) The thioredoxin-1 system is essential for fueling DNA synthesis during T-cell metabolic reprogramming and proliferation. Nat Commun 9:1851
Na HK, Surh YG (2008) Modulation of Nrf2-mediated antioxidant and detoxifying enzyme induction by the green tea polyphenol EGCG. Food Chem Toxicol 46:1271–1278
Nicolussi A, D'Inzeo S, Capalbo C, Giannini G, Coppa A (2017) The role of peroxiredoxins in cancer. Mol Clin Oncol 6:139–153
Pae HO, Chung HT (2009) Heme oxygenase-1: its therapeutic roles in inflammatory diseases. Immune Netw 9:12–19
Paraskeuas V, Fegeros K, Palamidi I, Theodoropoulos G, Mountzouris KC (2016) Phytogenic administration and reduction of dietary energy and protein levels affects growth performance, nutrient digestibility and antioxidant status of broilers. J Poult Sci 53:264–273
Paraskeuas V, Fegeros K, Hunger C, Theodorou G, Mountzouris KC (2017a) Dietary inclusion level effects of a phytogenic characterized by menthol and anethole on broiler growth performance, biochemical parameters including total antioxidant capacity and gene expression of immunerelated biomarkers. Anim Prod Sci 57:33–41
Paraskeuas V, Fegeros K, Palamidi I, Hunger C, Mountzouris KC (2017b) Growth performance, nutrient digestibility, antioxidant capacity, blood biochemical biomarkers and cytokines expression in broiler chickens fed different phytogenic levels. Anim Nutr 3:114–120
Patel D, Sen DJ (2013) Xenobiotics: An essential precursor for living system. Am J Adv Drug Deliv 1:262–270
Patlevič P, Vašková J, Švorc P Jr, Vaško L, Švorc P (2016) Reactive oxygen species and antioxidant defense in human gastrointestinal diseases. Integr Med Res 5:250–258
Polat U, Yesilbag D, Eren M (2011) Serum biochemical profile of broiler chickens fed diets containing rosemary and rosemary volatile oil. J Biodivers Environ Sci 5:23–30
Puentes-Pardo JD, Moreno-SanJuan S, Carazo Á, León J (2020) Heme oxygenase-1 in gastrointestinal tract health and disease. Antioxidants 9:1214
Rajput SA, Sun L, Zhang NY, Khalil MM, Ling Z, Chong L, Wang S, Rajput IR, Bloch DM (2019) Grape seed proanthocyanidin extract alleviates AflatoxinB1-induced immunotoxicity and oxidative stress via modulation of NF-κB and Nrf2 signaling pathways in broilers. Toxins 11:1–15
Roofchaee A, Irani M, Ebrahimzadeh MA, Akbari MR (2011) Effect of dietary oregano (Origanum vulgare L.) essential oil on growth performance, cecal microflora and serum antioxidant activity of broiler chickens. Afr J Biotechnol 10:6177–6183
Ross D, Siegel D (2017) Functions of NQO1 in cellular protection and CoQ10 metabolism and its potential role as a redox sensitive molecular switch. Front Physiol 8:595
Sahin K, Orhan C, Tuzcu Z, Tuzcu M, Sahin N (2012) Curcumin ameloriates heat stress via inhibition of oxidative stress and modulation of Nrf2/HO-1 pathway in quail. Food Chem Toxicol 50:4035–4041
Sahin K, Orhan C, Smith MO, Sahin N (2013) Molecular targets of dietary 575 phytochemicals for the alleviation of heat stress in poultry. Worlds Poult Sci J 69:113–124
Shah BR, Xu W, Mraz J (2019) Cytochrome P450 1B1: role in health and disease and effect of nutrition on its expression. RSC Adv 9:21050–21062
Shin S, Wakabayashi N, Misra V, Biswal S, Lee GH, Agoston ES, Yamamoto M, Kensler TW (2007) NRF2 modulates aryl hydrocarbon receptor signaling: influence on adipogenesis. Mol Cell Biol 27:7188–7197

Singh R, Chandrashekharappa S, Bodduluri SR, Baby BV, Hegde B, Kotla NG, Hiwale AA, Saiyed T, Patel P, Vijay-Kumar M, Langille MGI, Douglas GM, Cheng X, Rouchka EC, Waigel SJ, Dryden GW, Alatassi H, Zhang HG, Haribabu B, Vemula PK, Jala VR (2019) Enhancement of the gut barrier integrity by a microbial metabolite through the Nrf2 pathway. Nat Commun 10:1–18

Song ZH, Cheng K, Zheng XC, Ahmad H, Zhang LL, Wang T (2018) Effects of dietary supplementation with enzymatically treated Artemisia annua on growth performance, intestinal morphology, digestive enzyme activities, immunity, and antioxidant capacity of heat-stressed broilers. Poult Sci 97:430–437

Stefanson AL, Bakovic M (2014) Dietary regulation of Keap1/Nrf2/ARE pathway: focus on plant-derived compounds and trace minerals. Nutrients 6:3777–3801

Strange RC, Spiteri MA, Ramachandran S, Fryer AA (2001) Glutathione-S-transferase family of enzymes. Mutat Res 482:21–26

Sun L, Xu G, Dong Y, Li M, Yang L, Lu W (2020) Quercetin protects against lipopolysaccharide-induced intestinal oxidative stress in broiler chickens through activation of Nrf2 pathway. Molecules 25:1053–1065

Tabet F, Touyz RM (2007) Reactive oxygen species, oxidative stress, and vascular biology in hypertension. Comprehensive Hypertension 2:337–347

Tong C, Lia P, Yua LH, Li L, Li K, Chena Y, Yanga SH, Long M (2020) Selenium-rich yeast attenuates ochratoxin A-induced small intestinal injury in broiler chickens by activating the Nrf2 pathway and inhibiting NF-κB activation. J Funct Foods 16:103784

Tukey RH, Strassburg CP (2000) Human UDP-glucuronosyltransferases: metabolism, expression, and disease. Annu Rev Pharmacol Toxicol 40:581–616

Vomund S, Schäfer A, Parnham MJ, Brüne B, von Knethen A (2017) Nrf2, the master regulator of anti-oxidative responses. Int J Mol Sci 18:2772

Vrzal R, Ulrichová J, Dvorák Z (2004) Aromatic hydrocarbon receptor status in the metabolism of xenobiotics under normal and pathophysiological conditions. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub 148:3–10

Wang Y, Branicky R, Noë A, Hekimi S (2018a) Superoxide dismutases: dual roles in controlling ROS damage and regulating ROS signaling. J Cell Biol 217:1915–1928

Wang H, Muhammad I, Li W, Sun X, Cheng P, Zhang X (2018b) Sensitivity of arbor acres broilers and chemoprevention of aflatoxin B1-induced liver injury by curcumin, a natural potent inducer of phase-II enzymes and Nrf2. Environ Toxicol Pharmacol 59:94–104

Wang H, Pan L, Zhang X, Ji R, Si L, Cao Y (2020) The molecular mechanism of AhR-ARNT-XREs signaling pathway in the detoxification response induced by polycyclic aromatic hydrocarbons (PAHs) in clam Ruditapes philippinarum. Environ Res 183:109165

Wu J, Ibtisham F, Niu YF, Wang Z, Li GH, Zhao Y, Nawab A, Xiao M, An L (2019) Curcumin inhibits heat-induced oxidative stress by activating the MAPK Nrf2/ARE signaling pathway in chicken fibroblasts cells. J Therm Biol. 79:112–119

Wullaert A, Bonnet MC, Pasparakis M (2011) NF-kappaB in the regulation of epithelial homeostasis and inflammation. Cell Res 21:146–158

Yasui T, Kim EY, Iwata H, Franks DG, Karchner SI, Hahn ME, Tanabe S (2007) Functional characterization and evolutionary history of two aryl hydrocarbon receptor isoforms (AhR1 and AhR2) from avian species. Toxicol Sci 99:101–117

Ye W, Chen R, Chen X, Huang B, Lin R, Xie X, Chen J, Jiang J, Deng Y, Wen J (2019) AhR regulates the expression of human cytochrome P450 1A1 (CYP1A1) by recruiting Sp1. FEBS J 286:4215–4231

Younus H (2018) Therapeutic potentials of superoxide dismutase. Int J Health Sci (Qassim) 12:88–93

Zhang JF, Bai KW, Su WP, Wang AA, Zhang LL, Huang KH, Wang T (2018) Curcumin attenuates heat-stress-induced oxidant damage by simultaneous activation of GSH-related antioxidant enzymes and Nrf2-mediated phase II detoxifying enzyme systems in broiler chickens. Poult Sci 97:1209–1219

Zhang H, Pan S, Zhang K, Michiels J, Zeng O, Ding X, Wang J, Peng H, Bai J, Xuan Y, Su Z, Bai S (2020) Impact of dietary manganese on intestinal barrier and inflammatory response in broilers challenged with Salmonella Typhimurium. Microorganisms 8:757

Zhao Y, Fu Y, Sun Y, Zou M, Peng X (2019) Transcriptional regulation of gga-miR-451 by AhR: Arnt in mycoplasma gallisepticum (HS strain) infection. Int J Mol Sci 20:3087

Immunological Mechanisms of Probiotics in Chickens

13

Graham A. J. Redweik and Melha Mellata

Abstract

Probiotics are live microorganisms, mainly bacteria (e.g., *Lactobacillus*, *Bifidobacterium*, *Bacillus*, *Enterococcus*) and yeast (e.g., *Saccharomyces*). These microorganisms are orally delivered, usually included in diets as supplements, to confer health benefits to the host. Given their effects on improved feed conversion and direct pathogen exclusion mechanisms, probiotics are widely considered as feasible alternatives to antibiotics. Notably, probiotics are potent activators of the host immune system, having effects on local (i.e., intestinal) and extraintestinal immune responses. However, the mechanisms in which probiotics signal to the host to elicit these immune responses are poorly understood in poultry. This chapter summarizes the basic mechanisms in which bacteria trigger avian immune responses with a focus on probiotics. These pathways are typically stimulated via (1) binding of conserved microbial ligands to host pattern recognition receptors (PRRs) or (2) bacterial secretion of immunomodulatory metabolites. The further elucidation of these immunological pathways will expand the criteria scientists and producers use when selecting probiotic candidates.

Keywords

Avian immunity · PRRs · Metabolites · SCFAs · Neurochemicals

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13.1 Introduction

Historically, agricultural antibiotics have been used for removing harmful bacterial pathogens and improving weight gain in food animals (Dibner and Richards 2005). However, in the face of changing regulations, probiotics, or live microbes that when administered in sufficient amounts improve host health (Hill et al. 2014), have emerged as good, low-risk alternative and are now widely implemented in poultry industry (Lutful Kabir 2009; Khan and Naz 2013; Patterson and Burkholder 2003). Probiotics are delivered orally, typically through feed or water, and can directly compete against bacterial pathogens via antimicrobial substances and competitive exclusion (Stein 2005; Vieco-Saiz et al. 2019), improve feed conversion via digestive enzyme production (Liao and Nyachoti 2017), and modulate the composition of the gut microbiota (Yadav and Jha 2019), which is crucial for maximizing the health, welfare, and performance in poultry (Stanley et al. 2014). Given this wide range of functions, probiotics are uniquely poised in their ability to improve animal health.

Probiotics confer their primary modes of action in the intestinal tract, a uniquely complex tissue system that simultaneously performs an array of tasks like absorbing nutrients from the lumen, tolerating the commensal microbiota, maintaining barrier homeostasis, and triggering inflammatory immune responses to harmful microbes (Broom and Kogut 2018). The intestinal immune system is generally organized into two compartments: an epithelium, composed of a barrier of functionally diverse epithelial cells and intraepithelial lymphocytes (IELs) that separates host tissues from the intestinal lumen, and a lamina propria, which underlies the epithelium and is composed of professional phagocytes and lymphocytes and is largely important for local adaptive immune responses. These compartments work together to regulate virtually every intestinal task by reacting to host and microbial signals (Broom 2019). Gut bacteria communicate with the host intestinal system through conserved microbial ligands (e.g., lipopolysaccharide or LPS) and secreted signals or metabolites (e.g., short-chain fatty acids or SCFAs). Thus, a feasible strategy of optimizing gut health and, by extension, productivity is to modulate the chicken intestinal environment via supplementation with probiotics.

Probiotics are potent activators of the intestinal immune system but do so without inducing excessive inflammation (Tarradas et al. 2020), which is detrimental to animal performance (Berghman 2016; Klasing 2007). Given that probiotics are typically administered before chickens are physiologically mature, this situates probiotics in a unique position to instruct or "train" the host immune system in early life. Thus, identifying the microbial "pioneers" to colonize the chicken intestine and properly induce immune maturation is crucial for optimizing animal productivity. We recently described commercial probiotics used in poultry and summarized their health benefits and impact on the immune system (Redweik et al. 2020a, b). This chapter overviews research studies on host pattern recognition receptor (PRR)-activation by microbe associated molecular patterns (MAMPs), with an interest on those stimulated by probiotics. Additionally, this chapter discusses signals and metabolites directly secreted by probiotics to modulate the avian immune

system. Lastly, this chapter briefly describes how probiotics can modulate both local (i.e., intestinal) and extraintestinal immune responses.

13.2 Probiotics and PRRs: Immunomodulation via Innate Signaling Receptors

Broadly, animal cells have the capacity to detect microorganisms like bacteria via ancestral signaling receptors specific to motifs and components conserved in microbes. These innate signaling systems, called pattern recognition receptors (PRRs) help animal cells the exact proximity of bacteria (i.e., intra- or extracellular) based on spatial distribution of these PRRs as well as the structural composition of the bacterium (e.g., Gram-negative via lipopolysaccharide or LPS detection). PRRs can be split into three classes: membrane-bound, cytoplasmic, and soluble. Although PRRs are functionally conserved in animal cells, their molecular composition and ligand specificity widely vary between species. This section will solely focus on avian PRRs with a focus on probiotic-mediated expression and activities.

Avian Toll-Like Receptors Toll-like receptors (TLRs) are membrane-associated, functionally homologous PRRs conserved in all multicellular organisms (Carpenter and O'Neill 2009; Roach et al. 2005) and are the best characterized PRRs in chickens. These receptor proteins possess 19–27 conserved leucine-rich repeat (LRR) with variations in glycosylation and LRR locations in the protein, which in combination enables these receptors to bind specific microbial ligands. Interestingly, TLRs can distinguish members of the commensal microbiota from pathogenic bacteria (Velová et al. 2018). For example, in mammalian studies, probiotics which contain DNA suppressor motifs (TTAGG or TCAAGCTGA) activate TLR9 apically expressed on the intestinal epithelium, but this activation reduces inflammatory signaling via expansion of regulatory T cells (Tregs) (Bouladoux et al. 2012; de Kivit et al. 2011; Lee et al. 2006). However, birds evolved to utilize TLR21 and lack TLR9, so this mechanism has yet to be demonstrated in chickens.

To date, ten TLRs have been characterized in avian species (Alcaide and Edwards 2011). TLR1-like protein (TLR1L) and TLR2 each possesses two isoforms in chickens (i.e., TLR1LA/TLR1LB and TLR2t1/TLR2t2) and collectively form a complex to detect di- and triacylated lipopeptides. Interestingly, TLR1LA, otherwise known as TLR16, is a novel chicken TLR which carries ligand specificity of mammalian TLR1 and TLR6 in a single receptor (Keestra et al. 2007). TLR3 and TLR7 are endosomal membrane-bound TLRs with binding specificity to viral dsRNA and ssRNA, respectively (Keestra et al. 2013). Notably, TLR7 is the sole chicken TLR which does not activate MyD88, an adaptor protein crucial in many innate signaling cascades (Kawai and Akira 2007). TLR4 binds to the highly conserved lipid A motif in lipopolysaccharide (LPS), a major component of the outer cell membrane in Gram-negative bacteria like *Escherichia coli* and *Salmonella enterica*. Although mammalian TLR4 activation can induce MyD88-dependent (i.e., adaptor proteins MyD88 and TIRAP) and independent (i.e., adaptor proteins TRAM

and TRIF) pathways, chickens lack a TRAM ortholog and thus are limited to MyD88-dependent signaling only (Lynn et al. 2003). Thus, this could be in part why chickens are relatively resistant to the endotoxic effects of LPS (Iliev et al. 2007) as well as why intestinal *Salmonella* colonization is less inflammatory in chickens versus mammals (Wigley 2014; Kogut et al. 2016).

Chicken TLR5 is functionally analogous to its mammalian homolog via binding specificity to flagellin, the protein component of bacterial flagella. TLR5 is potently responsive to *Salmonella* flagellin and is critical for anti-*Salmonella* immunity (Keestra et al. 2008; Iqbal et al. 2005). TLR7 is typically associated with viral immunity, binding to TLR15 is a structurally and functionally unique avian TLR absent in fish and mammals (Ramasamy et al. 2012; Roach et al. 2005). which binds to fungal and bacterial proteases (de Zoete et al. 2011). Furthermore, TLR15 is responsive to infection from both Gram-positive and Gram-negative pathogens (Higgs et al. 2006; Shaughnessy et al. 2009; Nerren et al. 2010). Lastly, TLR21, the functional analog of mammalian TLR9, binds to CpG DNA, a mimic for motifs commonly found in bacterial DNA (Brownlie and Allan 2011; Keestra et al. 2010). However, TLR21 is unique in its relatively broader recognition of bacterial chromosomal DNA versus TLR9 (Bauer et al. 2001; Keestra et al. 2010). Altogether, these TLRs serve as immunological sentinels, poised to direct innate, cellular responses against microbial exposure.

Non-TLR Pattern Recognition Receptors Although non-TLR PRRs are present in birds, they receive much less focus versus their TLR counterparts. Nucleotide-binding oligomerization domain (NOD)-like receptors are a family of cytoplasmic PRRs that, when activated, play a major role in inflammasome formation (Pétrilli et al. 2007). Although NOD-1 is present in chickens, a homolog of mammalian NOD-2 is absent (Chen et al. 2013). Several soluble PRRs like C-reactive protein (CRP) and serum amyloid A (SAA) are found in chickens (Marques et al. 2017). Also known as acute phase proteins (APPs), these soluble PRRs are produced upon response to inflammatory stimuli and have a wide range of functions like improving tissue integrity, toxin neutralization, antioxidant activities, and antibacterial responses (i.e., complement activation or enhanced phagocytosis) (Juul-Madsen et al. 2014). Notably, APP production widely varies among tissues (Marques et al. 2017).

Avian PRRs and Probiotics Among studies investigating the impact of probiotics on poultry PRRs, TLRs are by far the best-characterized. When *Lactobacillus fermentum* and *Saccharomyces cerevisiae* were fed to non-challenged broilers, TLR2 and TLR4 expression was increased in the foregut (Bai et al. 2013). When *L. plantarum* was supplemented in laying hens, TLR4 expression was enhanced in the ileum. However, this improvement in TLR4 expression did not impact fecal shedding of *Salmonella* Enteritidis in challenged birds (Adhikari et al. 2019). Interestingly, although *Enterococcus faecium* strain AL41 did not change intestinal TLR expression in non-challenged birds, ceca TLR4 and TLR21 expression was increased in *E. faecium*-treated birds challenged with *Campylobacter jejuni*, albeit

this was not associated with a reduction in *C. jejuni* (Karaffova et al. 2017). This suggests that some probiotics might require a synergistic bacterial inoculation or inflammatory stimuli to induce changes in TLR expression. This synergism in enhancing TLR expression does necessarily require co-inoculation with a bacterial challenge. However, the probiotic products Lactofeed (*L. acidophilus, L. casei, E. faecium, Bifidobacterium thermophilum*) and Pediguard (*Pediococcus acidilactici*) individually increased TLR2 and TLR4 expression in the foregut, TLR expression was further increased when these probiotics were combined (Aalaei et al. 2019). Overall, these studies demonstrate a consistent increase in TLR4 expression induced by probiotics. This observation is peculiar given that none of these probiotics possess LPS (the ligand for chicken TLR4) as part of their outer envelope. Thus, these probiotics may make intestinal cells more-responsive to Gram-negative commensals in the gut microbiota, though the mechanism of how this would occur in chickens is unclear. Given that intestinal TLR4 expression did not affect the clearance of *C. jejuni* nor *Salmonella* (Adhikari et al. 2019; Karaffova et al. 2017), the immunological benefit, if any, of improving intestinal TLR4 expression in chickens for protection against foodborne disease-causing bacteria is questionable.

Importantly, not all probiotic studies report increases in TLR expression, as *Lactobacillus acidophilus* LA-5 did not change TLR expression in the ceca tonsils in 21-day-old broilers (Asgari et al. 2018). Some probiotics have been reported to decrease intestinal TLR expression, as a multistrain probiotic of *L. acidophilus, L casei, Streptococcus faecium, and Bacillus subtilis* decreased TLR4 expression in the ceca tonsils (Yitbarek et al. 2015). Thus, changes in TLR expression are dependent on the probiotic used. Although most studies use a mixture of probiotic microorganisms, this makes it impossible to determine which microbes may individually or in-combination drive changes in TLR expression. Furthermore, TLR expression varies between chicken lines (Ramasamy et al. 2010; Abasht et al. 2009; Sławińska et al. 2013), meaning that chickens may be more sensitive or resistant to probiotic-based immunological stimulation depending on their genetics. Thus, probiotic composition and chicken breed are crucial factors to consider when studying probiotic-induced TLR expression in poultry.

Probiotics also impact the signaling and expression of non-TLR PRRs in chickens. Similar to TLR expression, probiotics alter soluble PRR levels in chickens. *Bacillus subtilis* supplementation in *Salmonella*-challenged broilers lowered serum haptoglobin, an APP that binds to free hemoglobin to reduce oxidative stress. This observation was associated with reduced tissue severity induced by *Salmonella* (Park and Kim 2015). However, *B. subtilis* did not reduce circulatory haptoglobin levels in non-challenged animals (Park and Kim 2014). Similarly, only in *Salmonella*-challenged birds did *Lactobacillus salivarius* or *Bifidobacterium animalis* reduced circulatory ceruloplasmin (Bielecka et al. 2010), an APP that oxidizes iron into a non-toxic form (Murata et al. 2004). In addition, a multi-species probiotic (*L. plantarum, L. acidophilus, L. bulgaricus, L. rhamnosus, B. bifidum, Streptococcus thermophilus, E. faecium, Aspergillus oryzae, Candida pintolopesii*) reduced C-reactive protein in heat-stressed birds (Sohail et al. 2010). Altogether, these

findings suggest that an inflammatory agent or stressor needs to accompany probiotics to affect circulatory APPs in chickens. Although NOD1 receptor signaling was increased in *Salmonella* Pullorum-challenged birds (Tao et al. 2017), changes in NOD-like receptor activity have not been reported in response to probiotic supplementation. Chicken NOD1 is activated by iE-DAP, a component of the peptidoglycan cell wall (Tao et al. 2017), suggesting there is potential for probiotics to stimulate NOD-like receptor signaling in chickens.

13.3 Metabolite-Based Immune Signaling by Probiotics

The intestinal microbiota uses metabolites synthesized via fiber fermentation or other biochemical pathways to communicate with host immune cells. Although these molecules may have either direct action on other microbes, this section will focus on the effect of these metabolites on host immune pathways.

Short-Chain Fatty Acids The major end products of anaerobic, complex carbohydrate fermentation by the gut microbiota, short-chain fatty acids (SCFAs) exert multiple beneficial effects in the intestine, including improved gut immunological homeostasis, regulation of microbiota composition via pH reduction, improved gut motility, and enhanced feed conversion and productivity (Sunkara et al. 2011; Ricke 2003; Cherbut et al. 1997; Wu et al. 2018). Therefore, probiotics that increase the production of SCFAs (via directly or by modulating the resident microbiota) are desirable for poultry production. The three major straight-chain SCFAs differ only by the length in their carbon backbone: acetate (two carbons), propionate (three carbons), and butyrate (four carbons) (Cummings and Macfarlane 1997). These bacterial SCFAs traverse the gut epithelium to signal underlying immune cell populations via enterocyte monocarboxylate transporters, although butyrate is used by epithelial cells to maintain barrier homeostasis via increased tight junction protein abundance (Melhem et al. 2019; Yan and Ajuwon 2017). SCFAs can interact with immune cell populations via metabolite-sensing G-protein coupled receptors (GPCRs) on various host cells. Ligand binding to the GPCR causes the G protein α subunit to dissociate and trigger downstream intracellular responses (Melhem et al. 2019). GPR43, a GPCR responsive to acetate, was highly expressed in $CD4^+CD8^-CD25^+$ regulatory T cells and its activation was shown to be crucial for Treg expansion in chickens (Lee et al. 2018). SCFAs are also involved in the regulation of lipid and glucose metabolisms via binding to the GPCRs GPR41 and GPR43 (Zhang et al. 2019). Nutrient metabolism plays a significant role in immunological outcomes in the gut, as *Salmonella* elevates oxidative phosphorylation and fatty acid catabolism to persist in the intestine (Arsenault et al. 2013). Additionally, butyrate inhibits nitric oxide production by LPS-stimulated chicken macrophage cells, reducing the expression of cytokines, such as IL-1β, IL-6, IFN-γ, and IL-10 (Zhou et al. 2014). Acetate, propionate, butyrate, and other SCFAs have also been reported to increase antimicrobial gene expression. Thus, SCFAs are crucial for regulating the immunological status of the chicken intestine. Although SCFAs have

been reported to affect dendritic cell maturation, IgA secretion, and mucus production in the mammalian intestine (Melhem et al. 2019), these studies are lacking in poultry.

Several probiotics can increase SCFA production, either through direct SCFA synthesis or through precursor molecules. For example, *Bifidobacterium* and *Streptococcus* species can produce acetate, whereas some *Lactobacillus* species can produce acetate, propionate, and/or butyrate (Markowiak-Kopeć and Śliżewska 2020). Additionally, many *Lactobacillus, Lactococcus,* and *Pediococcus* species produce lactic acid, an organic acid that can be converted to butyrate or other SCFAs by other gut commensals (Flint et al. 2015). Several studies have been done in chickens describing probiotic-mediated changes in intestinal SCFA production. For example, synbiotic preparations containing several *Lactobacillus* species (e.g., *L. plantarum, L. reuteri, L. pentosus, L. rhamnosus, L. paracasei*) and *S. cerevisiae* increased lactic acid SCFAs by affecting the composition of the microbiota, which led to protection against pathogenic bacteria, e.g. *Clostridium* species and *Escherichia coli* (Śliżewska et al. 2020). Additionally, *Bacillus* species were shown to increase acetate production in the chicken ceca, whereas treatment with *Pediococcus pentosaceus* elevated levels of SCFAs propionate and butyrate. These SCFA increases were positively correlated between the abundance of *Bacteroidetes* (i.e., propionate and butyrate) and *Firmicutes* (i.e., acetate) (Wang et al. 2017). Thus, probiotics can be used to improve SCFA production in the gut, although which SCFAs are affected is species-specific. To our knowledge, no studies have studied probiotic-induced effects on the chicken immune system via SCFA signals. Given that butyrate has been shown to upregulate TLRs like TLR4 in mammalian intestinal cells (Xiao et al. 2018), this is a feasible possibility for poultry researchers to consider when elucidating mechanisms for probiotic-stimulated immunity.

Neurochemicals A recently emerging field in poultry research, microbial endocrinology, or the ability of microbes to secrete and respond to neurochemicals, has major implications on mammalian and avian health (Lyte 2016; Villageliu and Lyte 2017). Interestingly, many probiotics like *Lactobacillus* directly synthesize neurochemicals like GABA, making probiotics a direct vehicle for neurochemical delivery in the gut (Lyte 2011). This is an important characteristic to consider when discussing host immune responses, as the neuroimmunological axis is a crucial component of mammalian responses to infectious pathogens (Nutma et al. 2019). Mammalian lymphocytes and antigen-presenting cells are reported to express neurochemical receptors (Kerage et al. 2019). This has been consistent with chicken immune cells, as chicken lymphocytes also express beta-adrenergic receptors, which respond to catecholamines like dopamine, epinephrine, and norepinephrine (Motobu et al. 2003). Chicken macrophages also likely possess these receptors, as dopamine, epinephrine, and norepinephrine all increased *E. coli* phagocytosis and Fc-receptor expression (Ali et al. 1994). Currently, only one study has evaluated the effect of probiotics on neurochemical production in chickens (Redweik et al. 2019). In this study, norepinephrine production was significantly correlated with

Enterobacteriaceae abundances in the ceca, both of which were highest in the group treated solely with a probiotic mix, which supports previous studies finding *E. coli* to avidly generate catecholamine availability in the gut (Asano et al. 2012). Furthermore, IgA production against *E. coli* antigens was increased in the probiotic group only (Redweik et al. 2019). It is unclear whether increased norepinephrine improved intestinal IgA production against *E. coli* or whether norepinephrine levels were inconsequential in the immune response.

13.4 Probiotics and Extraintestinal Immunity

Although probiotics are delivered orally, they and other gut microbes instruct immune function outside of the intestinal tract. Whether it is through direct translocation of bacteria and their products into circulation (Belkaid and Hand 2014) as well as mobilization of leukocyte populations from the intestine to other sites like the lung (Enaud et al. 2020), immune activation in the gut impacts much more than local responses. Contrary to mammals that rely on the mesenteric lymphatic system to transport bacteria, TLR agonists, and metabolites between the gut and lung mucosa (Trompette et al. 2014; Bingula et al. 2017; McAleer et al. 2016), chickens have a much less sophisticated lymphatic system, lacking encapsulated lymph nodes. This deficiency is compensated with diffuse lymphoid tissues (Nochi et al. 2018). However, it is likely this physiological difference in lymphoid structure may impact the efficiency for bacteria, antigen, metabolites, and host immune cells to systemically circulate and induce extraintestinal immune responses. Regardless, gut microbes and probiotics have been well-characterized in impacting immune responses outside the intestine in chickens.

This chapter has already discussed the effect probiotics can have on soluble PRRs in circulation (Park and Kim 2014, 2015; Bielecka et al. 2010; Sohail et al. 2010). However, many probiotics required an accompanying stimulus, such as bacterial inflammation or stressor, for differences to be observed, suggesting these outcomes are indirectly related to probiotic treatments. Broilers fed *Lactobacillus plantarum* with fructooligosaccharides exhibited improved serum antibody levels and extraintestinal resistance to avian pathogenic *Escherichia coli* (APEC), a major cause of colibacillosis and mortality in chickens (Ding et al. 2019). Similarly, vaccine responses against APEC infection were greatly improved by the addition of dietary probiotics (Redweik et al. 2020a, b). Furthermore, administration of Nissle 1917, a human *E. coli* probiotic, in birds reduced mortality in responses to APEC challenge in vivo (Huff et al. 2006). Using genetic engineering, *Lactococcus lactis* expressing influenza neuraminidase induced mucosal (intestinal and respiratory tracts) and circulatory antibody production as well as conferred complete, influenza protection in treated birds (Lei et al. 2015). Altogether, these findings suggest that probiotics are a feasible means of improving extraintestinal immunity to pathogenic microbes.

13.5 Conclusion

Altogether, it is clear that gut microbes are major drivers of the chicken immune system, both local and extraintestinal, and probiotics can be used to calibrate host immunity. However, many of the mechanisms underlying these immunoregulatory functions of probiotics are unclear, as these benefits could arise from indirect modulation of the gut microbiota. Furthermore, genetic differences in PRR distribution between avian species (i.e., turkeys versus chickens), breed (i.e., broilers versus layers), and strain (i.e., fast- versus slow-growing) likely impact how effective certain probiotics are in birds. In addition, strain-specific differences between probiotic species also play a major role in the induction of the immune response (McFarland et al. 2018). Future research in these areas are crucial, as immunological factors should be prioritized when selecting for probiotic candidates for commercial poultry.

References

Aalaei M, Khatibjoo A, Zaghari M, Taherpou K, Akbari-Gharaei M, Soltani M (2019) Effect of single- and multi-strain probiotics on broiler breeder performance, immunity and intestinal toll-like receptors expression. J Appl Anim Res 47:236–242. https://doi.org/10.1080/09712119.2019.1618311

Abasht B, Kaiser MG, van der Poel J, Lamont SJ (2009) Genetic lines differ in Toll-like receptor gene expression in spleens of chicks inoculated with *Salmonella enterica* serovar Enteritidis. Poult Sci 88:744–749. https://doi.org/10.3382/ps.2008-00419

Adhikari P, Lee CH, Cosby DE, Cox NA, Kim WK (2019) Effect of probiotics on fecal excretion, colonization in internal organs and immune gene expression in the ileum of laying hens challenged with *Salmonella* Enteritidis. Poult Sci 98:1235–1242. https://doi.org/10.3382/ps/pey443

Alcaide M, Edwards SV (2011) Molecular evolution of the toll-like receptor multigene family in birds. Mol Biol Evol 28:1703–1715. https://doi.org/10.1093/molbev/msq351

Ali RA, Qureshi MA, McCorkle FM (1994) Profile of chicken macrophage functions after exposure to catecholamines in vitro. Immunopharmacol Immunotoxicol 16(4):611–625. https://doi.org/10.3109/08923979409019742

Arsenault RJ, Napper S, Kogut MH (2013) *Salmonella enterica* serotype typhimurium infection causes metabolic changes in chicken muscle involving AMPK, fatty acid and insulin/mTOR signaling. Vet Res 44:35–50. https://doi.org/10.1186/1297-9716-44-35

Asano Y, Hiramoto T, Nishino R, Aiba Y, Kimura T, Yoshihara K, Koga Y, Sudo N (2012) Critical role of gut microbiota in the production of biologically active, free catecholamines in the gut lumen of mice. Am J Physiol Gastrointest Liver Physiol 303:G1288–G1295. https://doi.org/10.1152/ajpgi.00341.2012

Asgari F, Falak R, Teimourian S, Pourakbari B, Ebrahimnezhad S, Shekarabi M (2018) Effects of oral probiotic feeding on toll-like receptor gene expression of the Chicken's Cecal Tonsil. Rep Biochem Mol Biol 6:151–157

Bai SP, Wu AM, Ding XM, Lei Y, Bai J, Zhang KY, Chio JS (2013) Effects of probiotic-supplemented diets on growth performance and intestinal immune characteristics of broiler chickens. Poult Sci 92:663–670. https://doi.org/10.3382/ps.2012-02813

Bauer S, Kirschning CJ, Häcker H, Redecke V, Hausmann S, Akira S, Wagner H, Lipford GB (2001) Human TLR9 confers responsiveness to bacterial DNA via species-specific CpG motif recognition. Proc Natl Acad Sci U S A 98:9237–9242. https://doi.org/10.1073/pnas.161293498

Belkaid Y, Hand TW (2014) Role of the microbiota in immunity and inflammation. Cell 157 (1):121–141. https://doi.org/10.1016/j.cell.2014.03.011

Berghman LR (2016) Immune responses to improving welfare. Poult Sci 95:2216–2218. https://doi.org/10.3382/ps/pew159

Bielecka M, Smoragiewicz W, Siwicki AK, Wójcik R, Biedrzycka E, Orłowski A, Kask S, Jankowski J, Karska-Wysocki B, Ham D (2010) The effect of various probiotic strains or Avilamycin feed additive on immune defense markers and acute-phase response to *salmonella* infection in chickens. Probiotics Antimicrob Proteins 2:175–185. https://doi.org/10.1007/s12602-010-9054-3

Bingula R, Filaire M, Radosevic-Robin N, Bey M, Berthon JY, Bernalier-Donadille A, Vasson MP, Filaire E (2017) Desired turbulence? Gut-lung axis, immunity, and lung cancer. J Oncol 2017: 5035371. https://doi.org/10.1155/2017/5035371

Bouladoux N, Hall JA, Grainger JR, dos Santos LM, Kann MG, Nagarajan V, Verthelyi D, Belkaid Y (2012) Regulatory role of suppressive motifs from commensal DNA. Mucosal Immunol 5: 623–634. https://doi.org/10.1038/mi.2012.36

Broom LJ (2019) Host-microbe interactions and gut health in poultry-focus on innate responses. Microorganisms 7. https://doi.org/10.3390/microorganisms7050139

Broom LJ, Kogut MH (2018) The role of the gut microbiome in shaping the immune system of chickens. Vet Immunol Immunopathol 204:44–51. https://doi.org/10.1016/j.vetimm.2018.10.002

Brownlie R, Allan B (2011) Avian toll-like receptors. Cell Tissue Res 343:121–130. https://doi.org/10.1007/s00441-010-1026-0

Carpenter S, O'Neill LA (2009) Recent insights into the structure of Toll-like receptors and post-translational modifications of their associated signalling proteins. Biochem J 422:1–10. https://doi.org/10.1042/BJ20090616

Chen S, Cheng A, Wang M (2013) Innate sensing of viruses by pattern recognition receptors in birds. Vet Res 44:82. https://doi.org/10.1186/1297-9716-44-82

Cherbut C, Aubé AC, Blottière HM, Galmiche JP (1997) Effects of short-chain fatty acids on gastrointestinal motility. Scandinavian J Gastroenterol 32:58–61. https://doi.org/10.1080/00365521.1997.11720720

Cummings JH, Macfarlane GT (1997) Role of intestinal bacteria in nutrient metabolism. J Parenter Enter Nutr 21:357–365. https://doi.org/10.1177/0148607197021006357

de Kivit S, van Hoffen E, Korthagen N, Garssen J, Willemsen LE (2011) Apical TLR ligation of intestinal epithelial cells drives a Th1-polarized regulatory or inflammatory type effector response in vitro. Immunobiology 216:518–527. https://doi.org/10.1016/j.imbio.2010.08.005

de Zoete MR, Bouwman LI, Keestra AM, van Putten JP (2011) Cleavage and activation of a Toll-like receptor by microbial proteases. Proc Natl Acad Sci U S A 108:4968–4973. https://doi.org/10.1073/pnas.1018135108

Dibner JJ, Richards JD (2005) Antibiotic growth promoters in agriculture: history and mode of action. Poult Sci 84:634–643. https://doi.org/10.1093/ps/84.4.634

Ding S, Wang Y, Yan W, Li A, Jiang H, Fang J (2019) Effects of *Lactobacillus plantarum* 15-1 and fructooligosaccharides on the response of broilers to pathogenic *Escherichia coli* O78 challenge. PLoS One 14:e0212079. https://doi.org/10.1371/journal.pone.0212079

Enaud R, Prevel R, Ciarlo E, Beaufils F, Wieërs G, Guery B, Delhaes L (2020) The gut-lung axis in health and respiratory diseases: a place for inter-organ and inter-kingdom crosstalks. Front Cell Infect Microbiol 10:9. https://doi.org/10.3389/fcimb.2020.00009

Flint HJ, Duncan SH, Scott KP, Louis P (2015) Links between diet, gut microbiota composition and gut metabolism. Proc Nutr Soc 74:13–22. https://doi.org/10.1017/S0029665114001463

Higgs R, Cormican P, Cahalane S, Allan B, Lloyd AT, Meade K, James T, Lynn DJ, Babiuk LA, O'farrelly C (2006) Induction of a novel chicken Toll-like receptor following *Salmonella enterica* serovar typhimurium infection. Infect Immun 74:1692–1698. https://doi.org/10.1128/IAI.74.3.1692-1698.2006

Hill C, Guarner F, Reid G, Gibson GR, Merenstein DJ, Pot B, Morelli L, Canani RB, Flint HJ, Salminen S, Calder PC, Sanders ME (2014) Expert consensus document. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nat Rev Gastroenterol Hepatol 11(8):506–514. https://doi.org/10.1038/nrgastro.2014.66

Huff GR, Huff WE, Enders C, Sonnenborn U, Rath NC, Farnell MB, Solis de los Santos F., & Donoghue A.M. (2006) Oral treatment with the probiotic *Escherichia coli* Nissle 1917 improves body weight and modulates the stress response of poultry in respiratory challenges with avian pathogenic *E. coli*. In: Proceeding of the XII European poultry conference, September. Verona, Italy, pp 10–14

Iliev I, Matteoli G, Rescigno M (2007) The yin and yang of intestinal epithelial cells in controlling dendritic cell function. J Exp Med 204:2253–2257. https://doi.org/10.1084/jem.20062535

Iqbal M, Philbin VJ, Withanage GS, Wigley P, Beal RK, Goodchild MJ, Barrow P, McConnell I, Maskell DJ, Young J, Bumstead N, Boyd Y, Smith AL (2005) Identification and functional characterization of chicken toll-like receptor 5 reveals a fundamental role in the biology of infection with *Salmonella enterica* serovar typhimurium. Infect Immun 73:2344–2350. https://doi.org/10.1128/IAI.73.4.2344-2350.2005

Juul-Madsen HR, Viertlböeck B, Härtle S, Smith AL, Göbel TW (2014) Chapter 7—Innate immune responses. In: Schat KA, Kaspers B, Kaiser P (eds) Avian immunology, 2nd edn. Academic Press, Boston, MA, pp 121–147

Karaffova V, Marcinkova E, Bobikova K, Herich R, Revajova V, Stasova D, Kavul'ova A et al (2017) TLR4 and TLR21 expression, MIF, IFN-β, MD-2, CD14 activation, and sIgA production in chickens administered with EFAL41 strain challenged with *Campylobacter jejuni*. Folia Microbiol (Praha) 62:89–97. https://doi.org/10.1007/s12223-016-0475-6

Kawai T, Akira S (2007) TLR signaling. Semin Immunol 19:24–32. https://doi.org/10.1016/j.smim.2006.12.004

Keestra AM, de Zoete MR, van Aubel RA, van Putten JP (2007) The central leucine-rich repeat region of chicken TLR16 dictates unique ligand specificity and species-specific interaction with TLR2. J Immunol 178:7110–7119. https://doi.org/10.4049/jimmunol.178.11.7110

Keestra AM, de Zoete MR, van Aubel RA, van Putten JP (2008) Functional characterization of chicken TLR5 reveals species-specific recognition of flagellin. Mol Immunol 45:1298–1307. https://doi.org/10.1016/j.molimm.2007.09.013

Keestra AM, de Zoete MR, Bouwman LI, van Putten JP (2010) Chicken TLR21 is an innate CpG DNA receptor distinct from mammalian TLR9. J Immunol 185:460–467. https://doi.org/10.4049/jimmunol.0901921

Keestra AM, de Zoete MR, Bouwman LI, Vaezirad MM, van Putten JP (2013) Unique features of chicken Toll-like receptors. Dev Comp Immunol 41:316–323. https://doi.org/10.1016/j.dci.2013.04.009

Kerage D, Sloan EK, Mattarollo SR, McCombe PA (2019) Interaction of neurotransmitters and neurochemicals with lymphocytes. J Neuroimmunol 332:99–111. https://doi.org/10.1016/j.jneuroim.2019.04.006

Khan R, Naz S (2013) The applications of probiotics in poultry production. Worlds Poult Sci J 69 (3):621–631. https://doi.org/10.1017/S0043933913000627

Klasing KC (2007) Nutrition and the immune system. Br Poult Sci 48:525–537. https://doi.org/10.1080/00071660701671336

Kogut MH, Swaggerty CL, Byrd JA, Selvaraj R, Arsenault RJ (2016) Chicken-specific Kinome array reveals that *Salmonella enterica* Serovar Enteritidis modulates host immune signaling pathways in the cecum to establish a persistence infection. Int J Mol Sci 17(8). https://doi.org/10.3390/ijms17081207

Lee J, Mo JH, Katakura K, Alkalay I, Rucker AN, Liu YT, Lee HK, Shen C, Cojocaru G, Shenouda S, Kagnoff M, Eckmann L, Ben-Neriah Y, Raz E (2006) Maintenance of colonic homeostasis by distinctive apical TLR9 signalling in intestinal epithelial cells. Nat Cell Biol 8:1327–1336. https://doi.org/10.1038/ncb1500

Lee IK, Gu MJ, Ko KH, Bae S, Kim G, Jin GD, Kim EB, Kong YY, Park TS, Park BC, Jung HJ, Han SH, Yun CH (2018) Regulation of $CD4^+CD8^-CD25^+$ and $CD4^+CD8^+CD25^+$ T cells by gut microbiota in chicken. Sci Rep 8(1):8627. https://doi.org/10.1038/s41598-018-26763-0

Lei H, Peng X, Ouyang J, Zhao D, Jiao H, Shu H, Ge X (2015) Protective immunity against influenza H5N1 virus challenge in chickens by oral administration of recombinant *Lactococcus lactis* expressing neuraminidase. BMC Vet Res 11:85. https://doi.org/10.1186/s12917-015-0399-4

Liao SF, Nyachoti M (2017) Using probiotics to improve swine gut health and nutrient utilization. Anim Nutr 3:331–343. https://doi.org/10.1016/j.aninu.2017.06.007

Lutful Kabir SM (2009) The role of probiotics in the poultry industry. Int J Mol Sci 10:3531–3546. https://doi.org/10.3390/ijms10083531

Lynn D.J., Lloyd A.T., O'Farrelly C. (2003) *In silico* identification of components of the Toll-like receptor (TLR) signaling pathway in clustered chicken expressed sequence tags (ESTs). Vet Immunol Immunopathol 93:177–184. https://doi.org/10.1016/s0165-2427(03)00058-8

Lyte M (2011) Probiotics function mechanistically as delivery vehicles for neuroactive compounds: microbial endocrinology in the design and use of probiotics. BioEssays 33:574–581. https://doi.org/10.1002/bies.201100024

Lyte M (2016) Microbial endocrinology: an ongoing personal journey. Adv Exp Med Biol 874:1–24. https://doi.org/10.1007/978-3-319-20215-0_1

Markowiak-Kopeć P, Śliżewska K (2020) The effect of probiotics on the production of short-chain fatty acids by human intestinal microbiome. Nutrients 12. https://doi.org/10.3390/nu12041107

Marques AT, Nordio L, Lecchi C, Grilli G, Giudice C, Ceciliani F (2017) Widespread extrahepatic expression of acute-phase proteins in healthy chicken (*Gallus gallus*) tissues. Vet Immunol Immunopathol 190:10–17. https://doi.org/10.1016/j.vetimm.2017.06.006

McAleer JP, Nguyen NL, Chen K, Kumar P, Ricks DM, Binnie M, Armentrout RA, Pociask DA, Hein A, Yu A, Vikram A, Bibby K, Umesaki Y, Rivera A, Sheppard D, Ouyang W, Hooper LV, Kolls JK (2016) Pulmonary Th17 antifungal immunity is regulated by the gut microbiome. J Immunol 197:97–107. https://doi.org/10.4049/jimmunol.1502566

McFarland LV, Evans CT, Goldstein E (2018) Strain-specificity and disease-specificity of probiotic efficacy: a systematic review and meta-analysis. Front Med 5. https://doi.org/10.3389/fmed.2018.00124

Melhem H, Kaya B, Ayata CK, Hruz P, Niess JH (2019) Metabolite-sensing G protein-coupled receptors connect the diet-microbiota-metabolites axis to inflammatory Bowel disease. Cells:8. https://doi.org/10.3390/cells8050450

Motobu M, El-Abasy M, Na KJ, Vainio O, Toivanen P, Hirota Y (2003) Effects of 6-hydroxydopamine on the development of the immune system in chickens. J Vet Med Sci 65:35–42. https://doi.org/10.1292/jvms.65.35

Murata H, Shimada N, Yoshioka M (2004) Current research on acute phase proteins in veterinary diagnosis: an overview. Vet J 168:28–40. https://doi.org/10.1016/S1090-0233(03)00119-9

Nerren JR, He H, Genovese K, Kogut MH (2010) Expression of the avian-specific toll-like receptor 15 in chicken heterophils is mediated by gram-negative and gram-positive bacteria, but not TLR agonists. Vet Immunol Immunopathol 136:151–156. https://doi.org/10.1016/j.vetimm.2010.02.017

Nochi T, Jansen CA, Toyomizu M, van Eden W (2018) The well-developed mucosal immune systems of birds and mammals allow for similar approaches of mucosal vaccination in both types of animals. Front Nutr 5:60. https://doi.org/10.3389/fnut.2018.00060

Nutma E, Willison H, Martino G, Amor S (2019) Neuroimmunology - the past, present and future. Clin Exp Immunol 197:278–293. https://doi.org/10.1111/cei.13279

Park JH, Kim IH (2014) Supplemental effect of probiotic *Bacillus subtilis* B2A on productivity, organ weight, intestinal *Salmonella* microflora, and breast meat quality of growing broiler chicks. Poult Sci 93:2054–2059. https://doi.org/10.3382/ps.2013-03818

Park JH, Kim IH (2015) The effects of the supplementation of *Bacillus subtilis* RX7 and B2A strains on the performance, blood profiles, intestinal Salmonella concentration, noxious gas

emission, organ weight and breast meat quality of broiler challenged with *salmonella typhimurium*. J Anim Physiol Anim Nutr (Berl) 99:326–334. https://doi.org/10.1111/jpn.12248
Patterson JA, Burkholder KM (2003) Application of prebiotics and probiotics in poultry production. Poult Sci 82:627–631. https://doi.org/10.1093/ps/82.4.627
Pétrilli V, Dostert C, Muruve DA, Tschopp J (2007) The inflammasome: a danger sensing complex triggering innate immunity. Curr Opin Immunol 19:615–622. https://doi.org/10.1016/j.coi.2007.09.002
Ramasamy KT, Reddy MR, Raveendranathan DN, Murugesan S, Chatterjee RN, Ullengala R, Haunshi S (2010) Differential expression of Toll-like receptor mRNA in White Leghorn and indigenous chicken of India. Vet Res Commun 34:633–639. https://doi.org/10.1007/s11259-010-9431-0
Ramasamy KT, Reddy MR, Verma PC, Murugesan S (2012) Expression analysis of Turkey (Meleagris gallopavo) toll-like receptors and molecular characterization of avian specific TLR15. Mol Biol Rep 39:8539–8549. https://doi.org/10.1007/s11033-012-1709-6
Redweik GAJ, Daniels K, Severin AJ, Lyte M, Mellata M (2019) Oral treatments with probiotics and live *Salmonella* vaccine induce unique changes in gut neurochemicals and microbiome in chickens. Front Microbiol 10:3064. https://doi.org/10.3389/fmicb.2019.03064
Redweik GAJ, Stromberg ZR, Van Goor A, Mellata M (2020a) Protection against avian pathogenic *Escherichia coli* and *Salmonella* Kentucky exhibited in chickens given both probiotics and live *Salmonella* vaccine. Poult Sci 99:752–762. https://doi.org/10.1016/j.psj.2019.10.038
Redweik GAJ, Jochum J, Mellata M (2020b) Live bacterial prophylactics in modern poultry. Front Vet Sci 28. https://doi.org/10.3389/fvets.2020.592312
Ricke SC (2003) Perspectives on the use of organic acids and short chain fatty acids as antimicrobials. Poult Sci 82:632–639. https://doi.org/10.1093/ps/82.4.632
Roach JC, Glusman G, Rowen L, Kaur A, Purcell MK, Smith KD, Hood LE, Aderem A (2005) The evolution of vertebrate toll-like receptors. Proc Natl Acad Sci U S A 102:9577–9582. https://doi.org/10.1073/pnas.0502272102
Shaughnessy RG, Meade KG, Cahalane S, Allan B, Reiman C, Callanan JJ, O'Farrelly C (2009) Innate immune gene expression differentiates the early avian intestinal response between *Salmonella* and *Campylobacter*. Vet Immunol Immunopathol 132:191–198. https://doi.org/10.1016/j.vetimm.2009.06.007
Sławińska A, D'Andrea M, Pilla F, Bednarczyk M, Siwek M (2013) Expression profiles of toll-like receptors 1, 2 and 5 in selected organs of commercial and indigenous chickens. J Appl Genet 54:489–492. https://doi.org/10.1007/s13353-013-0161-1
Śliżewska K, Markowiak-Kopeć P, Żbikowski A, Szeleszczuk P (2020) The effect of synbiotic preparations on the intestinal microbiota and her metabolism in broiler chickens. Sci Rep 10:4281. https://doi.org/10.1038/s41598-020-61256-z
Sohail MU, Ijaz A, Yousaf MS, Ashraf K, Zaneb H, Aleem M, Rehman H (2010) Alleviation of cyclic heat stress in broilers by dietary supplementation of mannan-oligosaccharide and *Lactobacillus*-based probiotic: dynamics of cortisol, thyroid hormones, cholesterol, C-reactive protein, and humoral immunity. Poult Sci 89:1934–1938. https://doi.org/10.3382/ps.2010-00751
Stanley D, Hughes RJ, Moore RJ (2014) Microbiota of the chicken gastrointestinal tract: influence on health, productivity and disease. Appl Microbiol Biotechnol 98:4301–4310. https://doi.org/10.1007/s00253-014-5646-2
Stein T (2005) *Bacillus subtilis* antibiotics: structures, syntheses and specific functions. Mol Microbiol 56:845–857. https://doi.org/10.1111/j.1365-2958.2005.04587.x
Sunkara LT, Achanta M, Schreiber NB, Bommineni YR, Dai G, Jiang W, Lamont S, Lillehoj HS, Beker A, Teeter RG, Zhang G (2011) Butyrate enhances disease resistance of chickens by inducing antimicrobial host defense peptide gene expression. PLoS One 6:e27225. https://doi.org/10.1371/journal.pone.0027225
Tao Z, Zhu C, Song W, Xu W, Zhang S, Liu H, Li H (2017) Inductive expression of the NOD1 signalling pathway in chickens infected with *Salmonella pullorum*. Br Poult Sci 58:242–250. https://doi.org/10.1080/00071668.2017.1280771

Tarradas J, Tous N, Esteve-Garcia E, Brufau AJ (2020) The control of intestinal inflammation: a major objective in the research of probiotic strains as alternatives to antibiotic growth promoters in poultry. Microorganisms 8. https://doi.org/10.3390/microorganisms8020148

Trompette A, Gollwitzer ES, Yadava K, Sichelstiel AK, Sprenger N, Ngom-Bru C, Blanchard C, Junt T, Nicod LP, Harris NL, Marsland BJ (2014) Gut microbiota metabolism of dietary fiber influences allergic airway disease and hematopoiesis. Nat Med 20:159–166. https://doi.org/10.1038/nm.3444

Velová H, Gutowska-Ding MW, Burt DW, Vinkler M (2018) Toll-like receptor evolution in birds: gene duplication, pseudogenization, and diversifying selection. Mol Biol Evol 35:2170–2184. https://doi.org/10.1093/molbev/msy119

Vieco-Saiz N, Belguesmia Y, Raspoet R, Auclair E, Gancel F, Kempf I, Drider D (2019) Benefits and inputs from lactic acid bacteria and their bacteriocins as alternatives to antibiotic growth promoters during food-animal production. Front Microbiol 10:57. https://doi.org/10.3389/fmicb.2019.00057

Villageliu DN, Lyte M (2017) Microbial endocrinology: why the intersection of microbiology and neurobiology matters to poultry health. Poult Sci 96:2501–2508. https://doi.org/10.3382/ps/pex148

Wang Y, Sun J, Zhong H, Li N, Xu H, Zhu Q, Liu Y (2017) Effect of probiotics on the meat flavour and gut microbiota of chicken. Sci Rep 7:6400. https://doi.org/10.1038/s41598-017-06677-z

Wigley P (2014) *Salmonella enterica* in the chicken: how it has helped our understanding of immunology in a non-biomedical model species. Front Immunol 5:482. https://doi.org/10.3389/fimmu.2014.00482

Wu W, Xiao Z, An W, Dong Y, Zhang B (2018) Dietary sodium butyrate improves intestinal development and function by modulating the microbial community in broilers. PLoS One 13: e0197762. https://doi.org/10.1371/journal.pone.0197762

Xiao T, Wu S, Yan C, Zhao C, Jin H, Yan N, Xu J, Wu Y, Li C, Shao Q, Xia S (2018) Butyrate upregulates the TLR4 expression and the phosphorylation of MAPKs and NK-κB in colon cancer cell in vitro. Oncol Lett 16(4):4439–4447. https://doi.org/10.3892/ol.2018.9201

Yadav S, Jha R (2019) Strategies to modulate the intestinal microbiota and their effects on nutrient utilization, performance, and health of poultry. J Anim Sci Biotechnol 10:2. https://doi.org/10.1186/s40104-018-0310-9

Yan H, Ajuwon KM (2017) Butyrate modifies intestinal barrier function in IPEC-J2 cells through a selective upregulation of tight junction proteins and activation of the Akt signaling pathway. PLoS One 12:e0179586. https://doi.org/10.1371/journal.pone.0179586

Yitbarek A, Echeverry H, Munyaka P, Rodriguez-Lecompte JC (2015) Innate immune response of pullets fed diets supplemented with prebiotics and synbiotics. Poult Sci 94:1802–1811. https://doi.org/10.3382/ps/pev147

Zhang JM, Sun YS, Zhao LQ, Chen TT, Fan MN, Jiao HC, Zhao JP, Wang XJ, Li FC, Li HF, Lin H (2019) SCFAs-induced GLP-1 secretion links the regulation of gut microbiome on hepatic lipogenesis in chickens. Front Microbiol 10:2176. https://doi.org/10.3389/fmicb.2019.02176

Zhou ZY, Packialakshmi B, Makkar SK, Dridi S, Rath NC (2014) Effect of butyrate on immune response of a chicken macrophage cell line. Vet Immunol Immunopathol 162(1–2):24–32. https://doi.org/10.1016/j.vetimm.2014.09.002

Pre- and Probiotic Effects on Innate Immunity and Metabolism in Cattle and Swine

14

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Abstract

Various nutritional and non-nutrient products can be used to improve animal health and alter the host microbiota. Prebiotics and probiotics can be used to aid in the development of a healthy microbiota through seeding of the GI tract with beneficial bacteria, as well as providing nutrients directly to these beneficial bacteria to maintain a healthy symbiotic relationship with the host. While conferring benefits to the host through reductions in pathogenic bacteria, and improvements in dry matter intake and intestinal integrity, other benefits outside of the gut have been elucidated. These include benefits to the immune system and, more recently, changes in metabolism in livestock supplemented with pre- and/or probiotic products. It is estimated that approximately 70% of the immune system is associated with the GI tract; yet, changes in the immune system by pre- and probiotics have not been observed systemically. Additionally, metabolic changes

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associated with supplementation of pre- and probiotics suggest that there may be an alteration or shift in energy utilization when cattle and swine are fed these supplements. This chapter explores the role of pre- and probiotics in livestock production, focusing on the mode of action as well as the influence on the innate immune response and changes in metabolism in cattle and swine.

Keywords

Cattle · Innate immunity · Livestock · Metabolism · Prebiotics · Probiotics

14.1 Introduction

The gut is a complex system, consisting of tissues and organs tasked with digesting and absorbing nutrients from consumed feedstuffs and removing harmful waste products. In addition, the network of microbiota living within the gastrointestinal (GI) tract helps not only in digestion of feedstuffs such as indigestible fibers, but also aids in maintaining physiological homeostasis through regulation of the hormone milieu. The microbiota compete for limited resources within the GI tract and react quickly to changes in the GI environment stemming from dietary changes and/or stressors which may ultimately lead to microbial imbalances (Callaway et al. 2008). Often overlooked is the presence of the immune system within the GI tract. In fact, it is estimated that 70% of the body's immune system is associated with the GI tract (Isolauri et al. 2001; Vighi et al. 2008). Thus, activation of the immune system associated with the GI tract, whether due to clinical or subclinical infection, is associated with activating a vast portion of the immune system as well as an increased energetic demand by the activated immune system.

When the immune system is activated during a clinical infection, there is a substantial increase in the energetic demand associated with providing sufficient amounts of energy for the immune system to defend against invading pathogens (Kvidera et al. 2016; Huntley et al. 2017; Humphrey and Klasing 2004). However, what is less frequently recognized is the energetic demands associated with subclinical infections. During subclinical infections, which are often associated with persistent inflammation, the immune system continually pulls energy away from other bodily systems. Thus, keeping immune system activation and inflammation at a minimum greatly benefits the animal as a whole as more energy can be directed toward production, health, and overall well-being.

However, genetic selection over the past several decades has focused on rapid lean tissue accretion, increased efficiency, and enhanced production parameters such as milk, eggs, and progeny with little focus on genetic selection for disease resistance. Thus, there has been a gradual depression of immunological resistance such that livestock are much more susceptible to immune challenges than 25 years ago (van der Most et al. 2010; Colditz and Hine 2016; Rauw 2012). In the past,

antibiotics have been used to prevent infection and improve performance. However, there has been increased scrutiny, both social and legislative, on the use of antibiotics in food-producing animals. Therefore, producers are in need of different management practices, feedstuffs, and other technologies that help support the immune system of their livestock while maintaining overall productivity.

One way to improve livestock health is to establish and maintain a healthy symbiotic relationship between the GI tract and the microbiota. This may be accomplished through supplementation of beneficial gut microorganisms as well as substances that support the maintenance and growth of these microorganisms. These supplements, termed pre- and probiotics, respectively, come in many different forms and have been demonstrated to benefit the host through modulation of immune function, both in the gut and systemically, and more recently through altering metabolism. This chapter covers an overview of pre- and probiotics and their suggested mode of action and further discusses the role of these products on the innate immune response and metabolism with a focus on cattle and swine.

14.2 Pre- and Probiotics: A Primer

By definition, probiotics are live microorganisms that are fed to enhance the health of an organism when supplied in a great enough quantity (Uyeno et al. 2015; Fuller 1989). These live microorganisms can be bacterial strains, typically gram-positive strains, as well as various yeast strains (Angelakis 2017) (Table 14.1). Examples of bacterial strains that are considered to have a host benefit include *Bifidobacterium*, *Enterococcus*, and *Lactobacillus* (Uyeno et al. 2015). Prebiotics, on the other hand, have been defined as being non-digestible feedstuffs that provide a benefit to the host through their positive effects on selective bacteria within the gut (Uyeno et al. 2015). Typically, these are fibers or starches that are not digested or broken down by the host but are utilized exclusively by specific microorganisms in the gut (Table 14.2). Many prebiotics are found naturally in various cereal grains. However, due to the nature of the commercial livestock production system, access to these grains is often limited. Commercially, pre- and probiotic products are sold in many different forms, including live or dried bacteria and yeast, bacterial spores, and bacterial and yeast cultures or fermentation products. Additionally, products containing both prebiotics and probiotics are available, commonly referred to as synbiotics (Hong et al. 2005). Yeast can be used as both a pre- and probiotic. To be deemed a probiotic, yeast must be live (live yeast or yeast culture). Yeast culture provides yeast as well as micronutrients produced by yeast fermentation that are thought to be beneficial to the animal. However, there are differences in effectiveness of yeast products based on differences in strain and type of yeast used (Thrune et al. 2009). Additionally, yeast fermentation products may also differ in composition, consistency, and effectiveness based on the growth conditions and substrates provided during the growth process, which have the potential to change based on availability and price of certain commodities. However, it's important to note that not all pre- and probiotics confer the same specific benefits within the host. Differential physiological, immunological,

Table 14.1 Probiotic products used in cattle and swine

Product	Species	References
L. acidophilus, L. plantarum	Dairy calves	Al-Saiady (2010)
L. acidophilus	Dairy calves	Fomenky et al. (2018)
L. casei, L. salivarius, P. acidilactici	Dairy calves	Frizzo et al. (2010)
B. subtilis	Dairy calves	Broadway et al. (2020)
		Sun et al. (2010)
S. cerevisiae	Dairy calves	Fomenky et al. (2018)
		Galvao et al. (2005)
		Garcia Diaz et al. (2018)
S. cerevisiae	Dairy cows	AlZahal et al. (2014)
		Pinloche et al. (2013)
		Thrune et al. (2009)
L. plantarum, E. faecium, C. butyricum	Dairy cows	Goto et al. (2016)
B. brevis	Weaned pigs	Che et al. (2016)
P. acidilactici	Weaned pigs	Di Giancamillo et al. (2008)
L. brevis	Weaned pigs	Liu et al. (2015)
B. licheniformis and *S. cerevisiae*	Weaned pigs	Pan et al. (2017)
L. rhamnosus	Weaned pigs	Zhang et al. (2010)
S. cerevisiae	Weaned pigs	Bontempo et al. (2006)
	Sows	Di Giancamillo et al. (2007)

and metabolic changes in the host animal stemming from the diversity in pre- and probiotic products and their mode of action are often observed.

Prebiotics and probiotics often work hand-in-hand together to create a balanced and healthy microbiota. The benefits of feeding probiotics to livestock is lengthy, including improved performance, balancing gut pH, reducing colonization of pathogenic bacteria, and improving immune function. In fact, much of the effects of probiotics in livestock initially focused on the growth-promoting effects, where numerous studies have reported increases in body weight gain and feed efficiency. Additionally, probiotics have been identified as alternatives to in-feed antimicrobials to promote growth (Angelakis 2017). Prebiotics support the beneficial microorganisms in probiotics by providing organic acids, peptides and proteins, and other growth factors that enhance this specific population of microbes. While there is a benefit to providing both pre- and probiotic products to livestock, much of the available literature shows that supplementing with either a prebiotic or probiotic can benefit the host. Additionally, pre- and probiotics can be targeted for specific benefits depending on the stage of production (e.g., nursery piglets, early lactation dairy cow, beef calf feedlot arrival).

Cattle are considered ruminants, yet the young calf is in a pre-ruminant state where the rumen is not fully functional. Probiotics fed during this time period can help to reduce colonization of the lower digestive tract with pathogenic bacteria, while increasing the number of beneficial bacteria, such as *Lactobacillus* species, that typically decrease as the calf ages (Uyeno et al. 2015). Additionally,

Table 14.2 Prebiotic products used in cattle and swine

Product	Species	References
Mannanoligosaccharide	Dairy calves	Garcia Diaz et al. (2018)
Cellulooligosaccharide	Dairy calves	Hasunuma et al. (2011)
L. gasseri and *P. freudenreichii* fermentation product	Dairy calves	Heinrichs et al. (2009)
Inulin, lactulose	Dairy calves	Masanetz et al. (2011)
S. cerevisiae fermentation product	Beef steers	Burdick Sanchez et al. (2020)
S. cerevisiae cell wall	Beef heifers	Burdick Sanchez et al. (2014)
S. cerevisiae fermentation product	Weaned pigs	Burdick Sanchez et al. (2018)
L. acidophilus fermentation product	Weaned pigs	Burdick Sanchez et al. (2019a)
Mannanoligosaccharide	Weaned pigs	Kim et al. (2000)
β-Glucan	Weaned pigs	Li et al. (2006)
Chitooligosaccharide	Weaned pigs	Liu et al. (2008)
Fructooligosaccharide, transgalactooligosaccharide	Weaned pigs	Mikkelsen and Jensen (2004)
Galactooligosaccharide	Weaned pigs	Smiricky-Tjardes et al. (2003)
		Tzortzis et al. (2005)
Isomaltooligosaccharide	Weaned pigs	Wang et al. (2016)
β-Glucan	Gilts	Xiao et al. (2004)
S. cerevisiae fermentation product	Sows	Shen et al. (2011)

supplementation of young cattle and swine may help to establish a healthy gut early in their life, perhaps reducing colonization of pathogenic bacteria such as *Escherichia coli* or *Salmonella* in the GI tract and reducing the negative impacts associated with the transitions between production stages (i.e., weaning, lactation (Chaucheyras-Durand and Durand 2010)).

It is interesting to note that the benefit or success of supplementing livestock with pre- and probiotics appears to be dependent on the general health of the animals prior to beginning supplementation. For example, groups of calves or pigs that are generally healthy, have limited stressor exposure, and with no persistent infections may not appear to benefit from supplementation with pre- or probiotics from a performance or immune perspective (Uyeno et al. 2015; Heinrichs et al. 2009). However, less thrifty animals, or those that are considered "high-risk" (i.e., increased stressor exposure, changes in feeding, and pathogen exposure) may receive the

greatest benefit in performance and health when supplemented with pre- or probiotics (Timmerman et al. 2005). Further, supplementation with pre- and probiotics will not alleviate issues associated with poor management of livestock, such as poor sanitation or inappropriate feeding strategies. Additionally, there is a general lack of information about the effect of removal of supplementation of these products on the benefits they provide. For example, when weaned pigs are supplemented with 5–7% plasma protein, abrupt removal of the plasma protein from the diet significantly reduces performance and increases susceptibility to diseases. Thus, it is clear that there is a continuing need for research into pre- and probiotics supplementation in livestock production systems.

14.2.1 Probiotic Mode of Action

There is a wide range of probiotic products available for use in livestock production systems. These products include yeast (live or dried; typically, *Saccharomyces cerevisiae*), *Lactobacillus*, and *Bifidobacterium* among others (Yan and Polk 2011) (Table 14.1). For example, *Lactobacillus* species have been noted to colonize the intestine of animals and humans and play a role in reducing concentrations of pathogenic bacteria such as *E. coli* and *Salmonella*, which are a major problem in terms of calf and piglet early life diarrhea (Frizzo et al. 2010). More recently, spore-producing bacteria, such as *Bacillus subtilis*, have emerged as potential probiotic products (Mingmongkolchai and Panbangred 2018) used to control *Salmonella* populations in livestock (Broadway et al. 2020). As discussed above, there are various benefits to the host when probiotics are supplemented. These include enhancing intestinal barrier function, altering microbial populations within the GI tract, and production of antimicrobial agents. Through promoting a healthy gut microbiome, colonization of pathogenic organisms may be reduced through competitive exclusion activities (Isolauri et al. 2001).

14.2.1.1 Enhancing Intestinal Barrier Function

Probiotics improve growth and immunity through maintaining and enhancing the intestinal barrier, thus reducing the migration of pathogenic bacteria and/or toxins to the outside of the GI tract as well as reducing colonization of the lumen by pathogenic microorganisms. Goblet cells within the epithelial layer of the intestine produce mucins, which are responsible for providing protection to the intestinal epithelial, but also allows for beneficial bacteria to bind (Ma et al. 2018). Feeding a probiotic blend of *Streptococcus*, *Bifidobacteria*, and *Lactobacillus* strains increased the number of mucin-producing goblet cells within the duodenum, cecum, and colon of supplemented pigs (Desantis et al. 2019). Additionally, differences in the thickness of the mucus layer have been found in yeast-supplemented pigs where yeast supplementation decreased mucus thickness compared to control pigs (Bontempo et al. 2006; Di Giancamillo et al. 2007). The authors suggested that this may be due to an increased number of pathogenic bacteria present in the gut of control pigs such that greater mucin was produced. Such an increase in mucus may be detrimental to

nutrient absorption within the GI tract, and the decreased mucus in yeast-supplemented pigs suggests this may result in increased nutrient absorption and thus pig performance (Bontempo et al. 2006). The greater amounts of acid glycoconjugates within the mucin in yeast-supplemented pigs suggest these pigs may experience greater resistance against bacterial infections (Bontempo et al. 2006).

Changes in intestinal permeability have also been observed with probiotic supplementation. Increased permeability, due to changes in cell-to-cell interactions (e.g., tight junctions), has been implicated in "leaky gut," leading to increased translocation of pathogenic bacteria and toxins from the GI tract into circulation and an increase in inflammation in the GI tract (Moeser et al. 2017). This ultimately results in increased systemic inflammation (Rodriguez-Jimenez et al. 2019). Additionally, villus height and crypt depth within the mucosa of the intestine can be used as a measurement of intestinal health, as cells within the crypts and villi are associated with nutrient absorption (Pluske et al. 1997). Improvement of tight junctions and barrier function has been observed when animals were supplemented with probiotics. Specifically, weaned pigs fed a probiotic containing *L. plantarum*, *Pediococcus acidilactici,* or *S. cerevisiae* had increased villus height and crypt depth within sections of the intestine (Di Giancamillo et al. 2007, 2008; Shin et al. 2019). A study in pigs fed a combination of *Bacillus licheniformis* and *S. cerevisiae* found reduced small intestine permeability compared to control pigs when challenged with *E. coli* K88, including an increase in occluding protein, associated with tight junctions, within the mucosa of the jejunum (Pan et al. 2017). Additionally, probiotic supplementation negated any negative effects of the *E. coli* K88 challenge on changes in small intestine morphology such that the morphology of supplemented pigs appeared similar to non-challenged pigs (Pan et al. 2017). Similarly, in vitro studies utilizing a porcine intestinal epithelial cell line model (IPEC-J2) found pre-treating the cells with *Lactobacillus* prevented an LPS or *E. coli* K88-induced decrease in transepithelial electrical resistance and reduction in tight junction proteins (Yang et al. 2015; Wu et al. 2016). Thus, improvement in barrier function may result in less leakage of pathogenic bacteria and toxins out of the GI tract, ultimately leading to reduced systemic and localized inflammation and increased nutrient absorption. Unfortunately, there is limited information in cattle on the ability of probiotics to modulate barrier function within the GI tract. However, similar enhancement of intestinal integrity can be assumed based on the findings of reduced bacterial translocation from the GI tract to the periphery in cattle supplemented with various probiotics (Broadway et al. 2020).

14.2.1.2 Altering Gastrointestinal Microbe Populations

In addition to enhancing the barrier within the GI tract, probiotics have been demonstrated to alter the microbial population within the gut. Feeding a *L. plantarum* probiotic to weaned pigs increased microbial diversity and richness compared to control pigs (Shin et al. 2019), suggesting probiotics may be beneficial in reducing the changes in the microbiome that occur following weaning. Additionally, decreases in fecal coliforms and increases in *Lactobacilli* and *Bifidobacteria*

were found in weaned pigs supplemented with *Lactobacillus rhamnosus* and challenged orally with *E. coli* K88 (Zhang et al. 2010). Similar results were observed with supplementation of *Lactobacillus brevis* in weaned pigs in the absence of a challenge, where decreased coliforms and increased *Lactobacillus* were found in feces (Liu et al. 2015). Supplementation of weaned dairy calves with a *Bacillus subtilis* product was able to not only reduce the shedding of an orally dosed *Salmonella typhimurium*, but reduced colonization of the bacteria within the GI tract and mesenteric lymph nodes (Broadway et al. 2020). Probiotics change the GI microbiome through several mechanisms, including altering the pH of the GI tract, competing for nutrients with pathogenic bacteria, and releasing antimicrobial substances.

Yeast, mainly of the *S. cerevisiae* strain, are the most common probiotic supplements given to dairy cows (Thrune et al. 2009; AlZahal et al. 2014). Feeding of live yeast to cattle has been found to improve rumen stability through increasing rumen pH (Desnoyers et al. 2009). Supplementation of dairy cows with live yeast increased rumen pH, increased the concentrations of the volatile fatty acids (VFA) propionate and butyrate, and reduced lactate and ammonia concentrations (Pinloche et al. 2013). Additionally, feeding a mixture of direct fed microbials to cannulated lactating dairy cows increased rumen pH (Nocek et al. 2002). When feeding cereal grains there is an increased likelihood of the production of lactic acid in the rumen, which drives down pH and can result in acidosis (Lean et al. 2000). This acidotic state is also detrimental to beneficial bacteria such as lactate producing bacteria and leads to a microbial imbalance within the gut, ultimately reducing fiber digestion (Goto et al. 2016). These studies demonstrate that feeding of probiotics can prevent the drop in pH and subsequent acidosis when fed to cattle. In contrast, a lower colonic pH and greater concentrations of lactic acid were observed when pigs were supplemented with *Lactobacillus reuteri* (Hou et al. 2015). The greater concentrations of lactic acid inhibit the growth of pathogenic bacteria within the pig GI tract (Gresse et al. 2017). This is a result of increased fermentation and concentrations of short chain fatty acids (SCFA) (Liu et al. 2018; Smiricky-Tjardes et al. 2003).

Active dry yeast may play a role in maturing the microbiota of the rumen by increasing the number of cellulolytic bacteria (Chaucheyras-Durand et al. 2008). For example, an increase in lactate-utilizing and fibrolytic bacteria were observed in cows supplemented with live yeast (Pinloche et al. 2013). This is proposed as one of the modes of action of yeast and may result in a more stabilized pH as noted earlier. Increases in fibrolytic and cellulolytic bacteria promote digestion of fiber-based feedstuffs which not only stabilizes rumen pH but also increases the utilization of ingested fibrous feedstuffs.

Beneficial bacteria can also compete with pathogens for nutrients as well as epithelial binding sites, a process known as competitive exclusion (Ma et al. 2018; Callaway et al. 2017). Yeast are able to scavenge oxygen in the rumen, and the resulting anaerobic environment is more suitable for fiber-digesting microorganisms within the rumen (Pinloche et al. 2013; Alnaimy Mostafa Habeeb 2017). Also, live yeast may utilize lactic acid, creating a more suitable environment for beneficial

microorganisms as too much lactic acid within the rumen may suppress bacterial growth (Alnaimy Mostafa Habeeb 2017). Yeast can proliferate in the rumen, and this increase was associated with increases in fiber-degrading bacteria including *A. lipolytica, R. albus*, and *F. succinogenes* when active dry live yeast was supplemented to lactating dairy cows (AlZahal et al. 2014). This is likely the result of the release of growth factors and organic acids from the proliferating yeast, which can be used by beneficial gut bacteria.

In addition to the direct effects of these products on the beneficial microbes in the gut, there is a subsequent negative impact on pathogenic bacteria within the microbiome that may be just as important (Uyeno et al. 2015). For example, feeding of active dry live yeast to dairy cows resulted in a 2.2-fold reduction in *Prevotella albensis*, a gram negative bacterium (AlZahal et al. 2014). Not only does this provide a benefit to the host but has also food safety implications such that a reduction within the gut may reduce the risk of horizontal transfer of pathogenic bacteria to food products.

Further, yeast have been found to directly bind to pathogenic bacteria, including *Salmonella*, *E. coli*, *Clostridium*, *Listeria,* and *Fusobacterium*, and the interaction between the yeast and bacteria was strain-specific (Posadas et al. 2017). Additionally, different *Lactobacillus* strains have been shown to decrease the attachment of pathogenic bacteria to the intestinal epithelium, which may be due to an increase in expression of certain mucins (Mack et al. 1999). This suggests that one mechanism by which probiotics inhibit pathogen binding and colonization within the GI tract is through an increased production of mucins, which protect the intestinal epithelium.

14.2.1.3 Production of Antimicrobial Compounds

Probiotic bacteria negatively influence pathogenic bacteria through the release of antimicrobial substances such as organic acids, proteins, and peptides that exhibit bacteriostatic or bactericidal properties, as well as hydrogen peroxide (Hong et al. 2005; Ma et al. 2018; Al-Saiady 2010). Certain strains of *Bacillus* produce bacteriocins, with a specific strain of *Bacillus subtilis* producing aminocoumacin A, an antibiotic effective against bacteria such as *Staphylococcus aureus*, *Shigella flexneri,* and *Helicobacter pylori* (Hong et al. 2005). Cell-free supernatant from different *Lactobacillus* isolates inhibited the growth of several cattle pathogens, including *E. coli* O157:H7, *Salmonella dublin*, *Salmonella typhimurium,* and *Salmonella enteritidis* (Lin et al. 2020).

14.2.2 Prebiotic Mode of Action

Prebiotics, as indigestible feed stuffs, are utilized by beneficial microbes in the GI tract ultimately resulting in benefits to the host. Examples include fructooligosaccharides (FOS), mannanoligosaccharides (MOS), inulin, lactulose, and cell walls of yeast and other fungi (Angelakis 2017) (Table 14.2). Thus, prebiotics elicit a majority of their effects through supplying beneficial microbes with a readily available substrate. Commercially available prebiotic products come

in many different forms, including purified FOS or MOS, bacterial or yeast culture by-products or fermentation products, as well as purified bacterial or yeast cell wall products. Additionally, various cereal grain brans, including barley and oat, naturally have high concentrations of prebiotics such as β-glucans (Carroll et al. 2012). Thus, feeding prebiotics may be used to counteract negative effects of a high grain diet that lacks natural prebiotics found in forages. Studies have found prebiotics elicit their effects by many of the same actions of probiotics, including altering pH, increasing SCFA concentrations, and preventing the binding of pathogenic bacteria to intestinal epithelium.

As mentioned in the previous section, yeast culture or yeast fermentation products contain nutrients that can be utilized by beneficial bacteria in the gut. A study by Callaway and Martin (1997) found that adding yeast culture to media containing *Selenomonas ruminantium* and DL-lactate resulted in stimulation of the growth of the bacteria while also increasing acetate and total VFA concentrations. It is believed that the nutrients, including B vitamins, organic acids, oligosaccharides, and amino acids, allow for rumen bacteria to better utilize lactate. A reduction in lactate concentrations prevents decreases in rumen pH which can lead to acidosis and microbial imbalance. An environment that is less acidic is beneficial to the fiber-digesting microorganisms within the rumen.

In pigs, the readily fermentable carbohydrates in FOS, MOS, and inulin result in an increase in lactate and SCFA and a subsequent decrease in intestinal pH (Liu et al. 2018). In cattle, supplementation with MOS resulted in greater ruminal pH and tended to increase SCFA compared to non-supplemented cattle (Garcia Diaz et al. 2018). The production of SCFA, as a result of prebiotic fermentation by gut bacteria, can have varying effects based on the concentration. For example, lower concentrations of SCFA can influence the regulation of virulence genes in pathogenic bacteria (Vogt et al. 2015). Additionally, SCFA play a role in reducing expression of adhesion and/or invasion factors associated with pathogenic bacteria (Tran et al. 2018; Lawhon et al. 2002). Studies in pigs have found increases in SCFA production (i.e., propionate and butyrate) as well as increases in *Bifidobacteria* and *Lactobacilli* when pigs were fed galactooligosaccharide (Smiricky-Tjardes et al. 2003; Tzortzis et al. 2005).

Studies have found feeding prebiotics to cattle can reduce not only fecal shedding of *E. coli*, but also reduce the virulence of the excreted *E. coli* (Grispoldi et al. 2017). Supplementation of weaned pigs with either FOS or transgalactooligosaccharides (TOS) increased the number of yeast within the GI tract (Mikkelsen and Jensen 2004). Further, there were greater concentrations of butyric acid yet less acetic acid in the cecum and proximal colon of pigs supplemented with FOS (Mikkelsen and Jensen 2004). Additionally, lactulose supplementation increased concentrations of *Lactobacilli* within the colon while decreasing butyric acid percentage, and increased the villus height within the ileum of weaned pigs (Guerra-Ordaz et al. 2014). Supplementation of weaned pigs with chitooligosaccharide increased fecal *Lactobacillus* yet decreased *E. coli* counts compared to control pigs, while also increasing the villus height and villus:crypt ratio within the intestine (Liu et al.

2008). Interestingly, supplementation of calves with lactulose decreased ileal villus height and also decreased crypt depth (Fleige et al. 2007).

The increase in beneficial bacteria in the gut, due to supplementation with prebiotics, prevents the binding and colonization of pathogenic bacteria within the GI tract, resulting in a reduction of these microorganisms. Therefore, prebiotics promote and substantiate the effects of probiotics and naturally occurring beneficial bacteria within the GI tract. Additionally, similar to live yeast, yeast cell wall products have been found to bind to pathogenic bacteria, yet to a lesser extent than live yeast (Posadas et al. 2017). It is estimated that approximately 50–60% of the polysaccharides in the cell wall of yeast are β-D-glucans (Kogan and Kocher 2007). Studies have indicated that FOS prevents pathogenic bacteria, including *Salmonella* and *E. coli*, from binding to the intestinal epithelium. Additionally, FOS is selectively fermented by *Bifidobacteria* and results in *Bifidobacteria* proliferation within the GI (Kawaguchi et al. 1993). It has been suggested that the binding of pathogenic bacteria to prebiotics prevents the ability of the bacteria to bind and colonize within the intestine, and thus the bacteria are flushed out of the system.

Regardless of whether or not prebiotics are fed with probiotics, these products have the potential to alter the GI tract microbiome in ways that can greatly benefit the host. Often the focus of effects of pre- and probiotics is on production traits such as growth and milk yield. However, there is an increasing amount of data to suggest these products can directly and/or indirectly influence other biological aspects, including immunity and metabolism. In fact, changes in immunity, such as reductions in inflammation, leading to a reduction in energy utilized by the immune system, may be one of the factors driving changes in animal performance.

14.3 Effect of Pre- and Probiotics on Innate Immune Function

One leading cause of disease in livestock is an unbalanced or altered microbiota (Brugman et al. 2018). Shifts in the dynamics of different bacteria populations, whether due to diet, environmental changes, or stress, can result in increased growth of pathogenic bacteria, increased toxin production, increased intestinal permeability, and ultimately increased inflammation. While the direct impact of these effects may be primarily considered to be a local effect within the GI tract, the greater physiological impact may be associated with reduced systemic immune function and reduced animal performance. One example of such disruption in cattle is subacute ruminal acidosis, which can leave cattle more susceptible to other illnesses and may often go undetected. Therefore, keeping the microbiota within the GI tract balanced has substantial benefits to the performance, health, and overall well-being of livestock. As discussed above, pre- and probiotics are products that have been proven to help maintain a healthy gut microbiome, thus perhaps providing a universal biological benefit to the host.

There are many reported benefits of pre- and probiotics in humans and animals, with effects ranging from anti-inflammatory to anti-allergic, and anti-cancer to anti-obesity (Azad et al. 2018). Initially the study of pre- and probiotics in livestock

focused on the benefits to performance such as weight gain and milk production. However, with changes in the availability and rules governing the use of antimicrobials, research efforts have shifted to determining if these products can be used as alternatives to antimicrobials. Indeed, both pre- and probiotics have been documented to alter immunity, including stimulating production of antibodies or reducing clinical sickness scores, but it is only recently that the mechanisms behind these responses have been studied in greater detail. It is believed that some of the documented effects may be a result of the products acting within the gut, where increased barrier function and decreased pathogenic bacteria may lead to an overall reduction in systemic inflammation. However, specific responses outside of the gut have been identified as well.

When disease occurs, the first line of defense against the invading pathogen is the innate immune response, the body's non-specific arm of the immune system. Innate immune defenses include physical (e.g., epithelial cell barriers), cellular (e.g., leukocytes), and secreted (e.g., cytokines, complement) components. When a pathogen is detected, secretion of cytokines by epithelial cells or resident leukocytes stimulates the recruitment of other leukocytes in an effort to destroy the pathogen without further activation of the immune system (i.e., adaptive immunity). The increase in local cytokine production also results in inflammation, leading to vasodilation and a further influx of leukocytes. Other factors are also stimulated, such as production of acute phase proteins and complement. While the innate immune response is much more complex, two main areas where pre- and probiotic supplementation can influence are leukocyte populations and cytokine production. In the next sections, the effects of pre- and probiotics on these aspects of innate immunity in cattle and swine are discussed.

14.3.1 Actions on Leukocytes

Leukocytes, also referred to as white blood cells, are the primary effector cells of the immune response and consist of 5 main cell types of differing functions: lymphocytes, neutrophils, monocytes (macrophages), eosinophils, and basophils. Although approximately 70% of the immune system is associated with the GI tract, significant influences of pre- and probiotics on circulating leukocytes have been identified. For example, greater concentrations of white blood cells were observed in dairy steers supplemented with different probiotics (Al-Saiady 2010). However, supplementation of Holstein calves with hydrolyzed yeast, a prebiotic, prevented any change in leukocyte populations in response to a live vaccine challenge (Kim et al. 2011). Lactulose supplementation in dairy calves reduced total leukocyte concentration compared to non-supplemented calves as well as calves supplemented with inulin (Masanetz et al. 2011). Sows supplemented with a *S. cerevisiae* fermentation product during gestation and lactation were reported to have reduced concentrations of neutrophils and white blood cells (Shen et al. 2011). Thus, the leukocyte response to supplementation appears to be dependent upon the pre- or probiotic used.

Changes in specific leukocyte populations have also been observed. Greater populations of lymphocytes, including T helper cells, cytotoxic T cells, and B lymphocytes, were observed in calves supplemented with a probiotic containing β-glucan and MOS (Szymanska-Czerwinska et al. 2009). However, the opposite response was observed in pigs, where MOS supplemented pigs had reduced concentrations of T helper and cytotoxic T cells compared to non-supplemented pigs (Kim et al. 2000). Lymphocytes are considered a part of the adaptive immune response, which produces a targeted response specifically against the invading pathogen. When weaned pigs were supplemented with yeast cell wall and subsequently challenged with *Salmonella typhimurium*, concentrations of total leukocytes, neutrophils, lymphocytes, and the neutrophil:lymphocyte ratio were reduced compared to non-supplemented pigs (Burdick Sanchez et al. 2019a). Similar results were observed following an LPS challenge in weaned pigs supplemented with a *Lactobacillus acidophilus* fermentation product (Burdick Sanchez et al. 2019b). Neutrophils are inflammatory leukocytes, and the neutrophil:lymphocyte ratio can be used as an index of inflammation with greater values indicative of greater inflammation. Increases or decreases in leukocyte populations may be a result of changes in production within primary lymphoid tissues or may be due to changes in movement of leukocytes from circulation into tissues. In support of this, supplementation with a yeast-based probiotic in Holstein heifers increased expression of the adhesion molecule L-selectin in neutrophils, which helps neutrophils and other leukocytes move from circulation and into tissues (Ryman et al. 2013).

Changes in immune cell function have also been observed. Neutrophils isolated from Holstein calves supplemented with yeast products had increased oxidative burst and phagocytic capacities, suggestive of increased cellular function (Ryman et al. 2013; Fomenky et al. 2018). Additionally, feeding yeast culture to Holstein calves tended to increase phagocytosis and killing of bacteria by neutrophils, and reduced the overall mortality rate of the calves during the first 70 days of life (Magalhaes et al. 2008). Thus, it appears that while changes in leukocyte numbers differ in response to pre- and probiotic supplementation, the functional aspects of the innate immune response may be increased regardless of which pre- or probiotic is supplemented. However, this is an area that requires further investigation to fully elucidate the multifaceted immunological responses associated with pre- and probiotic supplementation.

14.3.2 Activation of Cytokine Production

Cytokine production is the main regulator of an immune response, and depending on the type of cytokines released, can result in an inflammatory [Tumor necrosis factor-α (TNF-α), Interleukin-6 (IL-6), Interferon-γ (IFN-γ)], anti-inflammatory (IL-4, IL-12) or tolerogenic responses [Transforming growth factor-β (TGF-β), IL-10)]. Cytokines are also important for stimulation of complement and acute phase protein production. Further, cytokines are responsible for signaling recruitment of leukocytes and regulate many of the systemic responses observed in response to

illness, including fever, malaise, and anorexigenic responses. Thus, changes in cytokine production can direct the duration and magnitude of immune responses.

Various cytokine responses have been observed in pigs and cattle fed pre- and probiotic supplements. Weaned pigs supplemented with *Lactobacillus rhamnosus* GG and subsequently challenged with *E. coli* K88 had reduced serum concentrations of the pro-inflammatory cytokine IL-6 compared to challenged control pigs, with IL-6 values being similar in supplemented pigs compared to non-challenged control pigs (Zhang et al. 2010). In contrast, pigs supplemented with β-glucan and challenged with LPS had greater serum concentrations of the pro-inflammatory cytokines IL-6, TNF-α and the anti-inflammatory cytokine IL-10 compared to non-supplemented pigs (Li et al. 2006). It is possible that the difference in challenge type or the pre- or probiotic used may have resulted in the different cytokine responses observed between the aforementioned studies. Similarly, supplementation of weaned pigs with either a *Saccharomyces cerevisiae* fermentation product (SCFP) or a *Lactobacillus acidophilus* fermentation product had greater serum concentrations of TNF-α (SCFA only) and IL-6 following challenge with LPS (Burdick Sanchez et al. 2018, 2019b). Additionally, serum IL-6 and IL-2 concentrations increased in pigs supplemented with isomaltooligosaccharide (Wang et al. 2016). Liu et al. (2015) observed an increase in serum concentrations of IFN-γ yet a decrease in haptoglobin concentrations, an acute phase protein, in pigs supplemented with *Lactobacillus brevis*. Increased IFN-γ was observed in dairy calves supplemented with *Bacillus subtilis* natto, and there was a tendency for IL-4 concentrations to be decreased compared to control calves (Sun et al. 2010). Stimulation of monocytes isolated from pigs infected with porcine reproductive and respiratory virus with soluble β-glucan increased production of IFN-γ dose-dependently (Xiao et al. 2004). This suggests that prebiotics such as β-glucan may be able to enhance immunity against viruses. Supplementation of calves with a prebiotic blend of β-glucans and MOS increased serum concentrations of IL-1, IL-2, and IFN (Szymanska-Czerwinska et al. 2009). Similar to effects on leukocytes, these data suggest that there are differences in the production of some pro-inflammatory cytokines based on the model and product used.

Deciphering the benefit of cytokine concentrations can be difficult, especially when values are measured in a limited number of samples. The benefit or detriment of cytokines is based on the duration of the response, that is, the time values remain above basal concentrations, as well as the peak value attained. For example, greater concentrations of cytokines observed for a limited period of time and reduced concentrations of cytokines for an extended period of time may be examples of beneficial responses. Ultimately, however, the best determinant of whether a cytokine response is beneficial or not is observed by monitoring the recovery of the animal, both in the resolution of the immune response and the return to maintenance behaviors.

14.4 Metabolic Modulation

Activation of the immune system and maintaining an immunological response requires a significant amount of energy. As such, the effectiveness of an immune response may be reflective of the energy balance or energy stores available to the animal. Readily available glucose is necessary in order to provide sufficient energy to fuel an adequate immune response. In fact, studies have demonstrated that cattle and swine utilize over 1 kg of glucose during a 12-h period following an immune challenge with LPS (Kvidera et al. 2016, 2017). Thus, insufficient energy stores may prevent an adequate immune response and thus result in a prolonged or persistent immunological insult. Failure to resolve an immunological insult will further deplete energy stores within an animal, causing significant impacts on growth, productivity, and overall well-being. While much of the research using pre- and probiotics has focused on performance and immune benefits, recent studies are revealing that these products are impacting metabolism, which may ultimately be the key to understanding the changes observed in animal performance and immune function when these products have been supplemented. The remainder of this section will focus on how supplementing pre- and probiotics may be changing metabolic aspects with regard to energy availability within the animal.

The immune system requires glucose, a requirement that is substantially elevated upon activation. This is easily observed in the change in circulating glucose in response to an LPS challenge, where there is an initial rapid increase in circulating concentrations as glucose is being released from glycogen stores, and then a subsequent rapid decline in circulating glucose as it is taken up and utilized in various cells. Thus, differences in glucose concentrations during an immunological insult may indicate changes in energy availability for the immune system. Interestingly, differences in glucose concentrations have indeed been observed in animals supplemented with pre- or probiotics following an immune challenge. Specifically, steers supplemented with yeast cell wall displayed altered metabolic responses following LPS challenge, where greater increases in circulating concentrations of glucose and insulin were observed while non-esterified fatty acid concentrations were reduced compared to control calves (Burdick Sanchez et al. 2014). Similarly, greater glucose responses have also been observed in LPS-challenged steers supplemented with SCFP (Burdick Sanchez et al. 2020). Interestingly, following administration of a glucose tolerance test, steers supplemented with a probiotic were found to have reduced glucose yet greater insulin concentrations compared to non-supplemented steers, suggesting that these steers were more responsive to changes in glucose (Burdick Sanchez et al. 2019c) Similarly, Holstein calves with failure of passive transfer that were fed *Saccharomyces cerevisiae* were found to have greater glucose concentrations compared to non-supplemented calves (Galvao et al. 2005) and supplementing Holstein calves with cellooligosaccharide increased plasma insulin concentrations (Hasunuma et al. 2011). Collectively, these studies indicate that pre-/probiotic supplementation in cattle alters glucose availability during times of an immune challenge in a manner that may be beneficial to more rapidly resolve an immunological insult.

Beyond aiding in rapidly resolving an immunological insult, pre- and probiotic supplementation may also reduce the severity of catabolic actions on fat and lean tissue within the animal. For example, concentrations of non-esterified fatty acids, typically viewed as an indicator of fat catabolism, were reduced in weaned pigs supplemented with yeast cell wall (Burdick Sanchez et al. 2019a). Likewise greater total protein concentrations and decreased blood urea nitrogen were observed in pigs supplemented with *Lactobacillus brevis* in the absence of an immune challenge (Liu et al. 2015). Similar results were observed in pigs supplemented with *Brevibacillus brevis*, a spore-forming bacteria, where total protein was greater in supplemented pigs (Che et al. 2016). This may be indicative of improved protein status with decreased fat and protein degradation. There was a tendency for concentrations of plasma urea nitrogen to be reduced in sows fed a SCFP in gestation and lactation, suggesting this product may reduce protein catabolism (Shen et al. 2011). Therefore, it is possible that pre- and probiotics may influence metabolism by altering the catabolism of fat and protein.

The effect of pre- and probiotics on metabolism is an area that has not received a sufficient amount of attention. However, these products appear to significantly alter metabolism, potentially altering energy availability for growth, production traits as well as immune function. Thus, supplementation of livestock with pre- and probiotic products may be a way to improve animal health and ultimately productivity through modulation of metabolic pathways, and certainly requires further study.

14.5 Conclusion

Animal health is one area of livestock production that is in need of more focused attention. As our understanding of the microbiome and its influence on modulating animal health and disease grows, methods to modulate the microbiome to improve animal health and well-being are advancing. Decades of research has identified ways that pre- and probiotics alter the microbiota within the GI tract which can have local as well as systemic benefits to the animal. Harnessing these benefits allows livestock producers to also improve animal health and potentially metabolism and energy availability. Further, utilization of such products will also address the concerns of consumers who oppose the use of synthetic antimicrobials in livestock production systems.

References

Alnaimy Mostafa Habeeb A (2017) Importance of yeast in ruminants feeding on production and reproduction. Ecol Evol Biol 2(4):49–58

Al-Saiady MY (2010) Effect of probiotic bacteria on iinmunoglobulin G concentration and other blood components of newborn calves. J Anim Vet Adv 9(3):604–609

AlZahal O, Dionissopoulos L, Laarman AH, Walker N, McBride BW (2014) Active dry Saccharomyces cerevisiae can alleviate the effect of subacute ruminal acidosis in lactating dairy cows. J Dairy Sci 97(12):7751–7763

Angelakis E (2017) Weight gain by gut microbiota manipulation in productive animals. Microb Pathog 106:162–170

Azad MAK, Sarker M, Wan D (2018) Immunomodulatory effects of probiotics on cytokine profiles. Biomed Res Int 2018:8063647

Bontempo V, Di Giancamillo A, Savoini G, Dell'Orto V, Domeneghini C (2006) Live yeast dietary supplementation acts upon intestinal morpho-functional aspects and growth in weanling piglets. Anim Feed Sci Tech 129(3–4):224–236

Broadway PR, Carroll JA, Burdick Sanchez NC, Callaway TR, Lawhon SD, Gart EV et al (2020) Bacillus subtilis PB6 supplementation in weaned Holstein steers during an experimental salmonella challenge. Foodborne Pathog Dis 17(8):521–528

Brugman S, Ikeda-Ohtsubo W, Braber S, Folkerts G, Pieterse CMJ, Bakker P (2018) A comparative review on microbiota manipulation: lessons from fish, plants, livestock, and human research. Front Nutr 5:80

Burdick Sanchez NC, Young TR, Carroll JA, Corley JR, Rathmann RJ, Johnson BJ (2014) Yeast cell wall supplementation alters the metabolic responses of crossbred heifers to an endotoxin challenge. Innate Immun 20(1):104–112

Burdick Sanchez NC, Carroll JA, Broadway PR, Bass BE, Frank JW (2018) Modulation of the acute phase response following a lipopolysaccharide challenge in pigs supplemented with an all-natural Saccharomyces cerevisiae fermentation product. Livest Sci 208:1–4

Burdick Sanchez NC, Carroll JA, Corley JR, Broadway PR, Callaway TR (2019a) Changes in the hematological variables in pigs supplemented with yeast cell wall in response to a salmonella challenge in weaned pigs. Front Vet Sci. 6:246

Burdick Sanchez NC, Carroll JA, Broadway PR, Bass BE, Frank JW (2019b) Supplementation of a lactobacillus acidophilus fermentation product can attenuate the acute phase response following a lipopolysaccharide challenge in weaned pigs. Animal 13(1):144–152

Burdick Sanchez NC, Carroll JA, Broadway PR, Schell TH, Puntenney SB, McLean DJ (2019c) Supplementation of OmniGen-AF improves the metabolic response to a glucose tolerance test in beef heifers. Transl Anim Sci 3(4):1521–1529

Burdick Sanchez N, Carroll J, Broadway P, Edrington T, Yoon I, Belknap C (2020) Some aspects of the acute phase immune response to a lipopolysaccharide (LPS) challenge are mitigated by supplementation with a Saccharomyces cerevisiae fermentation product in weaned beef calves. Transl Anim Sci 4:txaa156

Callaway TR, Martin SA (1997) Effects of cellobiose and monensin on in vitro fermentation of organic acids by mixed ruminal bacteria. J Dairy Sci 80(6):1126–1135

Callaway TR, Edrington TS, Anderson RC, Harvey RB, Genovese KJ, Kennedy CN et al (2008) Probiotics, prebiotics and competitive exclusion for prophylaxis against bacterial disease. Anim Health Res Rev 9(2):217–225

Callaway TR, Edrington TS, Byrd JA, Nisbet DJ, Ricke SC (2017) Use of direct-fed microbials in layer hen production—performance response and salmonella control. In: Producing safe eggs. Elsevier, New York, pp 301–322

Carroll J, Sanchez N, Ballou M, Arthington J (2012) Phytochemicals as a preharvest pathogen reduction strategy, pp 167–182

Chaucheyras-Durand F, Durand H (2010) Probiotics in animal nutrition and health. Benef Microbes 1(1):3–9

Chaucheyras-Durand F, Walker ND, Bach A (2008) Effects of active dry yeasts on the rumen microbial ecosystem: past, present and future. Anim Feed Sci Tech. 145(1–4):5–26

Che J, Ye S, Liu B, Deng Y, Chen Q, Ge C et al (2016) Effects of Brevibacillus brevis FJAT-1501-BPA on growth performance, faecal microflora, faecal enzyme activities and blood parameters of weaned piglets. Antonie Van Leeuwenhoek 109(12):1545–1553

Colditz IG, Hine BC (2016) Resilience in farm animals: biology, management, breeding and implications for animal welfare. Anim Prod Sci 56(12):1961–1983

Desantis S, Mastrodonato M, Accogli G, Rossi G, Crovace AM (2019) Effects of a probiotic on the morphology and mucin composition of pig intestine. Histol Histopathol 34(9):1037–1050

Desnoyers M, Giger-Reverdin S, Bertin G, Duvaux-Ponter C, Sauvant D (2009) Meta-analysis of the influence of *Saccharomyces cerevisiae* supplementation on ruminal parameters and milk production of ruminants. J Dairy Sci 92(4):1620–1632

Di Giancamillo A, Bontempo V, Savoini G, Dellorto V, Vitari F, Domeneghini C (2007) Effects of live yeast dietary supplementation to lactating sows and weaning piglets. Int J Prob Preb 2(1):55–66

Di Giancamillo A, Vitari F, Savoini G, Bontempo V, Bersani C, Dell'Orto V et al (2008) Effects of orally administered probiotic Pediococcus acidilactici on the small and large intestine of weaning piglets. A qualitative and quantitative micro-anatomical study. Histol Histopathol 23(6):651–664

Fleige S, Preissinger W, Meyer HH, Pfaffl MW (2007) Effect of lactulose on growth performance and intestinal morphology of pre-ruminant calves using a milk replacer containing enterococcus faecium. Animal 1(3):367–373

Fomenky BE, Chiquette J, Lessard M, Bissonnette N, Talbot G, Chouinard YP et al (2018) Saccharomyces cerevisiae var. boulardii CNCM I-1079 and lactobacillus acidophilus BT1386 influence innate immune response and serum levels of acute-phase proteins during weaning in Holstein calves. Can J Anim Sci 98(3):576–588

Frizzo LS, Soto LP, Zbrun MV, Bertozzi E, Sequeira G, Armesto RR et al (2010) Lactic acid bacteria to improve growth performance in young calves fed milk replacer and spray-dried whey powder. Anim Feed Sci Tech. 157(3–4):159–167

Fuller R (1989) Probiotics in man and animals. J Appl Bacteriol 66(5):365–378

Galvao KN, Santos JE, Coscioni A, Villasenor M, Sischo WM, Berge AC (2005) Effect of feeding live yeast products to calves with failure of passive transfer on performance and patterns of antibiotic resistance in fecal Escherichia coli. Reprod Nutr Dev 45(4):427–440

Garcia Diaz T, Ferriani Branco A, Jacovaci FA, Cabreira Jobim C, Pratti Daniel JL, Iank Bueno AV et al (2018) Use of live yeast and mannan-oligosaccharides in grain-based diets for cattle: ruminal parameters, nutrient digestibility, and inflammatory response. PLoS One 13(11): e0207127

Goto H, Qadis AQ, Kim YH, Ikuta K, Ichijo T, Sato S (2016) Effects of a bacterial probiotic on ruminal pH and volatile fatty acids during subacute ruminal acidosis (SARA) in cattle. J Vet Med Sci 78(10):1595–1600

Gresse R, Chaucheyras-Durand F, Fleury MA, Van de Wiele T, Forano E, Blanquet-Diot S (2017) Gut microbiota dysbiosis in postweaning piglets: understanding the keys to health. Trends Microbiol 25(10):851–873

Grispoldi L, Bertero F, Franceschini S, Mastrosimone F, Sechi P, Iulietto MF et al (2017) Prevalence and characterisation of shigatoxigenic Escherichia coli isolated from beef cattle fed with prebiotics. Ital J Food Saf 6(4):6958

Guerra-Ordaz AA, Gonzalez-Ortiz G, La Ragione RM, Woodward MJ, Collins JW, Perez JF et al (2014) Lactulose and lactobacillus plantarum, a potential complementary synbiotic to control postweaning colibacillosis in piglets. Appl Environ Microbiol 80(16):4879–4886

Hasunuma T, Kawashima K, Nakayama H, Murakami T, Kanagawa H, Ishii T et al (2011) Effect of cellooligosaccharide or synbiotic feeding on growth performance, fecal condition and hormone concentrations in Holstein calves. Anim Sci J 82(4):543–548

Heinrichs AJ, Jones CM, Elizondo-Salazar JA, Terrill SJ (2009) Effects of a prebiotic supplement on health of neonatal dairy calves. Livest Sci 125(2–3):149–154

Hong HA, Duc Le H, Cutting SM (2005) The use of bacterial spore formers as probiotics. FEMS Microbiol Rev 29(4):813–835

Hou C, Zeng X, Yang F, Liu H, Qiao S (2015) Study and use of the probiotic lactobacillus reuteri in pigs: a review. J Anim Sci Biotechnol 6(1):14

Humphrey BD, Klasing KC (2004) Modulation of nutrient metabolism and homeostasis by the immune system. World Poultry Sci J 60(1):90–100

Huntley NF, Nyachoti CM, Patience JF (2017) Immune system stimulation increases nursery pig maintenance energy requirements. J Anim Sci 95:68–69

Isolauri E, Sutas Y, Kankaanpaa P, Arvilommi H, Salminen S (2001) Probiotics: effects on immunity. Am J Clin Nutr 73(2 Suppl):444S–450S

Kawaguchi M, Tashiro Y, Adachi T, Tamura Z (1993) Changes in intestinal condition, fecal microflora and composition of rectal gas after administration of fructooligosaccharide and lactulose at different doses. Bifidobacteria Microflora 12(2):57–67

Kim J, Hyun Y, Sohn K, Kim T, Woo H, Han I (2000) Effects of mannanoligosaccharide and protein levels on growth performance and immune status in pigs weaned at 21 days of age. Kor J Anin Sci 42(4):489–498

Kim MH, Seo JK, Yun CH, Kang SJ, Ko JY, Ha JK (2011) Effects of hydrolyzed yeast supplementation in calf starter on immune responses to vaccine challenge in neonatal calves. Animal 5(6):953–960

Kogan G, Kocher A (2007) Role of yeast cell wall polysaccharides in pig nutrition and health protection. Livest Sci 109(1–3):161–165

Kvidera SK, Horst EA, Abuajamieh M, Mayorga EJ, Sanz Fernandez MV, Baumgard LH (2016) Technical note: a procedure to estimate glucose requirements of an activated immune system in steers. J Anim Sci 94(11):4591–4599

Kvidera SK, Horst EA, Abuajamieh M, Mayorga EJ, Fernandez MV, Baumgard LH (2017) Glucose requirements of an activated immune system in lactating Holstein cows. J Dairy Sci 100(3):2360–2374

Lawhon SD, Maurer R, Suyemoto M, Altier C (2002) Intestinal short-chain fatty acids alter salmonella typhimurium invasion gene expression and virulence through BarA/SirA. Mol Microbiol 46(5):1451–1464

Lean IJ, Wade LK, Curtis MA, Porter J (2000) New approaches to control of ruminal acidosis in dairy cattle. Asian Austral J Anim Sci 13:266–269

Li J, Li DF, Xing JJ, Cheng ZB, Lai CH (2006) Effects of beta-glucan extracted from Saccharomyces cerevisiae on growth performance, and immunological and somatotropic responses of pigs challenged with Escherichia coli lipopolysaccharide. J Anim Sci 84(9):2374–2381

Lin WC, Ptak CP, Chang CY, Ian MK, Chia MY, Chen TH et al (2020) Autochthonous lactic acid bacteria isolated from dairy cow feces exhibiting promising probiotic properties and in vitro antibacterial activity against foodborne pathogens in cattle. Front Vet Sci 7:239

Liu P, Piao XS, Kim SW, Wang L, Shen YB, Lee HS et al (2008) Effects of chito-oligosaccharide supplementation on the growth performance, nutrient digestibility, intestinal morphology, and fecal shedding of Escherichia coli and lactobacillus in weaning pigs. J Anim Sci 86(10):2609–2618

Liu H, Ji HF, Zhang DY, Wang SX, Wang J, Shan DC et al (2015) Effects of lactobacillus brevis preparation on growth performance, fecal microflora and serum profile in weaned pigs. Livest Sci 178:251–254

Liu Y, Espinosa CD, Abelilla JJ, Casas GA, Lagos LV, Lee SA et al (2018) Non-antibiotic feed additives in diets for pigs: a review. Anim Nutr. 4(2):113–125

Ma T, Suzuki Y, Guan LL (2018) Dissect the mode of action of probiotics in affecting host-microbial interactions and immunity in food producing animals. Vet Immunol Immunopathol 205:35–48

Mack DR, Michail S, Wei S, McDougall L, Hollingsworth MA (1999) Probiotics inhibit enteropathogenic E. coli adherence in vitro by inducing intestinal mucin gene expression. Am J Phys 276(4):G941–G950

Magalhaes VJ, Susca F, Lima FS, Branco AF, Yoon I, Santos JE (2008) Effect of feeding yeast culture on performance, health, and immunocompetence of dairy calves. J Dairy Sci 91(4):1497–1509

Masanetz S, Preissinger W, Meyer HH, Pfaffl MW (2011) Effects of the prebiotics inulin and lactulose on intestinal immunology and hematology of preruminant calves. Animal 5(7):1099–1106

Mikkelsen LL, Jensen BB (2004) Effect of fructo-oligosaccharides and transgalacto-oligosaccharides on microbial populations and microbial activity in the gastrointestinal tract of piglets post-weaning. Anim Feed Sci Technol 117(1–2):107–119

Mingmongkolchai S, Panbangred W (2018) Bacillus probiotics: an alternative to antibiotics for livestock production. J Appl Microbiol 124(6):1334–1346

Moeser AJ, Pohl CS, Rajput M (2017) Weaning stress and gastrointestinal barrier development: implications for lifelong gut health in pigs. Anim Nutr 3(4):313–321

Nocek JE, Kautz WP, Leedle JAZ, Allman JG (2002) Ruminal supplementation of direct-fed microbials on diurnal pH variation and in situ digestion in dairy cattle. J Dairy Sci 85(2):429–433

Pan L, Zhao PF, Ma XK, Shang QH, Xu YT, Long SF et al (2017) Probiotic supplementation protects weaned pigs against enterotoxigenic Escherichia coli K88 challenge and improves performance similar to antibiotics. J Anim Sci 95(6):2627–2639

Pinloche E, McEwan N, Marden JP, Bayourthe C, Auclair E, Newbold CJ (2013) The effects of a probiotic yeast on the bacterial diversity and population structure in the rumen of cattle. PLoS One 8(7):e67824

Pluske JR, Hampson DJ, Williams IH (1997) Factors influencing the structure and function of the small intestine in the weaned pig: a review. Livest Prod Sci 51(1–3):215–236

Posadas GA, Broadway PR, Thornton JA, Carroll JA, Lawrence A, Corley JR et al (2017) Yeast pro-and paraprobiotics have the capability to bind pathogenic bacteria associated with animal disease. Transl Anim Sci. 1(1):60–68

Rauw WM (2012) Immune response from a resource allocation perspective. Front Genet 3:267

Rodriguez-Jimenez S, Horst EA, Mayorga EJ, Kvidera SK, Abeyta MA, Goetz BM et al (2019) The what, why, and physiologic cost of leaky gut syndrome. Proc Am Assoc Bovine Pract 52(2):165–171

Ryman VE, Nickerson SC, Kautz FM, Hurley DJ, Ely LO, Wang YQ et al (2013) Effect of dietary supplementation on the antimicrobial activity of blood leukocytes isolated from Holstein heifers. Res Vet Sci 95(3):969–974

Shen YB, Carroll JA, Yoon I, Mateo RD, Kim SW (2011) Effects of supplementing Saccharomyces cerevisiae fermentation product in sow diets on performance of sows and nursing piglets. J Anim Sci 89(8):2462–2471

Shin D, Chang SY, Bogere P, Won K, Choi JY, Choi YJ et al (2019) Beneficial roles of probiotics on the modulation of gut microbiota and immune response in pigs. PLoS One 14(8):e0220843

Smiricky-Tjardes MR, Grieshop CM, Flickinger EA, Bauer LL, Fahey GC Jr (2003) Dietary galactooligosaccharides affect ileal and total-tract nutrient digestibility, ileal and fecal bacterial concentrations, and ileal fermentative characteristics of growing pigs. J Anim Sci 81(10):2535–2545

Sun P, Wang JQ, Zhang HT (2010) Effects of Bacillus subtilis natto on performance and immune function of preweaning calves. J Dairy Sci 93(12):5851–5855

Szymanska-Czerwinska M, Bednarek D, Zdzisinska B, Kandefer-Szerszen M (2009) Effect of tylosin and prebiotics on the level of cytokines and lymphocyte immunophenotyping parameters in calves. Cent Eur J Immunol 34(1):1–6

Thrune M, Bach A, Ruiz-Moreno M, Stern MD, Linn JG (2009) Effects of Saccharomyces cerevisiae on ruminal pH and microbial fermentation in dairy cows yeast supplementation on rumen fermentation. Livest Sci 124(1–3):261–265

Timmerman HM, Mulder L, Everts H, van Espen DC, van der Wal E, Klaassen G et al (2005) Health and growth of veal calves fed milk replacers with or without probiotics. J Dairy Sci 88(6):2154–2165

Tran THT, Everaert N, Bindelle J (2018) Review on the effects of potential prebiotics on controlling intestinal enteropathogens salmonella and Escherichia coli in pig production. J Anim Physiol Anim Nutr (Berl) 102(1):17–32

Tzortzis G, Goulas AK, Gee JM, Gibson GR (2005) A novel galactooligosaccharide mixture increases the bifidobacterial population numbers in a continuous in vitro fermentation system and in the proximal colonic contents of pigs in vivo. J Nutr 135(7):1726–1731

Uyeno Y, Shigemori S, Shimosato T (2015) Effect of probiotics/prebiotics on cattle health and productivity. Microbes Environ 30(2):126–132

van der Most PJ, de Jong B, Parmentier HK, Verhulst S (2010) Trade-off between growth and immune function: a meta-analysis of selection experiments. Funct Ecol 25(1):74–80

Vighi G, Marcucci F, Sensi L, Di Cara G, Frati F (2008) Allergy and the gastrointestinal system. Clin Exp Immunol 153(Suppl 1):3–6

Vogt SL, Pena-Diaz J, Finlay BB (2015) Chemical communication in the gut: effects of microbiota-generated metabolites on gastrointestinal bacterial pathogens. Anaerobe 34:106–115

Wang XX, Song PX, Wu H, Xue JX, Zhong X, Zhang LY (2016) Effects of graded levels of isomaltooligosaccharides on the performance, immune function and intestinal status of weaned pigs. Asian-Austr J Anim Sci 29(2):250–256

Wu Y, Zhu C, Chen Z, Chen Z, Zhang W, Ma X et al (2016) Protective effects of lactobacillus plantarum on epithelial barrier disruption caused by enterotoxigenic Escherichia coli in intestinal porcine epithelial cells. Vet Immunol Immunopathol 172:55–63

Xiao Z, Trincado CA, Murtaugh MP (2004) β-Glucan enhancement of T cell IFNγ response in swine. Vet Immunol Immunop 102(3):315–320

Yan F, Polk DB (2011) Probiotics and immune health. Curr Opin Gastroenterol 27(6):496–501

Yang F, Hou C, Zeng X, Qiao S (2015) The use of lactic acid bacteria as a probiotic in swine diets. Pathogens 4(1):34–45

Zhang L, Xu YQ, Liu HY, Lai T, Ma JL, Wang JF et al (2010) Evaluation of lactobacillus rhamnosus GG using an Escherichia coli K88 model of piglet diarrhoea: effects on diarrhoea incidence, faecal microflora and immune responses. Vet Microbiol 141(1–2):142–148

Gut Microbiota and the Gut–Brain Axis in Neonatal Calves: Implications for Psychobiotic Usage for Stress Regulation

15

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Abstract

Psychobiotics are a type of probiotic that affect cognitive and behavioral functions of the host via the gut–brain axis. Proposed mechanisms of action of psychobiotics include the modulation via the hypothalamus–pituitary–adrenal axis (HPA) axis, direct immune effects, and various neural, hormonal, and metabolic pathways linked to gut microbiota. Growing evidence demonstrates that psychobiotics such as various *Lactobacillus, Bifidobacterium,* and *Streptococcus* species confer benefits for cognitive function, immunomodulation, and treatment or prevention of mental disorder such as depression, anxiety, and various altered mood or emotional states. There is a potential role for psychobiotics to improve immune function and development, production performances, and welfare during early life in livestock animals. Studies in rodents have analyzed the effects of probiotics on behavior and welfare, yet there are no studies on the potential use of psychobiotics in livestock animals for enhanced health and production. This chapter critically evaluates the potential use of psychobiotics as a method to reduce the effects of early life stress on neonatal ruminants and its impact on host susceptibility to infections. The effect of early feeding (colostrum, milk, psychobiotic administration) on the microbiome-GBA of dairy calves is discussed to understand the impact of early

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life stressors on postnatal development, along with relevant neuroendocrine interactions of host and microbes.

Keywords

Psychobiotics · Gut–brain axis · Gut microbiome · Calves

15.1 Introduction

The establishment of microbiome during early life plays a vital role in host health throughout life. The animal body can be considered as a superorganism in which eukaryotic cells and prokaryotic organisms interact and coexist, usually in a symbiotic state (Evrensel and Tarhan 2021). Extensive research in humans has reported that perinatal factors (immediately before and after birth) such as length of gestation (pre-mature vs. termed), labor duration, mode of delivery (C-section vs. vaginal), feeding method (breast-feeding vs. formula feeding), and exposure to antibiotics significantly affect the development and composition of the microbiome in newborn infants (Renz et al. 2017; Dong and Gupta 2019; Vu et al. 2021). Moreover, deviations in the establishment of microbial communities have been linked to an increased risk of developing microbiome-linked pathologies (allergies, asthma, obesity, cardiovascular disease, mental illness) later in life (Amenyogbe et al. 2017; Vu et al. 2021). The microbiome is not only a substantial element of immune activation and metabolism but also affects host behavior via the gut–brain axis (GBA) (Codagnone et al. 2019). While abnormal brain development and elevated stress responses are evident in germ-free mice (Sudo et al. 2004), early life stress has been linked to perturbed fecal microbial composition (O'Mahony et al. 2009; Park et al. 2021). These studies together indicate a bi-directional relationship that may lead to the co-development of microbiota and the neuroendocrine system during early life.

The establishment of microbiota in ruminant species (cattle, sheep, goats) is influenced by diet (Malmuthuge et al. 2015; Maynou et al. 2019; Fischer et al. 2018; Song et al. 2019; Bi et al. 2019) and exposure to the dam (Abecia et al. 2014, 2017). Similar to human studies, perturbed microbial communities during early life have been linked to neonatal calf diarrhea, also known as scours (Gomez et al. 2017). However, there is still a lack of understanding regarding the relationship between early microbial perturbations and GBA in neonatal calves.

Probiotics are defined as "live microorganisms that, when administered in adequate amounts, confer a health benefit to the host" (FAO 2001). Psychobiotics are probiotics that confer mental-health-specific benefits, especially in the regulation of neurofunctioning in response to gut dysbiosis and host disease states (Tremblay et al. 2021). As understanding grows regarding the interactions between microbes and the host via the GBA, the potential for a true "pursuit of happiness" or, more accurately, welfare improvement is realized by psychobiotic research performed in human and rodent models (Zhou and Foster 2015). The emerging evidence suggests that

psychobiotics could treat neuropsychiatric and physiological disorders related to depression, anxiety, early life stress, and abnormal emotional states to re-establish more neuro-normal ("healthy") physiological states in calves (Tremblay et al. 2021). This chapter will focus on the impact of early feeding (colostrum, milk, psychobiotic administration) on the microbiome-GBA of dairy calves to understand the impact of early life stressors on postnatal development. Psychobiotics will be explored as a potential regulator of stress response and disease incidence in the neonatal calf, and relevant interactions between the GBA will be discussed.

15.2 Neuroendocrine Hormone Production

Production of stress hormones is regulated via the neuroendocrine system by the activation of two central axes: the hypothalamus–pituitary–adrenal (HPA) axis and the sympathetic–adrenal–medullary (SAM) axis (Chen et al. 2015), which result in the release of glucocorticoids in the adrenal cortex and catecholamines (norepinephrine and epinephrine) in the adrenal medulla, respectively. In addition, human and rodent studies suggest extra-adrenal production of stress hormones such as glucocorticoids (GCs) also occurs in the intestinal mucosa and other tissues (Kostadinova et al. 2014; Noti et al. 2009; Talaber et al. 2013). The process of glucocorticoid production is initiated by the production of corticotropin-releasing hormone (CRH) and arginine vasopressin in the hypothalamus, which stimulates the production of adrenocorticotropic hormone (ACTH) in the pituitary gland (Charmandari et al. 2005; Chen et al. 2015). Once produced, ACTH triggers glucocorticoid and adrenergic production in the zona fasciculata in the adrenal cortex and adrenal medulla, respectively (Charmandari et al. 2005). Appropriate responses to stressors are crucial modulators of physiological maintenance and the overall performance of the host.

The activation of the HPA axis to produce glucocorticoids can be triggered by various environmental stressors and different types of agents such as neurotransmitters, cytokines, damage-associated molecular pattern (DAMPs), and microbe-associated molecular patterns (MAMPs) (Blalock and Smith 2007; Dantzer et al. 2008; Fleshner 2013; Matthews 2002). Glucocorticoid-induced signaling via the HPA axis has been shown to play an essential regulatory role in stress and immune response. Glucocorticoids have a stimulatory effect on immune response under acute stress by preventing inflammation, whereas exposure to chronic stress results in an immunosuppressive glucocorticoid-induced impact (Cruz-Topete and Cidlowski 2015; Dhabhar and McEwen 1999; Sorrells and Sapolsky 2007). During the first few days of life, GCs provide homeostatic feedback to support the underdeveloped immune system of neonates and prevent infectious diseases (Hulbert and Moisa 2016). In a rodent model, chronic (6 days) administration of corticosterone resulted in suppression of antigen-specific cell-mediated immunity, compared to acute (4 h) administration of the same dose of corticosterone, which had a stimulative effect on immune response (Dhabhar and McEwen 1999). Under normal physiological conditions, GCs have been shown to have an immune-enhancing

effect and stimulate the development of various organs such as GIT and respiratory system during the neonatal stage (Hulbert and Moisa 2016). For example, the glucocorticoid receptor gene knockout mice showed impaired lung maturation in neonates leading to respiratory failure and death (Kadmiel and Cidlowski 2013). Therefore, it is now evident that modulation of neuroendocrine system and GBA during early life is crucial for the neonatal development.

15.3 Role of the Neuroendocrine System in Neonatal Development

There has been an increasing interest in regulating energy metabolism and feed intake by the neuroendocrine system during the neonatal period. An in vitro study reported that stimulation of adrenergic receptors with epinephrine induced the intestinal secretion of the peptide GLP-1 in rodents (Claustre et al. 1999), which regulates metabolic processes. Connor et al. (2015) suggested that biological actions and properties of glucagon-like peptides in ruminants are similar to those in non-ruminants. Recent studies in calves reported that extended feeding colostrum increased the expression of plasma GLP-1 (Inabu et al. 2019) and GLP-2 (Pyo et al. 2020) as well as intestinal growth (Pyo et al. 2020) compared to whole milk feeding immediately postnatal. Adrenergic and serotonin receptors also regulate the secretion of GLP-2 by enteroendocrine L cells, which increases nutrient absorption and intestinal growth (Burrin et al. 2003; Connor et al. 2015; Drucker 2001). Schaff et al. (2015) reported that the binding capacity of glucocorticoid and β2-adrenergic receptors was lower in preterm calves than calves born at term, suggesting the expression of stress hormone receptors is dependent on the stage of maturation in neonatal calves.

Furthermore, the importance of stress hormones in the prenatal and postnatal development of various organs has been widely studied over the last few decades. GCs play a role in fetal and postnatal development of the brain, lungs, and GIT. Their physiological role is to regulate circadian and stress-associated feedback to maintain metabolic and homeostatic functions critical for life. However, stress hormones have unique roles in the regulation of immune responses during the neonatal period. Mammalian neonates typically have elevated concentrations of GCs in the first few days of life (Hulbert and Moisa 2016). Elevated GCs during the neonatal stage play a role in the maturation of the GIT and respiratory tract by modulating tight junction and formation of mucus layer (Hulbert and Moisa 2016). There is a need for a comprehensive investigation of the effect of stress hormones on neonatal development and their mutual influence on one another.

15.4 Gut–Brain Axis

Recent findings on the impact of gut microbiota on behavior, brain development, and the central nervous system have given rise to GBA theory. To maintain homeostasis within the GIT, the apical side of the intestinal epithelium is covered with a mucus layer that functions as a physical and chemical barrier to compartmentalize microbiota and other dietary antigens (Specian and Oliver 1991). Moreover, immune and epithelial cells of the intestinal wall are also equipped with various types of pattern recognition receptors (PRRs) located on membrane and cytoplasm, which allow them to recognize microbes based on specific molecular patterns produced by microbes (Abreu 2010). Recognition of these bacterial products triggers a cascade of either pro-inflammatory or anti-inflammatory responses (depending on the microbe recognized), which results in the production and secretion of cytokines, neurotransmitters, and GCs (Sandor and Buc 2005). PRRs also recognize damage-associated molecular patterns secreted by host cells following the activation of pro-inflammatory responses resulting from local tissue damage (Land 2015). Thus, the immune system is well equipped for microbial recognition, allowing efficient communication at the host–microbial interface.

Studies as early as the nineteenth century have demonstrated that bi-directional communication between the brain and the gut can alter emotional and physiological states as well as GIT functions in the host (Zhou and Foster 2015). Now as termed the GBA, it is evident that this results in profound effects on host behavior, physical health, and neural functioning (Toro-Barbosa et al. 2020). While mechanisms are not fully elucidated, this complex interplay involves multiple systems including the GIT and its microbiota, the central, autonomic, and enteric nervous systems, various immune functions, and the neuroendocrine system (Tremblay et al. 2021). It has been recently proposed that gut microbiota can contribute to this biochemical signaling by producing hormone-like metabolites, such as neurotransmitters and stress hormones, which can be used by host cells in systemic responses (Clarke et al. 2014). Moreover, some of these metabolites have been shown to stimulate bacterial proliferation. For example, catecholamines (epinephrine, norepinephrine) contribute adversely to the proliferation of gram-negative pathogens (Freestone et al. 2000, 2002).

An individual's microbial profile is influenced continually by a variety of factors including host gene expression, age, sex, diet, developmental state, and health status (Zhou and Foster 2015). Evidence suggests that both chronic and acute stress events can induce gut microbial dysbiosis, resulting in reduced gut motility and function imbalanced cytokine profiles, and increased permeability (Soldi et al. 2019). Oral administration of a multi-strain probiotic product (*Lactobacillus*, *Bifidobacterium*, *Bacillus*) has, however, improved barrier functions and reduced inflammation in stressed individuals (Soldi et al. 2019), indicating the potential to use probiotics to mitigate stress-driven negative effects. Lv et al. (2021) suggest that microbial shifts in the gut are related to increased bacterial infections, antibiotic administration, and altered cognitive stress responses. This gut microbiota shift can have profound effects on the host ranging from altered metabolic, immune, and neuropsychiatric

function to gastrointestinal disorder. Increasing evidence in germ-free animals has pointed toward the importance of microbiota in regulating neural function, development, and behavior. In a study performed by Luczynski et al. (2016), germ-free mice were shown to have significant morphological and structural abnormalities in the brain compared to normal mice, particularly in the amygdala and hippocampus regions. In these germ-free animals, maladaptive stress responses are those which cause undue physiological or psychological harm to the animal, such as self-harm behaviors, persistent chronic stress indicators (such as high blood cortisol), and psychological disorders such as severe aggressive, fear-based, or depressive symptoms. The previous study suggests that microbiota play a crucial role in typical central nervous system (CNS) development. The absence of microbiota may contribute to these maladaptive stress responses and behavioral profiles observed in germ-free animals.

Furthermore, Luczynski et al. (2016) and Sudo et al. (2004) highlighted that microbiota might play a crucial role in social and stress-related behaviors, as well as emotional and psychiatric disorders. Sarkar et al. (2016) described that the brain and gut together maintain host health, including physical processes (such as immunomodulation, inflammation, adiposity, and energy balance) and mental processes (such as motivation, emotional and cognitive function, and stress responses). In a study performed by Xie (2017), fecal microbiota transplantation from patients with major depressive disorder resulted in depressive and anxious behaviors in mice but not when transplantation was done using feces from healthy individuals (Xie 2017). This study indicates the importance of gut microbial composition in maintaining host behavior and health. These findings suggest gut microbiota may contribute to the alteration of host physiology through the GBA. However, few studies have investigated the role of gut microbiota in the GBA and the relationship of gut microbiota and host stress response in neonatal calves to date.

15.4.1 Role of Gut Microbiota in Gut–Brain Axis

Commensal microbiota protects the host from pathogens by competing for nutrients and space and by secreting antimicrobial substances such as SCFA or bacteriocins (Buffie and Pamer 2013; Tenaillon et al. 2010). The absence of gut microbiota and perturbed microbial communities have been associated with poor functioning of the immune system, reduced metabolism, underdevelopment of the GIT, and psychological disorder (Hanning and Diaz-Sanchez 2015), suggesting the importance of microbiota in early development due to the potential long-term health and physiological effects on the host. The histological and physiological changes of the GIT in the absence of gut microbiota have been widely studied on germ-free animals. Sudo et al. (2004) reported that exposure to microbes at an early stage of life is crucial for postnatal development of HPA axis, and neural pathways related to stress responses in mice can be modulated by gut microbiota. Furthermore, the colonization of germ-free mice using specific-pathogen-free microbiota or fecal microbiota produced serotonin (a neurotransmitter primarily synthesized in the GIT) in the gut (Hata

et al. 2017), indicating the importance of gut microbial colonization in modulating a wide range of neural activities. Serotonin is derived from tryptophan by the tryptophan hydroxylase (TPH1) enzyme. Plasma concentration of tryptophan, the precursor of serotonin, significantly increased under stress conditions in Bifidobacteria-treated rats (Desbonnet et al. 2008). In addition, Reigstad et al. (2015) reported that indigenous and human-derived gut microbiota increased TPH1 at both protein and gene expression levels in mice. Reigstad et al. (2015) also reported that microbiota-derived SCFAs could affect the expression of TPH1 protein and genes in human embryonal carcinoma (EC) cells. Besides serotonin, GC levels are higher in germ-free mice, and the administration of probiotics such as *Bifidobacterium infantis* reduces plasma GCs in treated animals (Sudo et al. 2004). There is a lack of studies to evaluate the effect of calf gut microbiota on neurotransmitters and stress hormones. However, recently we reported that the abundance of *Lactobacillus* and *E. coli* in the colon of dairy calves was positively correlated to the expression of serotonin receptor (*SLC4A4*) and α-adrenergic receptor (*ADRA1A*) (Hromádková et al. 2020), indicating potential associations between early life gut microbiota and GBA. While this study is based on correlation, it is vital to understand further the causal (bi-directional) relationship between early gut microbiota and stress in modulating the GBA of neonatal calves.

Furthermore, Luczynski et al. (2016) and Sudo et al. (2004) highlighted that microbiota may play a crucial role in social and stress-related behaviors, as well as emotional and psychiatric disorders. Sudo et al. (2004) demonstrated that, in response to stressors, germ-free mice had overactive HPA axis activity. Hyperactivity of the HPA axis is one of the most consistent findings in patients diagnosed with stress-related disorders such as anxiety and depression (Chen et al. 2015). Additionally, it is speculated that the HPA axis is susceptible to developmental reprogramming in early life (Chen et al. 2015). Sudo et al. (2004) found that this overactive HPA could be reversed by administering *Bifidobacterium infantis,* a commonly used probiotic, suggesting the potential use of probiotics as psychobiotics to reverse adverse effects of microbiota driven stress-related changes in the host. Depression and anxiety-related disorders have been associated with shifts in the production of neurotrophic factors.

In 2013, Dinan et al. proposed the term "psychobiotics" as a novel class of probiotics that could be utilized specifically for mental health. Some applications of psychobiotics performed to date are described in Table 15.1. Tian et al. (2020) proposed that treatment with *Bifidobacterium breve* alone or alongside antidepressants could be used to treat chronic depression. In this study, *B. breve* was shown to reverse chronic stress-induced depressive symptoms, microbial abnormalities, and dysbiosis while producing antidepressant-like effects pertaining to mood, memory, and motivation. The authors further reported that the administration of *B. breve* reduced the hyperactivity of the HPA axis and inflammation via modulating glucocorticoid receptors (Tian et al. 2020). Interestingly, the effect of *B. breve* on mitigating hyperactivity of the HPA axis was similar to that of chronically stressed mice receiving antidepressants. Desbonnet et al. (2010) performed a maternal separation model in 33 rat pups administered either a control, an SSRI, or

Table 15.1 Psychobiotic strains used to date, including behavioral, physiological, and measurable results from treatment groups in different animal models

Study model	Psychobiotic	Observation summary	References
ELS mice $N = 10$/group	*Lactobacillus plantarum PS128*	Increased locomotor activity, dopamine, and serotonin levels in prefrontal cortex Reduced anxiety and depression like behaviors, corticosterone levels	Liu et al. (2015)
Male SPF CRS rats $N = 22$/group	*Lactobacillus helveticus*	Increased serotonin and BDNF expression in hippocampus Reduced anxiety, depression, cognitive dysfunction, plasma cortisol, and acetylcholine levels	Liang et al. (2015)
Healthy male volunteers (human) $N = 22$	*Bifidobacterium longum* 1714	Improved hippocampus dependent memory performance Reduced stress	Allen et al. (2016)
Male BALB/c mice $N = 16$/group	*Lactobacillus rhamnosus*	Increased GABA expression in cortical regions Reduced anxiety, depression, plasma cortisol levels, GABA expression in hippocampus, amygdala, and locus coeruleus	Bravo et al. (2011)
Men and women $N = 55$ Male Wister rats $N = 12$/group	*Lactobacillus helveticus* and *Bifidobacterium longum* R0175	Rats: reduced anxiety like behavior Humans: reduced somatization, anxiety, depression, anger-hostility, urinary free cortisol level Increased problem solving and memory	Messaoudi et al. (2011)
961 women, mainly healthcare workers (20–71 years old)	*Lactobacillus delbrueckii* ssp. *bulgaricus OLL1073R-1*	Based on survey results, treatment groups reported and enhanced quality of life by improved quality of sleep and reduced incidence of gastrointestinal disease	Kinoshita et al. (2021)

the psychobiotic *Bifidobacterium infantis*. Control animals exhibited typical stress signs, including reduced performance (reduced feed intake and growth), increased inflammatory markers, and reduced immune function. In pups who received the psychobiotic, these effects were significantly reduced and even normalized in some animals, suggesting that major stressor events could have a minimized effect on host health with the administration of psychobiotics.

Psychobiotics have also been proposed as a method to regulate the GBA in early life. Microbial populations in the gut and central nervous system co-develop during the first few years of an animal's life, suggesting a role for psychobiotics as an early intervention to improve health and welfare. In humans, perturbations of microbiota establishment during early life (such as with antibiotic- or stress-induced-dysbiosis)

are linked to the development of a variety of mood disorders such as major depressive disorder, anxiety, post-traumatic stress disorder, and bipolar disorder later in life (Tremblay et al. 2021). Overall, the application of psychobiotics in human and rodent trials has shown promise in various applications, from improving cognitive functioning to increased gut health. Since psychobiotics are essentially probiotics with mental health-specific effects, specific probiotic strains (such as *Bifidobacterium longum* and *Saccharomyces cerevisiae)* already utilized in animal production can be combined for psychobiotic effect. Using the information from these trials, an application could translate to production animals such as dairy and beef calves to improve mental health parameters associated with animals of economic importance. Since there is a link between gut dysbiosis, mental disorder, and reduced immune function in animals, psychobiotics could act as a method to improve stress robustness and improve various production parameters overall.

15.4.2 Effect of Pathogenic Bacteria on Host Health and Development in GBA

Previous studies suggested an association between pathogenic bacteria and the production or alteration of the function of neurotransmitters such as catecholamines and serotonin. It has been proposed that eukaryotic neurotransmitters such as norepinephrine promote growth and virulence of pathogenic bacteria in the intestine (Freestone et al. 2000). This process can be regulated either via recognition of norepinephrine by adrenergic receptors on the basolateral side of the intestinal epithelium of the host (Green and Brown 2016) or through the bacterial adrenergic receptor, QseC sensor kinase (Clarke et al. 2006). Freestone et al. (2007) reported catecholamines produced by the enteric nervous system as host-derived signals stimulated the growth of pathogenic bacteria such as *E. coli*, *Salmonella enterica,* and *Yersinia enterocolitica*. Furthermore, it has been suggested that catecholamines such as epinephrine and norepinephrine can affect chemotaxis, biofilm formation, motility, gene expression, and growth of *E. coli* O157:H7 (Bansal et al. 2007), an economically important pathogen for the agriculture industry due to significant public health risks. Pasupuleti et al. (2014) described the principle of chemotaxis at which norepinephrine is converted into 3,4-dihydroxymandelic acid, a strong attractant for *E. coli*. Pasupuleti and colleagues (2014) suggested that norepinephrine may have an indirect role in chemotaxis by inducing the synthesis of bacterial enzymes that generate 3,4-dihydroxymandelic from norepinephrine.

Moreover, Enteropathogenic *E. coli* (EPEC) infection has been shown to affect the function and expression of the serotonin transporter (Esmaili et al. 2009). This transporter functions on sodium- and chloride-dependent mechanisms and has been localized on the apical and basolateral sides of the intestinal epithelium (Martel et al. 2003). Interestingly, activation of serotonin transporter decreased by 53% on the apical side of a human intestinal cell infected by EPEC infection. In contrast, the activity of this transporter on the basolateral was less affected (Esmaili et al. 2009). These findings suggest that serotonin is transported via the serotonin transporter

either from basal-to-apical or apical-to-basal sides of the enteric epithelium. Pathogenic *E. coli* can disrupt its transfer mainly by inhibiting serotonin transporter on the apical side. A recent study revealed that serotonin decreased the virulence of enterohemorrhagic *E. coli* (EHEC) by reducing the expression of enterocyte effacing gene that facilitates attachment and effacing to host epithelial cells (Kumar et al. 2020). Moreover, serotonin reduced the expression of the same gene in *Citrobacter rodentium* (enteric pathogen in mouse) (Kumar et al. 2020), suggesting that this neurotransmitter affects the pathogenesis of enteric pathogens. Enteric infection by *E. coli* (ETEC) is common in neonatal calves and it would be interesting to understand the role of stress and GBA in modulating calf resilience to enteric infections.

15.5 Proposed Mechanisms of Psychobiotics

As described above, psychobiotics have been proposed as a method to reduce or alleviate the effects of gut dysbiosis on mental health. Much of the psychobiotic research performed to date has been performed by inducing stress and utilizing behavioral analysis to assess motivation and emotional state in relation to that stressor (Cheng et al. 2019). Lower *Bifidobacterium* and *Lactobacillus* species are observed in patients with major depressive disorder and Alzheimer's disease, while decreased *Blautia, Roseburia,* and *Coprococcus* counts are apparent in individuals with Parkinson's disease (Cheng et al. 2019). A variety of psychobiotics (including but not limited to *Lactobacillus, Bifidobacterium,* and *Streptococcus*; Table 15.1) have been studied in human and animal trials, with varying efficacy. While psychobiotic effects have been proven in various studies to date, the exact mode of action in successful strains is not yet fully understood. Recently, Toro-Barbosa et al. (2020) proposed four different mechanisms that psychobiotics enhance host functioning by stimulating the enteric nervous system or immune system: (1) hypothalamic–pituitary–adrenal (HPA) axis stress response stimulation, (2) direct effects on the immune system and inflammation, (3) molecular secretions including neurotransmitters, proteins, and short-chain fatty acids (SCFAs), and (4) balancing the microbiome.

15.5.1 Effect on the Gut–Brain Axis

The HPA axis includes complex interactions between the hypothalamus, pituitary gland, adrenal cortex, regulatory inputs, and secreted factors and hormones (De Santis et al. 2017). Toro-Barbosa et al. (2020) suggest that gut microbiota dysbiosis can lead to the activation of the HPA axis, increasing the upregulation of stress molecules such as cortisol and corticosterone. This chronic stress leads to the overproduction of cortisol, increasing threat sensitivity, negative mood, impaired memory, and other cognitive functions. This further inhibits immune activity, increasing inflammation and gut permeability. As a result, neuroendocrine

functioning is altered with shifts in microbial populations in the gut. Such mechanisms have been detailed in the above sections. Therefore, utilizing psychobiotics to downregulate aspects of the HPA axis shows promise in treating neuropsychiatric disorders by addressing chronic stress indicators at the root.

15.5.2 Immune Function

Aberrant immune responses have historically been linked to gut microbial dysbiosis (Zhou and Foster 2015). Metabolites from gut microbes absorbed through intestinal barriers stimulate immune cells and pro-inflammatory members. Inflammation has been linked to various adverse disease events in the host, including higher psychological disorders and intestinal disease. Luang-In et al. (2020) performed a study on rats either by administering antibiotics alongside a psychobiotic or only antibiotics. Rats who received the antibiotic alone showed increased inflammation and aberrant behavior, including higher anxiety levels, altered social behaviors, and increased aggression. Administration of psychobiotic along with antibiotics, however, prevented behavior shifts and decreased inflammation compared to the antibiotic-only group. This suggests a neuro-health benefit was afforded to psychobiotic administered subjects based on interactions with the immune system.

15.5.3 Production of Molecules

Host and microbial production of molecules in the body (Table 15.2) may also provide evidence for the mechanical action of psychobiotics (Zhou and Foster 2015). Bacteria can communicate in a process known as quorum sensing, which utilizes hormone-like signals to mediate commensal and pathogenic relationships between gut microbes and mammalian hosts (Sudo 2019). Essentially, quorum sensing allows cell populations to respond to changes in their environment, resulting in an alteration of gene expression to the most beneficial phenotypes as a method of communication between the gut–brain axis. As a result, this communication mechanism can detect altered levels of host and microbial molecules and allow for microbes to upregulate or downregulate functions such as production or suppression of metabolites, hormones, proteins, and other psychiatric and gut regulating molecules. Molecules such as SCFA, serotonin, dopamine, BDNF, epinephrine can impact host-bacterial communication and result in mental changes associated with altered gene expression. Some strains of *Lactobacillus* and *Bifidobacterium* spp. produce GABA, serotonin, acetylcholine, and dopamine, and it has recently been found that serotonin synthesis in the gut is at least partially regulated by microbial activity (Cheng et al. 2019). Psychiatric conditions are often related to pro-inflammatory markers in the body, enhancing the blood–brain barrier's permeability, altering hormone levels in the brain (Cheng et al. 2019). Depression, in particular, is related to a decrease in serotonin and dopamine in the brain, and as a result, it makes sense that microbes might evolve to produce these molecules to ensure a healthy host environment for

Table 15.2 Molecules in the body related to disordered neurofunctioning or health in the host

Molecules	Effect on body	Where is it produced?	Contribution to disordered function in the host
Serotonin	Regulates behavioral and biological functions like mood and positive emotional processing (Toro-Barbosa et al. 2020) Contributes to the central nervous system (CNS) and peripheral tissue processes May promote normal gut function (such as intestinal motility, absorption, and transit)	Produced in neurons originating in the brain stem Enterochromaffin cells in intestinal epithelium produce serotonin to regulate intestinal fluid secretion in the gut (Alcaino et al. 2018) Produced by many *Lactobacillus* and *Bifidobacterium* spp. (Yano et al. 2015)	Decreased serotonin levels may contribute to psychiatric disorders like anxiety and depression (Jacobs 1994)
Dopamine	Linked to prefrontal cortex-dependent function regulators like attention, decision making, and inhibitory control. (Toro-Barbosa et al. 2020) Related to motivation and reward systems	Produced in hypothalamus Derived from tyrosine, an amino acid. Gut microbiota generate free dopamine in the lumen.	Altered dopamine levels may contribute to psychiatric disorders like anxiety and depression. (Needham et al. 2020)
Epinephrine	Linked to prefrontal cortex-dependent function regulators like attention, decision making, and inhibitory control (Toro-Barbosa et al. 2020) Related to homeostatic function in the body including cardiac and pulmonary output during times of stress	Produced in adrenal glands Derived from tyrosine, an amino acid Gut microbiota may generate free norepinephrine in the lumen	Altered stress and fear responses
Gamma-Aminobutyric Acid (GABA) and Glutamate	Coordinate to control excitatory and inhibitory neural transmission in the CNS (Toro-Barbosa et al. 2020) Glutamate excites neuron activity while GABA inhibits (Jacobs 1994) Important in learning, memory, synapse plasticity, and neural excitatory function	GABA made in brain cells from glutamate Produced by many *lactobacillus* strains (Samardzic et al. 2018)	Altered cognitive retention, learning, behavior

(continued)

Table 15.2 (continued)

Molecules	Effect on body	Where is it produced?	Contribution to disordered function in the host
Acetylcholine	Influences the brain via: affecting synaptic plasticity, reinforcing neuronal loops as well as cortical dynamics during learning, altered neuronal thresholds, and greatly increases neuron firing rate in response to environmental stimuli (Toro-Barbosa et al. 2020)	Synthesized in cytoplasm of nerve cells Produced in a strain of *Lactobacillus plantarum* (Liu et al. 2015)	Altered neuromodulation in the brain
Short Chain Fatty Acids (SCFAs)	Metabolic substrate to govern host cellular metabolism—specifically lipid metabolism and adipose tissues, increase structural integrity of epithelial barrier, maintain proper immune function (Toro-Barbosa et al. 2020) Increases blood-brain-barrier structural integrity, regulates neurotransmission, alters neurotrophic factors, and increases memory consolidation (Morrison and Preston 2016) New research suggests a role in gut-brain axis signaling (Silva et al. 2020)	Carbohydrates, mainly nondigestible, that escape small intestine digestion/absorption are fermented by gut microbiota Produced by many microbial spp. in the gut	May contribute to gut disorders in animals such as acidosis and bloat, which in turn can affect behavior
Brain-derived neurotrophic factor (BDNF)	Governs specific neuronal populations by affecting viability and functional integrity (Toro-Barbosa et al. 2020) Influences neuronal survival and differentiation and in the CNS Promotes survival after insults to cells	Produced in endoplasmic reticulum of brain cells	Altered levels can disrupt synaptic transmission and plasticity. Gut microbiota can change behavior by changing BDNF expression in the brain (Toro-Barbosa et al. 2020) Associated with psychiatric disorder May also affect normal

(continued)

Table 15.2 (continued)

Molecules	Effect on body	Where is it produced?	Contribution to disordered function in the host
			neurodevelopment such as neurogenesis, synaptogenesis, synaptic maturation, and neural activity (Needham et al. 2020)

colonial growth. Notable molecules are described in Table 15.2, including their effect on the host body, where it is produced, and how altered production in the body or via microbial activity could contribute to disordered function in the host.

15.5.4 Balancing the Microbiome

Gut dysbiosis and dysregulation in the microbiota have been linked to various psychiatric and physiological disorders, including irritable bowel syndrome, depression, anxiety, schizophrenia, attention-deficit/hyperactivity disorder (Martins-Silva et al. 2021). One of the mechanisms of psychobiotics is their direct impact on restore or rebalance of the microbial dysbiosis.

One important thing to consider is the capacity for colonization of the psychobiotics in the gut. Additionally, it is important to understand the interaction of psychobiotics with the resident microbiota and host-derived specificity. Host-derived specificity is a concept in which a probiotic strain originates from the animal species receiving the administered strain (host-specific psychobiotics) (Dogi and Perdigón 2006). For example, feeding *Bifidobacterium* isolated from mice may not work on calves. However, feeding calves with calf-derived *Bifidobacterium* strains are generally considered more likely to colonize. One crucial aspect to consider is whether psychobiotics genuinely need to take up residence in the gut to have a positive effect. Even disruptions in the established microbiota (caused by antibiotics or foodborne illness), the microbiome tends to show resilience to colonization from foreign microbes historically not endemic to the host environment (Leclercq et al. 2017). As a result, prolonged colonization of probiotics is not required long-term if benefits can be incurred by the production of metabolites, interactions with natural flora, promotion of immune responses against specific microbes, and direct stimulation of intestinal epithelium (Sanders 2011).

15.6 Models Used to Study Psychobiotics

To date, rodent and human models have dominated the psychobiotic research world. Increasing evidence in germ-free (i.e., sterile intestinal environment) animals has pointed toward the importance of microbiota in the regulation of neural function, development, and behavior. In a study performed by Luczynski et al. (2016), germ-free mice were shown to have significant morphological and structural abnormalities in the brain compared to normal mice, particularly in the amygdala and hippocampus regions. This suggests that microbiota plays a crucial role in normal CNS development, and the absence of normal flora may contribute to the maladaptive stress responses and behavioral profiles observed in germ-free animals. Links between alterations in neurotrophic factors and corresponding depression have been found in germ-free mice, with the implication that gut microbial shifts in the animal led to altered serotonin production, which in turn affected regions associated with mood, motivation, behavior, and neuro-disorder (Dinan and Cryan 2013). Mice genetically engineered to have altered BDNF signaling display enhanced aggression, altered cognitive behavior, and poor response to antidepressant treatments (Maynard et al. 2016). Since many antidepressant treatments are selective serotonin reuptake inhibitors (SSRIs), which promote the uptake of serotonin into the brain for regulation of mood and positive emotional processing, it is reasonable that knockout mice may have impaired serotonin uptake if BDNF levels are negatively affecting microbial populations in the gut.

In humans, a study performed by Soldi et al. (2019) in healthy adult volunteers with self-reported psychological stress showed that administration of Lactoflorene Plus (a probiotic composed mostly of various Lactobacillus strains) led to increased good bacteria such as *Bifidobacterium*, *Lactobacillus*, and *Faecalibacterium*, and decreased potentially pathogenic species like *Dialister*, *Escherichia*, and *Shigella* populations. In the volunteers, the administration of the probiotic led to improved immune function, increased SCFA producers, and decreased potentially harmful bacteria leading to lower intestinal inflammation and abdominal pain in volunteers (Soldi et al. 2019). Anti-inflammatory activity of psychobiotics and corresponding reductions in neuropsychiatric and intestinal disorder have been well established in humans. For example, treatment with the psychobiotic *Lactobacillus farciminis* was shown to attenuate the HPA stress response by reducing intestinal barrier permeability (Ait-Belgnaoui et al. 2012).

Overall, the application of psychobiotics in human and rodent has shown promise in a variety of applications from improving cognitive functioning to increased gut health. Using the information from these trials, there is an application which could translate to production and performance animals to improve welfare parameters associated with animals of economic importance.

15.7 Implications of Psychobiotics in Neonatal Calves

Despite the fact that some probiotic trials have analyzed the effects of probiotics on behavior and welfare in animal models, no studies to date have been performed indicating the potential use of psychobiotics in livestock animals for enhanced health and productivity measures. Probiotics have been utilized for many years for animal husbandry purposes, with benefits ranging from improved immunomodulation, nutrient digestibility, growth performance, and even as an alternative to antibiotics in reducing the incidence of disease in a herd or flock (Alayande et al. 2020). Livestock producers would have incentive to utilize psychobiotics via improved health, improved welfare, and reduced stress, which will lead to produce cost-effective and consumer-attractive products. In essence, the neuropsychiatric and physiological benefits would have to lead to marked improvements in production parameters.

Calves go through several stressful events during the pre-weaning period, including maternal separation, drastic changes in diet, dehorning, castration, transportation, and weaning, which will contribute to variations in the postnatal development of calves. Osorio (2020) provides an extensive review on how stressors during pregnancy (maternal nutritional restriction/over-nutrition, thermal stressors) and at birth (prolonged calving) modulate the intestinal development and maturation of calves. Nevertheless, the impacts of these stressors on establishing the gut microbiome and the GBA are still not clear. In dairy cattle, neuroendocrine hormones released during stress responses can affect calf health, food intake, and digestion (Freestone and Lyte 2010). Studies evaluating the prenatal stressors revealed that heat stress during the late gestation tended to increase blood cortisol levels of heifer calves at birth (Tao et al. 2012). Besides, the calves born to heat-stressed cows had altered immune functions than calves born to cows exposed to evaporating cooling (Strong et al. 2015). For example, the expression of TNFα and TLR2 in blood and the percentage of neutrophils were higher in calves born to heat-stressed cows than those born to cows exposed to cooling. Heat stress in dairy cows has been shown to affect the taxonomic and functional composition of fecal microbiota (Chen et al. 2018). It is, however, not clear whether heat stress during late gestation can affect the establishment of gut microbiota in neonatal calves. With the future changes in the climate and ambient temperature, understanding the impact of heat stress in calves gut microbiota, immune system, and GBA will be vital for cattle production systems. In pregnant rats, hypothermia (cold stress) has been linked to altered HPA axis responses, such as increased BDNF (Wang et al. 2019). Hypothermia is another stress event newborn calves can experience during winter, which needs to be investigated in detail to understand the effect of GBA during early life.

Transportation, another common stressor in the livestock industry, results in various physiological, immunological, and behavioral changes in the animal (Earley et al. 2012). Chen et al. (2015) present a critical review on the effect of transportation stress on host immune responses. Transportation stress has been linked to significant changes in the respiratory microbiome of calves, but there is a lack of understanding

on how the respiratory microbiome responds to stress. In beef calves, transportation stress often occurs together with weaning and co-mingling. It is essential to understand how these different stressors contribute to gut and respiratory microbiome changes, which might, in return, affect the host immune system. In veal calves, transportation usually happened during the first week of life when these neonatal calves go through critical development processes such as microbiome establishment, immune and neural system development. Nonetheless, stress in veal calves is not well understood yet. Dehorning and castration are two other stressful events that have been linked to altered gut microbial composition in dairy calves (Mir et al. 2019). Similar to dairy calves, both veal and beef calves go through similar stressors during early life; however, there is a lack of understanding of the impact of these management practices on GBA.

Psychobiotics are expected to benefit animals during these major stressors. As previously discussed, antibiotic- or stress-induced dysbiosis in the gut is related to various adverse physiological outcomes, including increased incidence of disease, psychological disorder, and increased stress and inflammation throughout the body. Therefore, optimizing the gut microbiota is essential for healthy calf rearing and as a method to reduce adverse health events as the animal ages. As a result, measurable aspects regarding improved production and health must be tangible and respond to stressors that would typically take part in the animal's life. These stress events may include castration, ear tagging, handling, transportation, weaning, or lameness. However, each animal may have different genetic predispositions, microbial population shifts, and behavioral outcomes in response to stress, thus, individual animals may respond to psychobiotic administration in a different way. Production parameters to measure in response to psychobiotic administration could include feed intake, average daily gain (ADG), disease incidence, appetite indicator levels, and behavioral scores in genetically similar animals. According to Uyeno et al. (2015), commensal *Lactobacilli* and *Bifidobacterium* communities decline in neonatal calves as animals undergo major stressor events. However, direct-fed psychobiotic *Lactobacillus* and *Bifidobacterium* can be administered to reduce the risk or severity of scours onset in calves, particularly concerning stress-induced dysbiosis. Further research is needed to understand the direct neuroendocrine effects current proposed psychobiotic species might have, including how these strains can directly affect host stress and immune responses.

15.7.1 Colostrum Management

Feeding management and sufficient nutrient intake play a significant role in neonatal development and can reduce the risk of mortality and morbidity in a herd (Hulbert and Moisa 2016). Studies in ruminants suggest that early dietary experiences have a more significant and longer-lasting effect than those occurring later in life (Distel et al. 1994; Eckert et al. 2015; Soberon et al. 2012; Soberon and Van Amburgh 2017; Yanez-Ruiz et al. 2010). Colostrum, the first milk produced postpartum, contains high concentrations of immunoglobulins and innate immune components, which

protect neonates against infectious agents (Sears et al. 2017). An adequate supply of colostrum is important for neonates since it contributes to various physiological changes, mainly related to metabolic and endocrine pathways. Bovine colostrum supplies the calf several components vital for the development of immunity and GIT. Colostrum contains nutritional components that consist of minerals, trace elements, vitamins, essential fatty and amino acids, and non-nutrient components including immunoglobulins and eukaryotic cells such as lactocytes, leucocytes, and erythrocytes (Blum 2006). Colostrum provides innate immune cells that can secrete various immune-related components such as cytokines, antimicrobial proteins, and peptides (e.g., lactoferrin, defensins, and transferrin) and passively acquired maternal immunoglobulins, which are essential for host-defensive mechanisms against potential pathogens (Stelwagen et al. 2009). Numerous studies have reported that feeding colostrum has a positive effect on postnatal development. It has been suggested that colostrum feeding supports the development of GIT (Pyo et al. 2020) and the growth of commensal bacteria (Malmuthuge et al. 2015; Song et al. 2019). It has also been suggested that prolonged colostrum feeding can influence the number of binding sites of insulin receptors in the intestinal mucosa of neonatal calves (Hammon and Blum 2002).

Salivary cortisol (glucocorticoid) levels peaked within an hour of birth in calves (Kovács et al. 2021), indicating increased stress after birth. It would be interesting to understand the impact of the temporal variation of stress after birth on the initiation of gut colonization. Feeding colostrum or milk replacer to newborn calves at birth decreased cortisol level significantly within an hour after feeding (Gruse et al. 2015), indicating that feeding immediately after birth is vital for modulating the stress calves. A single feeding of colostrum within an hour of birth facilitated the colonization of bacteria in small and large intestines of newborn calves compared to colostrum deprivation or delayed feeding (Malmuthuge et al. 2015; Song et al. 2019). However, the role of colostrum-driven changes in the gut microbiome in the modulation of stress in calves and the GBA during this stressful time is unknown.

15.7.2 Future Directions of Psychobiotics Research in Neonatal Calves

There is a role for psychobiotics in applications related to standard production animal practices, resulting in improved health and welfare parameters. Ruminants such as cattle are prone to various production diseases in early life, which can be mitigated by encouraging the development of a healthy gut microbiome. Calf scours is the leading cause of mortality and morbidity during calf's early life. Therefore, the prevention of symptom onset is paramount in maintaining calf growth (Uyeno et al. 2015). Due to the stable nature of the microbial populations in adult ruminants in the absence of antibiotic administration, pre-ruminants (such as calves, kids, and lambs) may benefit most significantly from *Lactobacillus* and *Bifidobacterium*-based psychobiotic products. Early life microbial colonization has a profound effect on neural and CNS development in the young ruminant, affecting stress responses and

the development of normal behavior (Tremblay et al. 2021). As a result, producers have the potential for selecting for stress robustness in the early life of calves. This selection would allow for reduced disease incidence (including scours), improved immune function, and increased production.

Additionally, Carey et al. (2008) described that probiotic application with various *Bifidobacterium, Lactobacillus,* and *Pediococcus* strains has led to the downregulated expression of virulent factor Shiga toxin produced by *E. coli* O157: H7 strains, which are commonly found in the gastrointestinal tract of ruminants. While this strain is harmless to ruminants, it is linked to foodborne outbreaks, diarrhea, hemorrhagic colitis, and hemolytic uremic syndrome in humans (Carey et al. 2008). Since this *E. coli* strain is of public health importance, incentives exist for producers to reduce the microbial count and shedding from their herds into the environment. Increased disease in a herd can impact both animal and human health (via zoonotic disease transmission such as with *E. coli O157:H7*). Disease in a herd also can cause dysbiosis of the gut, leading to further physiological and neuropsychiatric health impacts down the line. As a result, psychobiotics can be administered to pre-ruminants alongside probiotic treatments to assist with one health principles related to environmental, animal health, and public health measures.

Feed intake is often reduced in animals with stressful or psychologically adverse events or conditions. As a result, significant efforts are employed by producers to increase feed intake (and therefore average daily gain) in their herds. One possible solution could be the use of psychobiotics to increase stress robustness and appetite indicators in the animal. For example, a study performed by Rocks et al. (2021) analyzed psychobiotic strains in fermented foods in the treatment of stress-induced anorexia nervosa in humans. While anorexia nervosa is typically a highly comorbid and treatment-resistant psychological disorder, Rocks proposed that psychobiotics had the potential to restore weight, appetite, assist with nutritional recovery, and improve psychiatric functioning in affected patients. A reduction in feed intake is often seen in relation to maternal separation, transportation, and co-mingling in beef and veal calves. Despite that reduced feed intake in ruminants is not typically linked to a psychological eating disorder like anorexia nervosa, an opportunity exists to study hormones related to hunger, satiety, stress, and metabolic functioning to determine if psychobiotics are viable options to address weaning-related anorexia in calves. Since some trials previously mentioned associate the use of psychobiotic to improved growth, these positive effects may result from a behavioral shift from the administered product; however, more research is needed in this area.

Stereotypies are defined as repetitive, seemingly purposeless tasks which offer little to no identifiable benefit to the animal. Cattle species are prone to several oral stereotypies, including tongue playing, manipulation of objects, and conspecifics (Schneider et al. 2020). The presence of stereotypies in captive animals typically indicates a high level of stress or some restrictions on animal welfare. In dairy and veal production systems, calves are separated from the dam at birth, while in beef calves, they are typically weaned between 6 and 9 months old. Perturbations in microbiota related to stress from maternal separation can lead to a reduction in animal welfare, and a predisposition to the development of stereotypies. Aside from

weaning, transportation and co-mingling can also increase stress levels of calves. We propose psychobiotics as a means to regulate stress during these events, leading to improved weight maintenance, faster gains, and a higher level of animal welfare via a reduction in fear, distress, and stress indicators.

Disease-associated costs are typically among the highest costs for producers. Early life scours (diarrhea disease) in beef operations can cost as much as $4000/year/100 head in a herd with 20% morbidity when considering calf death losses, treatment costs, and reduced performance (Beef Cattle Research Council 2019). Oral administration of *Faecalibacterium prausnitzii* resulted in reduced incidence of scours and calf death, demonstrating a role for microbial interventions which can both persist and influence gut health in the host (Malmuthuge and Guan 2017). It is evident that catecholamines (epinephrine, norepinephrine) function as potent stimulatory agents of pathogenic bacteria such as *E. coli* o157:H7, *Salmonella enterica,* and *Yersinia enterocolitica* (Freestone et al. 2007). Plasma catecholamine levels increase with the prolonged calving (dystocia) in calves (Borcher 1992). It is, however, not clear whether prolonged calving increases the risk of enteric infections in calves or whether it affects microbial establishment. Enteric infections are prevalent in neonatal calves pre-weaning and have been linked to altered microbial community composition (Gomez et al. 2017; Ma et al. 2020; Whon et al. 2021). Besides, weaning strategy has been shown to influence the gut microbial community of neonatal calves (Meale et al. 2017), which may be partly caused by the stress caused during the weaning process. However, there is a lack of understanding for the interrelationship among stressors that pre-weaned calves experience, the impact on the gut microbiome and GBA, and the risk of developing enteric infections. In the future, it will be interesting to understand the impact of elevated stress during calving on the initial gut colonization process and how early management practices can be optimized to decrease the favorable condition for potential enteric pathogens in the neonatal calf gut when the calves are under stress.

15.8 Conclusions

Psychobiotics are probiotic supplements administered with the purpose of improved neuropsychiatric performance and health. Psychobiotics are a potential therapeutic avenue for the modulation of the GBA, which affords the host and resident microbes a route for bi-directional communication. Human and rodent trials revealed a promising future for the utilization of psychobiotics to improve health by mitigating stress, which can also be applied in the livestock production. Neonatal calves that go through a series of stressful events provide an acceptable model to understand the use of psychobiotics in neonatal livestock species.

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References

Abecia L, Jimenez E, Martinez-Fernandaz G, Martin-Garcia AI, Ramos-Morales E, Pinloche E et al (2017) Natural and artificial management before weaning promotes different rumen microbial colonization but not differences in gene expression levels at the rumen epithelium of newborn goats. PLoS One 12:e0182235

Abecia L, Waddams KE, Martinez-Fernandaz G, Martin-Garcia AI, Ramos-Morales E, Newbold CJ et al (2014) Feeding management in early life influences microbial colonization and fermentation in the rumen of newborn goat kids. Anim Prod Sci 54:1449–1454

Abreu M (2010) Toll-like receptor signalling in the intestinal epithelium: how bacterial recognition shapes intestinal function. Nat Rev Immunol 10:131–144. https://doi.org/10.1038/nri2707

Ait-Belgnaoui A, Durand H, Cartier C, Chaumaz G, Eutamene H, Ferrier L, Houdeau E, Fioramonti J, Bueno L, Theodorou V (2012) Prevention of gut leakiness by a probiotic treatment leads to attenuated HPA response to an acute psychological stress in rats. Psychoneuroendocrinology 37(11):1885–1895. https://doi.org/10.1016/j.psyneuen.2012.03.024

Alayande KA, Aiyegoro OA, Ateba CN (2020) Probiotics in animal husbandry: applicability and associated risk factors. Sustainability 12(1087). https://doi.org/10.3390/su12031087

Alcaino C, Knutson KR, Treichel AJ, Yildiz G, Strege PR, Linden DR, Li JH, Leiter AB, Szurszewski JH, Farrugia G, Beyder A (2018) A population of gut epithelial enterochromaffin cells is mechanosensitive and requires Piezo2 to convert force into serotonin release. Proc Natl Acad Sci 115. https://doi.org/10.1073/pnas.1804938115

Allen AP, Hutch W, Borre YE, Kennedy PJ, Temko A, Boylan G, Murphy E, Cryan JF, Clarke G (2016) Bifidobacterium longum 1714 as a translational psychobiotic: modulation of stress, electrophysiology and neurocognition in healthy volunteers. Transl Psychiatry 6:e939. https://doi.org/10.1038/tp.2016.191

Amenyogbe N, Kollmann TR, Ben-Othman R (2017) Early-life host-microbiome interphase: the key frontier for immune development. Front Pediatr 5:111

Bansal T, Englert D, Le J, Hegde M, Wood TK, Jayaraman A (2007) Differential effects of epinephrine, norepinephrine, and indole on Escherichia coli O157:H7 chemotaxis, colonization, and gene expression. Infect Immun 75(9):4597–4607

Beef Cattle Research Council (2019) A simple system to fit the scourge of scours. Alberta Farmer. https://www.albertafarmexpress.ca/livestock/a-simple-system-to-help-reduce-scours-in-newborn-calves/

Bi Y, Cox MS, Zhang F, Suen G, Zhang N, Tu y, et al. (2019) Feeding modes shape the acquisition and structure of the initial gut microbiota in newborn lambs. Environ Microbiol 21:2333–2346

Blalock JE, Smith EM (2007) Conceptual development of the immune system as a sixth sense. Brain Behav Immun 21(1):23–33. https://doi.org/10.1016/j.bbi.2006.09.004

Blum JW (2006) Nutritional physiology of neonatal calves. J Anim Physiol Anim Nutr (Berl) 90(1–2):1–11. https://doi.org/10.1111/j.1439-0396.2005.00614.x

Borcher GM (1992) An evaluation of the endocrine response to dystocia in the primiparous cow and calf. Theses, Dissertations, & Student Scholarship: Agricultural Leadership, Education & Communication Department 80. https://digitalcommons.unl.edu/aglecdiss/80

Bravo JA, Forsythe P, Chew MV, Escaravage E, Savignac HM, Dinan TG et al (2011) Ingestion of lactobacillus strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve. Proc Natl Acad Sci USA 108:16050e5

Buffie CG, Pamer EG (2013) Microbiota-mediated colonization resistance against intestinal pathogens. Nat Rev Immunol 13(11):790–801. https://doi.org/10.1038/nri3535

Burrin DG, Stoll B, Guan X (2003) Glucagon-like peptide 2 function in domestic animals. Domest Anim Endocrinol 24(2):103–122

Carey CM, Kostrzynska M, Ojha S, Thompson S (2008) The effect of probiotics and organic acids on Shiga-toxin 2 gene expression in enterohemorrhagic *Escherichia coli* O157:H7. J Microbiol Methods 73:125–132

Charmandari E, Tsigos C, Chrousos G (2005) Endocrinology of the stress response. Annu Rev Physiol 67:259–284. https://doi.org/10.1146/annurev.physiol.67.040403.120816
Chen S, Wang J, Peng D, Li G, Chen J, Gu X (2018) Exposure to heat-stress environment affects the physiology, circulation levels of cytokines, and microbiome in dairy cows. Sci Report 8:14606
Chen Y, Arsenault R, Napper S, Griebel P (2015) Models and methods to investigate acute stress responses in cattle. Animals 5(4):1268–1295. https://doi.org/10.3390/ani5040411
Cheng LH, Liu YW, Wu CC, Wang S, Tsai YC (2019) Psychobiotics in mental health, neurodegenerative and neurodevelopmental disorders. J Food Drug Anal 27(3):632–648. https://doi.org/10.1016/j.jfda.2019.01.002
Clarke G, O'Mahony SM, Dinan TG, Cryan JF (2014) Priming for health: gut microbiota acquired in early life regulates physiology, brain and behaviour. Acta Paediatr 103:812–819
Clarke MB, Hughes DT, Zhu C, Boedeker EC, Sperandion V (2006) The QseC sensor kinase: a bacterial adrenergic receptor. Proc Natl Acad Sci U S A 103(27):10420–10425
Claustre J, Brechet S, Plaisancie P, Chayvialle JA, Cuber JC (1999) Stimulatory effect of beta-adrenergic agonists on ileal L cell secretion and modulation by alpha-adrenergic activation. J Endocrinol 162(2):271–278
Codagnone MG, Spichak S, O'Mahony SM, O'Leary OF, Clarke G, Stanton C, Dinan TG, Cryan JF (2019) Programming bugs: microbiota and the developmental origins of brain health and disease. Biol Psychiatry 85(2):150–163. https://doi.org/10.1016/j.biopsych.2018.06.014. Epub 2018 Jun 27
Connor EE, Evock-Clover CM, Walker MP, Elsasser TH, Kahl S (2015) Comparative gut physiology symposium: comparative physiology of glucagon-like peptide-2: implications and applications for production and health of ruminants. J Anim Sci 93(2):492–501. https://doi.org/10.2527/jas.2014-8577
Cruz-Topete D, Cidlowski JA (2015) One hormone, two actions: anti- and pro-inflammatory effects of glucocorticoids. Neuroimmunomodulation 22(1–2):20–32
Dantzer R, O'Connor JC, Freund GG, Johnson RW, Kelley KW (2008) From inflammation to sickness and depression: when the immune system subjugates the brain. Nat Rev Neurosci 9(1):46–56. https://doi.org/10.1038/nrn2297
De Santis G, Kavvadia M, Abd Almajeed Abbaas Alwardat N, Bigioni G, Zeppieri C, Cascapera S, De Lorenzo A (2017) Psychobiotics as integrative therapy for neuropsychiatric disorders with special emphasis on the microbiota-gut-brain axis. Biomed Prev Issues 2(111)
Desbonnet L, Garrett L, Clarke G, Bienenstock J, Dinan TG (2008) The probiotic *Bifidobacteria infantis*: an assessment of potential antidepressant properties in the rat. J Psychiatr Res 43(2):164–174. https://doi.org/10.1016/j.jpsychires.2008.03.009
Desbonnet L, Garrett L, Clarke G, Kiely B, Cryan JF, Dinan TG (2010) Effects of the probiotic *Bifidobacterium infantis* in the maternal separation model of depression. Neuroscience 170:1179–1188. https://doi.org/10.1016/j.neuroscience.2010.08.005
Dhabhar FS, McEwen BS (1999) Enhancing versus suppressive effects of stress hormones on skin immune function. Proc Natl Acad Sci USA 96(3):1059–1064
Dinan TG, Cryan JF (2013) Melancholic microbes: a link between gut microbiota and depression? Neurogastroenterol Motility 25(9):713–719. https://doi.org/10.1111/nmo.12198
Distel RA, Villalba JJ, Laborde HE (1994) Effects of early experience on voluntary intake of low-quality roughage by sheep. J Anim Sci 72(5):1191–1195
Dogi C, Perdigón G (2006) Importance of the host specificity in the selection of probiotic bacteria. J Dairy Res 73(3):357–366. https://doi.org/10.1017/S0022029906001993
Dong TS, Gupta A (2019) Influence of early life, diet, and the environment on the microbiome. Clin Gastroenterol Hepatol 17:231–242
Drucker DJ (2001) Glucagon-like peptide 2. J Clin Endocrinol Metab 86(4):1759–1764. https://doi.org/10.1210/jcem.86.4.7386
Earley B, Murray M, Prendiville DJ, Pintado B, Borque C, Canali E (2012) The effect of transport by road and sea on physiology, immunity and behaviour of beef cattle. Res Vet Sci 92:531–541. https://doi.org/10.1016/j.rvsc.2011.04.002

Eckert E, Brown HE, Leslie KE, DeVries TJ, Steele MA (2015) Weaning age affects growth, feed intake, gastrointestinal development, and behavior in Holstein calves fed an elevated plane of nutrition during the preweaning stage. J Dairy Sci 98(9):6315–6326. https://doi.org/10.3168/jds.2014-9062

Esmaili A, Nazir SF, Borthakur A, Yu D, Turner JR, Saksena S et al (2009) Enteropathogenic Escherichia coli infection inhibits intestinal serotonin transporter function and expression. Gastroenterology 137(6):2074–2083

Evrensel A, Tarhan KN (2021) Emerging role of gut-microbiota-brain axis in depression and therapeutic implication. Prog Neuro-Psychopharmacol Biol Psychiatry 106:110138. https://doi.org/10.1016/j.pnpbp.2020.110138

FAO (2001) Evaluation of health and nutritional properties of powder milk and live lactic acid bacteria. Food and Agriculture Organization of the United Nations and World Health Organization. [Accessed March 30th, 2021]. Available from: ftp://ftp.fao.org/es/esn/food/probio_report_en.pdf

Fischer AJ, Song Y, He Z, Haines DM, Steele MA (2018) Effect of delaying colostrum feeding on passive transfer and intestinal bacterial colonization in neonatal male Holstein calves. J Dairy Sci 101:3099–3109. https://doi.org/10.3168/jds.2017-13397

Fleshner M (2013) Stress-evoked sterile inflammation, danger associated molecular patterns (DAMPs), microbial associated molecular patterns (MAMPs) and the inflammasome. Brain Behav Immun 27(1):1–7. https://doi.org/10.1016/j.bbi.2012.08.012

Freestone PPE, Lyte M, Neal CP, Maggs AF, Haigh RD, Williams PH (2000) The mammalian neuroendocrine hormone norepinephrine supplies iron for bacterial growth in the presence of transferrin or lactoferrin. J Bacteriol 182(21):6091–6098

Freestone PP, Haigh RD, Lyte M (2007) Specificity of catecholamine-induced growth in *Escherichia coli* O157:H7, *Salmonella enterica* and *Yersinia enterocolitica*. FEMS Microbiol Lett 269(2):221–228. https://doi.org/10.1111/j.1574-6968.2006.00619.x

Freestone PP, Williams PH, Haigh RD, Maggs AF, Neal CP, Lyte M (2002) Growth stimulation of intestinal commensal *Escherichia coli* by catecholamines: a possible contributory factor in trauma-induced sepsis. Shock 18(5):465–470

Freestone P, Lyte M (2010) Stress and microbial endocrinology: prospects for ruminant nutrition. Animal 4(7):1248–1257. https://doi.org/10.1017/S1751731110000674

Gomez DE, Arroyo LG, Costa MC, Viel L, Weese JS (2017) Characterization of the fecal bacterial microbiota and healthy and diarrheic dairy calves. J Vet Intern Med 31:928–939

Green BT, Brown DR (2016) Interactions between bacteria and the gut mucosa: do enteric neurotransmitters acting on the mucosal epithelium influence intestinal colonization or infection? Adv Exp Med Biol 874:121–141

Gruse J, Görs S, Tuchscherer A, Otten W, Weitzel JM, Metges CC, Wolffram S, Hammon HM (2015) The effects of oral quercetin supplementation on splanchnic glucose metabolism in 1-week-old calves depend on diet after birth. J Nutr 145:2486–2495. https://doi.org/10.3945/jn.115.218271

Hammon HM, Blum JW (2002) Feeding different amounts of colostrum or only milk replacer modify receptors of intestinal insulin-like growth factors and insulin in neonatal calves. Domest Anim Endocrinol 22(3):155–168. https://doi.org/10.1016/S0739-7240(02)00122-4

Hanning I, Diaz-Sanchez S (2015) The functionality of the gastrointestinal microbiome in non-human animals. Microbiome 3:51. https://doi.org/10.1186/s40168-015-0113-6

Hata T, Asano Y, Yoshihara K, Kimura-Todani T, Miyata N, Zhang X-T et al (2017) Regulation of gut luminal serotonin by commensal microbiota in mice. PLoS One 12(7):e0180745. https://doi.org/10.1371/journal.pone.0180745

Hromádková J, Suzuki Y, Pletts S, Pyo J, Ma T, Chen Y, Steele MA, Guan LL (2020) Effect of colostrum feeding strategies on the expression of neuroendocrine genes and active gut mucosa-attached bacterial populations in neonatal calves. J Dairy Sci 103:8629–8642. https://doi.org/10.3168/jds.2019-17710

Hulbert LE, Moisa SJ (2016) Stress, immunity, and the management of calves. J Dairy Sci 99:3199–3216

Inabu Y, Pyo J, Pletts S, Guan LL, Steele MA, Sugino T (2019) Effect of extended colostrum feeding on plasma glucagon-like peptide-1 concentration in newborn calves. J Dairy Sci 102: 4619–4627. https://doi.org/10.3168/jds.2018-15616

Jacobs B (1994) Serotonin, motor activity and depression-related disorders. Am Sci 82(5):456–463. Retrieved June 9, 2021, from http://www.jstor.org/stable/29775282

Kadmiel M, Cidlowski JA (2013) Glucocorticoid receptor signaling in health and disease. Trends Pharmacol Sci 34(9):518–530. https://doi.org/10.1016/j.tips.2013.07.003

Kinoshita T, Maruyama K, Suyama K et al (2021) Consumption of OLL1073R-1 yogurt improves psychological quality of life in women healthcare workers: secondary analysis of a randomized controlled trial. BMC Gastroenterol 21(237). https://doi.org/10.1186/s12876-021-01793-7

Kostadinova F, Schwaderer J, Sebeo V, Brunner T (2014) Why does the gut synthesize glucocorticoids? Ann Med 46(7):490–497. https://doi.org/10.3109/07853890.2014.932920

Kovács L, Kézér FL, Bodó S et al (2021) Salivary cortisol as a non-invasive approach to assess stress in dystocic dairy calves. Sci Rep 11(1):6200. https://doi.org/10.1038/s41598-021-85666-9

Kumar A, Russell RM, Pifer R, Menezes-Garcia Z, Cuesta S, Narayanan S et al (2020) The serotonin neurotransmitter modulates virulence of enteric pathogens. Cell Host Microbe 28: 41–53.e8

Land WG (2015) The role of damage-associated molecular patterns in human diseases: Part I – promoting inflammation and immunity. Sultan Qaboos Univ Med J 15(1):e9–e21

Leclercq S, Mian FM, Stanisz AM, Bindels LB, Cambier E, Ben-Amram H, Koren O, Forsythe P, Bienenstock J (2017) Low-dose penicillin in early life induces long-term changes in murine gut microbiota, brain cytokines and behavior. Nat Commun 8:15062. https://doi.org/10.1038/ncomms15062

Liang S, Wang T, Hu X, Luo J, Li W, Wu X, Duan Y, Jin F (2015) Administration of *Lactobacillus helveticus* NS8 improves behavioral, cognitive, and biochemical aberrations caused by chronic restraint stress. Neuroscience 310:561e77

Liu WH, Yang CH, Lin CT, Li SW, Cheng WS, Jiang YP et al (2015) Genome architecture of lactobacillus plantarum PS128, a probiotic strain with potential immunomodulatory activity. Gut Pathog 7:22

Luang-In V, Katisart T, Konsue A, Nudmamud-Thanoi S, Narbad A, Saengha W, Wangkahart E, Pumriw S, Samappito W, Ma NL (2020) Psychobiotic effects of multi-strain probiotics originated from Thai fermented foods in a rat model. Food Sci Anim Resour 40(6): 1014–1032. https://doi.org/10.5851/kosfa.2020.e72

Luczynski P, Whelan SO, O'Sullivan C, Clarke G, Shanahan F, Dinan TG, Cryan JF (2016) Adult microbiota-deficient mice have distinct dendritic morphological changes: differential effects in the amygdala and hippocampus. Eur J Neurosci 44(9):2654–2666. https://doi.org/10.1111/ejn.13291

Lv T, Ye M, Luo F, Hu B, Wang A, Chen J, Yan J, He Z, Chen F, Qian C, Gao H, Zhang J, Liu Z, Ding Z (2021) Probiotics treatment improves cognitive impairment in patients and animals: a systematic review and meta-analysis. Neurosci Biobehav Rev 120:159–172. https://doi.org/10.1016/j.neubiorev.2020.10.027

Ma T, Villot C, Renaud D, Skidmore A, Chevaux A, Steele M et al (2020) Linking perturbations to temporal changes in diversity, stability, and compositions of neonatal calf gut microbiota: prediction of diarrhea. ISME J 14:2223–2235

Malmuthuge N, Guan LL (2017) Understanding the gut microbiome of dairy calves: opportunities to improve early-life gut health. J Dairy Sci 100(7):5996–6005. https://doi.org/10.3168/jds.2016-12239

Malmuthuge N, Chen Y, Liang G, Goonewardene LA, Guanle L (2015) Heat-treated colostrum feeding promotes beneficial bacteria colonization in the small intestine of neonatal calves. J Dairy Sci 98(11):8044–8053. https://doi.org/10.3168/jds.2015-9607

Martins-Silva T, Salatino-Oliveira A, Genro JP, Meyer FDT, Li Y, Rohde LA, Hutz MH, Tovo-Rodrigues L (2021) Host genetics influences the relationship between the gut microbiome and psychiatric disorders. Prog Neuro-Psychopharmacol Biol Psychiatry 106:110153. https://doi.org/10.1016/j.pnpbp.2020.110153

Martel F, Monteiro R, Lemos C (2003) Uptake of serotonin at the apical and basolateral membranes of human intestinal epithelial (Caco-2) cells occurs through the neuronal serotonin transporter (SERT). J Pharmacol Exp Ther 306(1):355

Matthews SG (2002) Early programming of the hypothalamo-pituitary-adrenal axis. Trends Endocrinol Metab 13(9):373–380

Maynard K, Hill J, Calcaterra N et al (2016) Functional role of BDNF production from unique promoters in aggression and serotonin signaling. Neuropsychopharmacology 41:1943–1955. https://doi.org/10.1038/npp.2015.349

Maynou G, Chester-Jones H, Bach A, Terre M (2019) Feeding pasteurized waste milk to pre-weaned dairy calves changes fecal and upper respiratory microbiota. Front Vet Sci 6:159

Meale SJ, Li SC, Azevedo P, Derakshani H, DeVries T et al (2017) Weaning age influences the severity of gastrointestinal microbiome shifts in dairy calves. Sci Report 7:198

Messaoudi M, Lalonde R, Violle N, Javelot H, Desor D, Nejdi A et al (2011) Assessment of psychotropic-like properties of a probiotic formulation (lactobacillus helveticus R0052 and Bifidobacterium longum R0175) in rats and human subjects. Br J Nutr 105:755e64. https://doi.org/10.1017/S0007114510004319

Mir RA, Kleinhenz MD, Coetzee JF, Allen HK, Kudva IT (2019) Fecal microbiota changes associated with dehorning and castration stress primarily affects light-weight dairy calves. PLoS One 14(1):e0210203. https://doi.org/10.1371/journal.pone.0210203

Morrison DJ, Preston T (2016) Formation of short chain fatty acids by the gut microbiota and their impact on human metabolism. Gut Microbes 7:189–200. https://doi.org/10.1080/19490976.2015.1134082

Needham BD, Kaddurah-Daouk R, Mazmanian SK (2020) Gut microbial molecules in behavioural and neurodegenerative conditions. Nat Rev Neurosci 21:717–731. https://doi.org/10.1038/s41583-020-00381-0

Noti M, Sidler D, Brunner T (2009) Extra-adrenal glucocorticoid synthesis in the intestinal epithelium: more than a drop in the ocean? Semin Immunopathol 31(2):237–248. https://doi.org/10.1007/s00281-009-0159-2

O'Mahony SM, Marchesi JR, Scully P, Coding C, Ceolho A-M, Quigley EMM et al (2009) Early life stress alters behavior, immunity, and microbiota in rats: implications for irritable bowel syndrome and psychiatric illnesses. Biol Psychiatry 65:263–267

Osorio JS (2020) Gut health, stress, and immunity in neonatal dairy calves: the host side of host-pathogen interactions. J Anim Sci Biotechnol 11:105

Park HJ, Kim SA, Kang WS, Kim JW (2021) Early-life stress modulates gut microbiota and peripheral and central inflammation in a sex-dependent manner. Int J Mol Sci 22:1899

Pasupuleti S, Sule N, Cohn WB, MacKenzie DS, Jayaraman A, Manson MD (2014) Chemotaxis of Escherichia coli to norepinephrine (NE) requires conversion of NE to 3,4-dihydroxymandelic acid. J Bacteriol 196(23):3992–4000

Pyo J, Hare K, Pletts S, Inabu Y, Haines D, Sugino T, Guan LL, Steele M (2020) Feeding colostrum or a 1:1 colostrum:milk mixture for 3 days postnatal increases small intestinal development and minimally influences plasma glucagon-like peptide-2 and serum insulin-like growth factor-1 concentrations in Holstein bull calves. J Dairy Sci 103:4236–4251. https://doi.org/10.3168/jds.2019-17219

Reigstad CS, Salmonson CE, Rainey JF 3rd, Szurszewski JH, Linden DR, Sonnenburg JL, Farrugia G, Kashyap PC (2015) Gut microbes promote colonic serotonin production through an effect of short-chain fatty acids on enterochromaffin cells. FASEB J 29(4):1395–1403. https://doi.org/10.1096/fj.14-259598

Renz H, Holt PG, Inouye M, Logan AC, Prescott SL, Sly PD (2017) An exposome perspective: early-life events and immune development in a changing world. J Allergy Clin Immunol 140: 24–40

Rocks T, West M, Hockey M, Aslam H, Lane M, Loughman A, Jacka FN, Ruusunen A (2021) Possible use of fermented foods in rehabilitation of anorexia nervosa: the gut microbiota as a modulator. Prog Neuro-Psychopharmacol Biol Psychiatry 107:110201. https://doi.org/10.1016/j.pnpbp.2020.110201

Samardzic J, Jadzic D, Hencic B, Jancic J, Strac DS (2018) Introductory chapter: GABA/glutamate balance: a key for normal brain functioning. In: Samardzic J (ed) GABA and glutamate—new developments in neurotransmission research. InTech, London

Sanders ME (2011) Impact of probiotics on colonizing microbiota of the gut. J Clin Gastroenterol 45:S115–S119. https://doi.org/10.1097/MCG.0b013e318227414a

Sandor F, Buc M (2005) Toll-like receptors. I. Structure, function and their ligands. Folia Biol (Praha) 51(5):148–157

Sarkar A, Lehto SM, Harty S, Dinan TG, Cryan JF, Burnet P (2016) Psychobiotics and the manipulation of bacteria-gut-brain signals. Trends Neurosci 39(11):763–781. https://doi.org/10.1016/j.tins.2016.09.002

Schaff CT, Rohrbeck D, Steinhoff-Wagner J, Kanitz E, Sauerwein H, Bruckmaier RM, Hammon HM (2015) Hepatic glucocorticoid and alpha1- and beta2-adrenergic receptors in calves change during neonatal maturation and are related to energy regulation. J Dairy Sci 98(2):1046–1056. https://doi.org/10.3168/jds.2014-8636

Schneider L, Kemper N, Spindler B (2020) Stereotypic behavior in fattening bulls. Animals 10(1): 40. https://doi.org/10.3390/ani10010040

Sears KT, Tennant SM, Reymann MK, Simon R, Konstantopoulos N, Blackwelder WC, Pasetti MF et al (2017) Bioactive immune components of anti-diarrheagenic enterotoxigenic *Escherichia coli* hyperimmune bovine colostrum products. Clin Vaccine Immunol 24(8). https://doi.org/10.1128/cvi.00186-16

Silva YP, Bernardi A, Frozza RL (2020) The role of short-chain fatty acids from gut microbiota in gut-brain communication. Front Endocrinol (Lausanne) 11:25. https://doi.org/10.3389/fendo.2020.00025

Soberon F, Van Amburgh ME (2017) Effects of preweaning nutrient intake in the developing mammary parenchymal tissue. J Dairy Sci 100(6):4996–5004

Soberon F, Raffrenato E, Everett RW, Van Amburgh ME (2012) Preweaning milk replacer intake and effects on long-term productivity of dairy calves. J Dairy Sci 95(2):783–793. https://doi.org/10.3168/jds.2011-4391

Soldi S, Tagliacarne SC, Valsecchi C, Perna S, Rondanelli M, Ziviani L, Milleri S, Annoni A, Castellazzi A (2019) Effect of a multistrain probiotic (Lactoflorene® Plus) on inflammatory parameters and microbiota composition in subjects with stress-related symptoms. Neurobiol Stress 10:100138. https://doi.org/10.1016/j.ynstr.2018.11.001

Song Y, Malmuthuge N, Li F, Guan LL (2019) Colostrum feeding shapes the hindgut microbiota of dairy calves during the first 12h of life. FEMS Microbiol Ecol 95. https://doi.org/10.1093/femsec/fiy203

Sorrells SF, Sapolsky RM (2007) An inflammatory review of glucocorticoid actions in the CNS. Brain Behav Immun 21(3):259–272. https://doi.org/10.1016/j.bbi.2006.11.006

Specian RD, Oliver MG (1991) Functional biology of intestinal goblet cells. Am J Phys 260(2 Pt 1): C183–C193. https://doi.org/10.1152/ajpcell.1991.260.2.C183

Stelwagen K, Carpenter E, Haigh B, Hodgkinson A, Wheeler TT (2009) Immune components of bovine colostrum and milk. J Anim Sci 87(13 Suppl):3–9. https://doi.org/10.2527/jas.2008-1377

Strong RA, Silva EB, Cheng HW, Eicher SD (2015) Acute brief heat stress in late gestation alters neonatal calf innate immune functions. J Dairy Sci 98:7771–7783

Sudo N, Chida Y, Aiba Y et al (2004) Postnatal microbial colonization programs the hypothalamic-pituitaryadrenal system for stress response in mice. J Physiol 558:263–275

Sudo N (2019) Biogenic amines: signals between commensal microbiota and gut physiology. Front Endocrinol 10. https://doi.org/10.3389/fendo.2019.00504

Talaber G, Jondal M, Okret S (2013) Extra-adrenal glucocorticoid synthesis: immune regulation and aspects on local organ homeostasis. Mol Cell Endocrinol 380(1–2):89–98. https://doi.org/10.1016/j.mce.2013.05.007

Tao S, Monteiro APA, Thompson IM, Hayen MJ, Dahl GE (2012) Effect of late-gestation maternal heat stress on growth and immune function of dairy calves. J Dairy Sci 95:7128–7136

Tenaillon O, Skurnik D, Picard B, Denamur E (2010) The population genetics of commensal *Escherichia coli*. Nat Rev Microbiol 8(3):207–217. https://doi.org/10.1038/nrmicro2298

Tian P, O'Riordan KJ, Lee Y-K, Wang G, Zhao J, Zhang H, Cryan JF, Chen W (2020) Towards a psychobiotic therapy for depression: Bifidobacterium breve CCFM1025 reverses chronic stress-induced depressive symptoms and gut microbial abnormalities in mice. Neurobiol Stress 12: 100216. https://doi.org/10.1016/j.ynstr.2020.100216

Toro-Barbosa M, Hurtado-Romero A, Garcia-Amezquita LE, García-Cayuela T (2020) Psychobiotics: mechanisms of action, evaluation methods and effectiveness in applications with food products. Nutrients 12(12):3896. https://doi.org/10.3390/nu12123896

Tremblay A, Lingrand L, Maillard M, Feuz B, Tompkins TA (2021) The effects of psychobiotics on the microbiota-gut-brain axis in early-life stress and neuropsychiatric disorders. Prog Neuro-Psychopharmacol Biol Psychiatry 105:110142. https://doi.org/10.1016/j.pnpbp.2020.110142

Uyeno Y, Shigemori S, Shimosato T (2015) Effect of probiotics/prebiotics on cattle health and productivity. Microbes Environ 30(2):126–132

Vu K, Lou W, Tun HM, Komya TB, Morales-Lizcano N, Chari RS et al (2021) From birth to overweight and atopic diseases: multiple and common pathways of the infant gut microbiome. Gastroenterology 160:128–144.e10

Wang D, Xu B, Wang J, Wang H, Guo J, Ji H et al (2019) Response of the maternal hypothalamus to cold stress during late pregnancy in rats. Brain Res 1722:146354

Whon TW, Kim HS, Shin N-R, Sung H, Kim M-S, Kim JY et al (2021) Calf diarrhea by prolonged expansion of autochthonus gut enterobacteriaceae and their lytic bacteriophages. mSytems 6: e00816–20

Xie P (2017) Alterating the gut microbiome by microbiota transplantation from depressed patients into germ-free mice results In depressive-like behaviors through a pathway mediated by the Host's metabolism. Eur Neuropsychopharmacol 27(3):S478–S479. https://doi.org/10.1016/j.euroneuro.2016.09.562

Yanez-Ruiz DR, Macias B, Pinloche E, Newbold CJ (2010) The persistence of bacterial and methanogenic archaeal communities residing in the rumen of young lambs. FEMS Microbiol Ecol 72(2):272–278

Yano JM, Yu K, Donaldson GP, Shastri GG, Ann P, Ma L, Nagler CR, Ismagilov RF, Mazmanian SK, Hsiao EY (2015) Indigenous bacteria from the gut microbiota regulate host serotonin biosynthesis. Cell 161(2):264–276. https://doi.org/10.1016/j.cell.2015.02.047

Zhou L, Foster JA (2015) Psychobiotics and the gut-brain axis: in the pursuit of happiness. Neuropsychiatr Dis Treat 16(11):715–723. https://doi.org/10.2147/NDT.S61997