Prebiotics and Probiotics in Feed and Animal Health

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Abstract

The ban of antimicrobial growth promoters (AGP) has been a challenge for animal nutrition, increasing the need to find alternative methods to control and prevent the colonization of pathogenic bacteria. The elimination of antibacterials in animal nutrition has had adverse consequences on the production, health, and welfare of animals. Much research has been focused on the development of antibiotic alternatives to maintain or improve animal health and performance. Modulation of the gut microbiota with zoo-technical feed additives such as prebiotics and probiotics for host protection to support animal husbandry, including livestock, poultry, and fish farming, is the key to maximize productivity and maintain animal health and welfare. This chapter describes the classes of available prebiotics, probiotics, and synbiotics alternatives to increase productivity and aid performance in several food-producing animals. For farm animals, optimal combinations of various alternatives coupled with good management and husbandry practice, better housing conditions, and improvement of biosecurity measures are essential.

Keywords

Prebiotics · Probiotics · Synbiotics · Feed · Animal health · Animal welfare

1 Introduction

The concept of improving animal health and welfare through enhanced gut health has been known in food animal production for decades; however, only now are the tools available to identify microbes in the intestine associated with improved performance (Ballou et al. 2016). Preserving the integrity of the intestinal barrier is also critical for animal health and welfare. As well as ensuring nutrient absorption, the intestinal barrier is important in protecting the animal immune system (i.e., mucus production, prevention against bacteria and toxins entering the bloodstream). The more important objective of animal husbandry now is to deliver foods safe for human consumption while taking into account animal welfare and respect for the environment (Gaggìa et al. 2010).

Prebiotic and probiotic approaches require using microbial food supplements that benefit the host by improving intestinal microbial balance (Gibson and Roberfroid 1995). Dietary administration of spore-forming bacteria can restore the natural balance of the animal gut microflora and return the gut to its normal nutritional, growth, and health status (Fuller 1989). Researchers have used the term synbiotic to describe the use of prebiotic and probiotic mixtures that may benefit animal or human gastrointestinal (GI) systems (Kolida and Gibson 2011).

2 Gastrointestinal Microbiota and Microbiome

The microbiota is considered a "super-organism" and is an integral part of the gastrointestinal tract (GIT). This concept refers to the close relationship between microbes residing in the GIT and the animal host developed during the long course of evolution (Ley et al. 2008). The GI microbiota is a complex population of microorganisms that are significant in health and disease. Numerous functions benefiting the host are ascribed to the gut microbiota of mammals, such as the digestion and fermentation of carbohydrates, production of vitamins, maintenance of normal intestinal villi function, regulation of immune responses, and protection from pathogenic bacteria. The functions of microbiota include "nutrition" [fermentation of nondigestible substrates (i.e., carbohydrates) to generate short-chain fatty acids (SCFA)],

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absorption of ions, production of amino acids and vitamins K, B_9, B_{12}, "protection" (the barrier effect that prevents invasion by nonnative microbes), and trophic effects on the intestinal epithelium and immune system, that is, the development and homeostasis of local and systemic immunity (Guarner 2007). Moreover, the intestinal bacteria are important in GI health. Resident commensal organisms (normal microflora, indigenous microbiota) promote gut health through the induction of mucus production and enterocyte turnover (Kamada et al. 2013) and also are important in host immunity, nutrient absorption, and metabolism. The resident commensal organisms of the gut flora both protect against invading organisms (e.g., enterotoxigenic *Escherichia coli* strains) within the GIT and are responsible for (1) the synthesis of vitamins; (2) the bioconversion of toxic compounds to nontoxic residues; (3) the stimulation of the immune system; (4) the maintenance of gut peristalsis and intestinal mucosal integrity, and (5) the provision of a barrier against colonization by pathogens. These effects as produced by resident commensal organisms may be mediated through direct competition for nutrients, stimulation of antimicrobial peptide production by the enterocyte, and host immunomodulation (Sancak et al. 2004).

Bacteria also promote self-tolerance by inducing hyporesponsiveness to the resident commensal organisms (Seepersadsingh et al. 2004). These important functions of health can be significantly impaired by bacterial dysbiosis (i.e., altered gut bacterial composition), which occurs when bacteria populations within the GIT become unbalanced. Dysbiosis is likely caused by an altered environment within the GIT, such as changes in pH, motility, oxygen level, and the presence of blood, and has been associated with the pathogenesis of many inflammatory diseases and infections.

The GI microbiota of domestic animals is a dense, large, and complex bacterial community, composed of bacteria, protozoa, fungi, Archaea, and viruses. The microbiota colonizes the gut with metabolic activity that affects the physiology and pathology of the host mucosal immune system. The microbiota located in the GIT of mammals can be considered a "metabolically active organ" whose composition and functions have been characterized for better understanding of the major contribution of the gut microbiota to animal nutrition. The GI microbiota is complex. Bacterial species of the genera *Lactobacillus* and *Bifidobacterium* have been shown to supply beneficial host effects of their metabolic function and end-products. Homeostasis regulation by the microbiota enhances its beneficial components, so it could be possible to treat various intestinal disorders and maintain host well-being (O'Hara and Shanahan 2007). Faecal microbial transplantation thus might be a novel therapy for dysbiosis in veterinary medicine (Redfern et al. 2017).

The GI microbiota promotes the supply, digestion, and fermentation of plant polymers in herbivorous animals and the absorption of nutrients, improves growth performance and prevents pathogen colonization, and maintains normal mucosal immunity. The importance of the GI microbiota and the host–microbe crosstalk is highlighted by the results of studies using germ-free animals.

In chickens, diet and environment affect the GIT microbial status. The microbiota of chickens varies according to factors such as diet, location, and age. Microbial richness and diversity increase with age, with dramatic changes in the microbial community as chickens grow older. Most studies examined the effect of time on the chicken caecum microbiota, as this is the organ with the greatest diversity and abundance in the entire intestinal tract. However, microbial diversity of the chicken microbiota is relatively low compared to the intestinal microbiota of other animals, which is attributed to the rapid transit of food through the digestive system, with short retention times. For example, a typical retention time for a 29-day-old broiler chicken is between 4 and 5 h, compared to humans, where the average is 20 h (Clavijo and Vives Flórez 2018).

Dirty litter and other animal management parameters affect GIT microbial composition both directly by providing a continuous source of bacteria and indirectly by influencing the physical condition and defence of the birds. Animals reared under conditions that prevent bacterial colonization display impaired intestinal immune system development and function (O'Hara and Shanahan 2007). This aspect demonstrates that there is a symbiotic relationship between host and microbiota. The intestinal microbiota is a highly complex milieu of more than 600 species, which may be present at levels up to 10^{11} colony-forming units of bacteria per gram of intestinal contents. Data show that bacterial densities in the ileum and caecum of broiler chickens 1 day after hatching already reach 10^8 and 10^{10} per gram of digesta, respectively. The numbers of microbes reach 10^{11} per gram of caecal digesta and 10^9 per gram of ileal digesta during the first 3 days post hatching and remain relatively stable for the following 30 days (Apajalahti et al. 2004). More than half the 640 species found in chickens represent previously unknown bacterial genera composing a healthy microbiota crucial for the health of the host.

The intestinal environment consists of microbiota, the mucosal immune system, and the gut structure and function, which affect host health and animal productivity. These aspects of the intestinal environment are all influenced by diet; thus, better understanding of the relationships between nutrition, the intestine, and host health is important for optimizing animal production. Because the GIT, particularly the large intestine (i.e., colon and caecum), is the most important site of fermentative activity, the importance of microflora activity (i.e., fermentation) must be clarified in relationship to host health. Although the intestinal contents pass through the human small intestine in only 2–4 h, the large bowel transit time is normally 20–80 h, so there is more

than enough time for the development and activity of the microflora (Williams et al. 2001).

As indicated previously, the intestinal tract, in addition to absorption and digestion, is also the body's largest organ of host defence. This organ represents the largest surface area in contact with the antigens of the external environment, and the dense wall-to-wall of the gut microbiota overlying the mucosa normally accounts for the largest proportion of the antigens presented to the resident immune cells and those stimulating the pattern recognition receptors such as toll-like receptor (TLRs) and NOD-like receptors (NLRs). Part of the intestinal mucosal barrier function is formed by a common mucosal immune system, which provides communication between the different mucosal surfaces of the body (Sekirov et al. 2010). The gut microbiota is intimately involved in numerous aspects of normal host physiology, from nutritional status to behavior and stress response, acting as a microbial organ. The indigenous microflora is host- and location specific, very complex in composition, and generally possesses properties that are beneficial to the host. Therefore, a major concern of antibiotic use is the long-term alteration of the normal healthy gut microbiota and horizontal transfer of resistance genes, which could result in a reservoir of organisms with a multidrug-resistant gene pool (Da Costa et al. 2013). Many environmental factors can affect the composition and function of gut microbiota in livestock animals. Feeding practices, animal diets, farm management, and productivity constraints also influence microbial balance in the GIT and consequently affect feed efficiency, digestive health, and animal welfare (Chaucheyras-Durand and Durand 2010).

In this regard, piglet weaning represents a critical period during which the still immature gut microbiota confronts a radical diet change, leading to increased susceptibility of the young animals to pathogen colonization. Multiple stressors encountered at piglet weaning induce transient anorexia, intestinal inflammation, and unbalanced gut microbiota (Gresse et al. 2017). The circumstances of piglet weaning transition often cause GI infections (e.g., colibacillosis diarrhea), and the potential use of feed supplements to achieve better animal health, welfare, and productivity through manipulation of the GIT microbial ecosystem has gained considerable attention.

Data from the rumen environment suggest it is home to a diverse population of microbes encompassing all three domains of life: Bacteria, Archaea, and Eukarya. Of the three domains of life inhabiting the rumen, the bacteria are predominant (10^{10}–10^{11} cells per gram of rumen content). Viruses are the most abundant biological entities, participating in microbe balance within an ecosystem and facilitating horizontal gene transfer.

Most feed ingredients of plant origin contain considerable amounts of fiber redefined to be soluble and insoluble nondigestible carbohydrates with three or more monomeric units, lignins that are intrinsic and intact in plants, and certain isolated and synthetic nondigestible carbohydrates with three or more monomeric units (Gibson et al. 2017). Insoluble fibers have traditionally been regarded as an inert nutrient diluent with little or no nutritive value in monogastric animal diets; however, they have further functions such as (1) improving gut health, (2) enhancing nutrient digestion, and (3) modulating animal behavior. It is suggested that monogastric animals need fiber because their gut development requires physical stimulation by hard, solid particles of feed (Hetland et al. 2004). So, Choct et al. (1996) demonstrated that addition of soluble non-starch polysaccharides (NSP) to a broiler chicken diet drastically increased volatile fatty acid (VFA) production in the ileum, which was easily reversed when the NSP were depolymerized with an enzyme. The VFA levels in the ileum were negatively correlated with apparent metabolisable energy and starch digestion. The microbiota also degrades polysaccharides that are not digestible by the host, thus increasing the nutritive value of the diet (Choct 2009). The functions of microbiota in pathogen exclusion and the synthesis of vitamins, minerals, and other biologically active compounds are well documented (Patterson and Burkholder 2003).

The link between nutrition and the microbiota is well established. Diet is considered as one of the main drivers in shaping the gut microbiota over the lifetime. The intestinal function and gut microflora of broiler chickens are influenced by cereal grain and microbial enzyme supplementation (Shakouri et al. 2009), dietary fat content, feed form, and NSP-degrading enzymes (Torok et al. 2008). The GIT of chickens harbor microbiomes important in (1) growth and development, including the production of energy-rich SCFA; (2) promotion of GIT villus and crypt morphology; (3) nutrient utilization, including reduction in luminal viscosity and deconstruction of dietary polysaccharides; (4) nutrient absorption; and (5) well-being of their chicken hosts, including detoxification. During the deconstruction of dietary polysaccharides, GIT bacteria produce SCFA. The composition and proportions of these SCFA vary depending on microbial composition, which is to some degree adaptable, and fine-tuned by the composition and structure of the fiber component of the chicken's diet. Acetate is the primary SCFA produced in most GIT environments, including the chicken, followed by propionate and butyrate. The chicken GIT is inhabited by various bacteria, methanogenic archaea, fungi, and viruses (Yeoman et al. 2012). The chicken GIT microbiome produces enzymes enabling the deconstruction of dietary polysaccharides (Beckmann et al. 2006). These enzymes are critical to host nutrition because chickens, similar to most animals, lack the genes for glycoside hydrolase, polysaccharide lyase, and carbohydrate esterase enzymes that are necessary to facilitate this process (Yeoman et al. 2012).

The gut "microbiome" (i.e., the natural intestinal microbial communities of the host, which refers to genetic elements

or the genome of the intestinal microbiota) contains more than 100 times the number of genes in our human genome and confers on us functional features that we have not evolved ourselves (Turnbaugh et al. 2007; Gibson et al. 2017). The GI microbiome is a diverse consortium of bacteria, archaea, fungi, protozoa, and viruses that inhabit the gut of all mammals. The microbiomes exist within greater systems that are organic in nature, including human, animal, plant, and invertebrate) or inorganic, such as soil, water, manufactured products, and the constructed environment. Dysfunctional microbiomes are associated with reduction in animal productivity, such as increases in antimicrobial resistance in livestock and poultry. Studies in humans and other mammals have implicated the microbiome in a range of physiological processes that are vital to host health including energy homeostasis, metabolism, gut epithelial health, immunological activity, and neurobehavioral development. The microbial genome confers metabolic capabilities exceeding those of the host organism alone, making the gut microbiome an active participant in host physiology (Barko et al. 2018).

Supporting an optimal gut microbiome may also prove beneficial in animal science as a means to manage stressful situations and to increase the productivity of farm animals. The microbiome and its genetics are an underestimated influence on animal health and growth. Although there remains a paucity of data about the intestinal microbiome in small animals, recent studies have helped characterize its role in host animal health and associated disease states (Barko et al. 2018). Microbiome characterization has progressed rapidly in recent years as DNA-based profiling technologies have verified and replaced traditional culture-based techniques. Using those newer techniques, it was found that 90% of the bacteria in the chicken GIT represent previously unknown species (Apajalahti et al. 2004).

As with humans, the microbiomes of plants and animals are necessary in plant and animal growth and development. Plants are constantly confronted by both abiotic and biotic stresses that seriously reduce their productivity. Recent evidence shows that a combination of abiotic and biotic stress can have a positive effect on plant performance by reducing susceptibility to biotic stress. Such an interaction between both types of stress suggests crosstalk between their respective signaling pathways. This synergistic or antagonistic crosstalk may include the involvement of phytohormones, transcription factors, kinase cascades, and reactive oxygen species (ROS). In certain cases, such crosstalk can lead to cross-tolerance and enhancement of plant resistance against pathogens (Rejeb et al. 2014).

Animal microbiomes are investigated and manipulated along with modern agricultural practices to increase productivity. The rumen microbiome reportedly contains up to 28,000 different viral genotypes obtained from each environment, with prophage sequences outnumbering potential lytic phages by approximately 2:1: the most abundant bacteriophage and prophage types are associated with members of the dominant rumen bacterial phyla Firmicutes and Proteobacteria (Berg et al. 2012). Viruses have been shown to be a driving factor in the evolution of microbiomes in various environments with important roles in controlling the numbers of microbes in an ecosystem, naturally selecting phage-resistant microbes, and facilitating horizontal gene transfer (Rohwer and Thurber 2009; Parsley et al. 2010).

The microbiome of a mature sow could contain between 10 and 100 trillion organisms. Every organ contains its own specific microbiome (lungs, skin, intestinal tract, etc.) (van Haandel 2016). There are different species of microbiomes and, within the species, there are genetic variations. The microbiome of the pig intestinal tract is dominated by two major groups, Firmicutes and *Bacteroides*, with the composition varying moderately from the beginning to the end of the GIT (Jensen 1998; Niu et al. 2015). The gut microbiome in young pigs is dramatically shaped by the composition of dietary glycans, reflected by the different functional capacities of the microbiome before and after weaning. Before weaning the microbial flora appears to be milk oriented. During the weaning phase the pig and its microbiome are subjected to a drastic period of transition, but during the nursing phase the microbial population is rather stable. After weaning (day 28 and beyond), populations of *Bacteroides* and *Enterobacteriaceae* decline and populations of *Lactobacillaceae*, *Ruminococcaceae*, *Veillonellaceae*, and *Prevotellaceae* increase (Frese et al. 2015). The microbiome adapts its bacterial composition to changes in the living environment or substrates in the diet (Lallès et al. 2007). The microbiome is in continuous symbiosis with its host, containing pathogens and other bacteria that are perfectly in balance in a healthy animal.

Little is known about the acquisition and development of the intestinal microbiome in dogs and cats, limited to a handful of studies in kittens and puppies. To date, only one longitudinal study of developing kittens has been published (Deusch et al. 2015). As in humans, the early faecal microbiome is characterized by a high degree of interindividual variation, and that intraindividual diversity and compositional stability increase with age. Also, similar to humans, the relative abundance of *Lactobacillus* and *Bifidobacterium* decreased with age, whereas *Bacteroides* and bacterial genes associated with the ability to metabolize complex carbon sources increased with age. However, there was no major change in bacterial gene repertoires between weeks 30 and 42 in these kittens (Barko et al. 2018).

Relative to the poultry microbiome and food safety, it should be highlighted that *Salmonella* affects people in many countries each year, giving rise to hospitalizations and deaths. This aspect is addressed by researchers who

studied the development of the chick microbiome from hatch to 28 days. The microbiome of growing chicks develops rapidly from days 1 to 3, and the microbiome is primarily *Enterobacteriaceae*, but Firmicutes increase in abundance and taxonomic diversity starting around day 7. Predicted metagenomic content suggests that, functionally, treatment may stimulate more differences at day 14, despite the strong taxonomic differences at day 28. These studies found that both vaccination and prebiotic use (i.e., microbial nutrients) with the chick diet help reduce the persistence of *Salmonella* in the challenged birds (Ballou et al. 2016). The GI microbiome in poultry differs ecologically from that of mammals in that colonization occurs primarily from the surrounding environment and individuals of the same age are reared in close proximity rather than in direct contact with adults. Colonization of poultry by microbes from environmental sources may have important biosecurity and management implications if human pathogens are transferred from environmental reservoirs through the poultry supply chain to consumers (Oakley et al. 2013). The microbial communities associated with agricultural animals are important for animal health, food safety, and public health. The use of high-throughput sequencing (HTS) to characterize the poultry microbiome across a series of farm-to-fork samples demonstrates the utility of HTS in monitoring the food supply chain and identifying sources of potential zoonoses and interactions among taxa in complex communities (Oakley et al. 2013). Similar approaches with other poultry and livestock may help prevent other food-borne diseases.

Growth performance that may differ between chicken breeds could be associated with the GIT microbiome. However, there may always be variation among individuals, probably because of initial bacterial colonization at posthatch. It was reported that the jejune microbiota was dominated by lactobacilli (more than 99% of jejune sequences) and showed no difference between birds with high and low feed conversion ratios (FCR), whereas the caecal microbial community displayed higher diversity with 24 unclassified bacterial species, significantly differentially more abundant between high-versus low-performing birds (Stanley et al. 2012). Many broiler chickens and microbiota studies contain only data from males or the sex of the broiler chickens was unknown. This sex bias in the literature might influence our understanding of the microbiota development in chickens, and therefore the sex of the chicken should always be reported (Stanley et al. 2012).

The genetic codes of a number of commensal bacteria, including *Lactobacillus acidophilus* and *L. johnsonii* (Pridmore et al. 2004), which produced SCFA, have recently been sequenced; this will facilitate future studies on microbial gene expression and improve our understanding of interactions between the host and the microbiome.

A healthy gut is the key to a healthy animal. An infected gut (i.e., by coccidiosis or by necrotic enteritis) is not a healthy gut, and is not efficient in digesting and transporting nutrients (Choct 2009). Thus, a balanced and diverse microbial composition is essential for optimal digestion and nutrient uptake. The most important tool for good gut health is to provide the best feed possible that meets the nutritional needs for the age category and stage of production.

The brain–gut–microbiota axis comprises an extensive communication network between the brain, the gut, and the microbiota (Wiley et al. 2017). Development of a diverse gut microbiota is crucial for multiple features of behavior and physiology, as well as many fundamental aspects of brain structure and function. Appropriate early-life assembly of the gut microbiota is also believed to influence subsequent emotional and cognitive development. If the composition, diversity, or assembly of the gut microbiota is impaired, this impairment can have a negative impact on host health and lead to disorders. Recent advances in DNA sequencing technology show that changes in the gastrointestinal microbiome are associated with diseases including inflammatory diseases, asthma, obesity, diabetes, cardiovascular disease, immune-mediated conditions, and even potentially neuropsychiatric illnesses including anxiety and depression (Wiley et al. 2017). Microbiomes represent one source of human and animal genetic and metabolic diversity. For example, aging predisposes humans and animals to a natural degeneration in GI function, epithelial barrier integrity, GI microbiota composition, and immune system function (adaptive and innate), elevating the risk of infections. Potentially pathogenic bacteria (i.e., enterobacteria and *Clostridia*) increase with aging, whereas *Bifidobacterium* species, which contribute to the protection of the intestinal tract, decrease. Little is known about the acquisition and development of the intestinal microbiome in dogs and cats. As in humans, the early faecal microbiome is characterized by a high degree of interindividual variation and that intraindividual diversity and compositional stability increase with age (Yatsunenko et al. 2012). Also similar to humans, the relative abundance of *Lactobacillus* and *Bifidobacterium* decreased with age, whereas *Bacteroides* and bacterial genes associated with the ability to metabolize complex carbon sources increased with age (Barko et al. 2018). In chickens, the microbiota varies according to diverse factors such as diet, location, and age. Dramatic changes have been described in the microbial community as chickens grow older. Most of the studies examined the effect of time on the chicken caecum microbiota (i.e., organ with the greatest diversity and abundance in the entire intestinal tract). However, the microbial diversity of the chicken microbiota is relatively low compared to the intestinal microbiota of other animals, which is attributed to the rapid transit of food through the digestive system, with short retention times; for example, a typical retention time for a

29-day-old broiler chicken is between 4 and 5 h, compared to humans, where the average is 20 h.

3 Classes of Alternatives to Antimicrobial Growth Promoters

The ban of antibiotic growth promoters (AGPs) has been a challenge for animal nutrition, increasing the need to find alternative methods to control and prevent the colonization of pathogenic bacteria (Anadón et al. 2006). Although the EU has banned antibiotics that are applied as growth promoters, they are still regularly used for therapeutic reasons. When antibiotics are used, the intestinal microbiota becomes unbalanced, and restrains the future intestinal health of the animal. Better intestinal health will have a positive impact on FCR and uniformity across all species. An ideal alternative should have the same benefits as AGP, ensure optimum animal performance, and increase nutrient availability (Huyghebaert et al. 2011). Considering the proposed mechanism of action of AGPs (microbiome and immunomodulating activities), a practical alternative should possess both these properties in addition to having a positive impact on FCR and growth (Huyghebaert et al. 2011; Seal et al. 2013). Applications of prebiotics and probiotics are needed not only for health- and welfare-promoting properties and performance, but also to displace the application of antibiotics in animal feed. Prebiotics and probiotics are regarded as components of strategies to reduce or even eliminate routine antimicrobial use in animal production and are seen as potential alternatives to in-feed antibiotics. Modulation of the gut microbiota with prebiotics and probiotics as the new feed additives for host-protecting functions to support animal health and welfare is an important issue in animal production.

3.1 Prebiotic Concept: Prebiotic Effects

Prebiotics are products that confer health benefits. Found naturally in many foods, prebiotics are also isolated from plants, or synthesized from lactose or sucrose by enzymatic methods, and promote the selective growth of certain indigenous gut bacteria. A prebiotic was defined by Gibson and Roberfroid (1995) as "a non-digestive food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health." These authors revised this concept and proposed a new prebiotic definition "as a selectively fermented ingredient that allows specific changes; both in the composition and/or activity in the GI microbiota that confers benefits upon host well-being and health" (Gibson et al. 2004; Roberfroid 2007). The latest definition equalizes "prebiotic" and "bifidogenic" and

includes in the definition the "prebiotic index" (i.e., gives the absolute increase of the faecal bifidobacteria concentrate per gram of daily consumed prebiotics). As the prebiotic effects, or rather "bifidogenic effects," depend on the type and concentration of the prebiotic and on the *Bifidobacterium* concentration in the intestine of the host, therefore there is not a simple dose–effect relationship. Other investigators have proposed definitions that preferentially emphasized one or more different functional characteristics. FAO (2007) describes prebiotics as "non-viable food components that confer a health benefit on the host associated with modulation of the microbiota." The definition arose from observations that particular dietary fibres bring about a specific modulation of the gut microbiota, particularly increased numbers of *Bifidobacterium* or *Lactobacillus* spp., and that a decrease in potential harmful bacteria is a sufficient criterion for health promotion.

The selectivity for bifidobacteria may be promoted by the ingestion of substances such as fructo-oligosaccharides (FOS) and inulin, *trans*-galactosylated oligosaccharides, and soybean oligosaccharides (Mitsuoka et al. 1987). Recently, an expert consensus document updated the definition of a prebiotic (target-associated microbiota) as a substrate that is selectively utilized by host microorganisms, conferring a health benefit (Gibson et al. 2017). Prebiotics as substrate are nondigestible feed ingredients that influence the microbiota in a manner favourable for host health by stimulating growth or activity of potentially positive microbial flora in the large intestine (Patterson and Burkholder 2003), Thus, a prebiotic is a substrate that is selectively utilized by the host microorganisms conferring a health benefit (Gibson et al. 2017). All prebiotics are fibre, but not all fibres are prebiotics. Beneficial animal health effects must be documented for a substance to be considered a prebiotic. Prebiotics for use by animals in which microbial-focused strategies to maintain health and prevent disease are as relevant as for humans (Gibson et al. 2017).

During the past two decades, prebiotics have been recognized for their ability to manipulate host microbiota for the benefit of the host. Prebiotics include a diversity of non-starch polysaccharides (NSP) or oligosaccharides including mannan-oligosaccharide (MOS), fructans (FOS and inulin), oligofructose, galactans [galacto-oligosaccharide (GOS)], malto-oligosaccharide, lactulose, lactitol, gluco-oligosaccharide, xylo-oligosaccharide, soya-oligosaccharide, isomalto-oligosaccharide (IOS), and pyrodextrins.

Nondigestible in the small intestine, FOS could be utilized only by a few bacterial species, notably *Bifidobacterium*, and including such oligosaccharides in the food increased the count of bifidobacteria in the intestine. It should be stated that is difficult to test the selective stimulation of individual bacterial strains among the more than 400 cultivable and nonculturable bacterial strains in the human gut; for that

reason, the prebiotic effect has been defined as "the selective stimulation of growth and/or activity(ies) of one or a limited number of microbial genus(era)/species in the gut microbiota that confer(s) health benefits to the host" (Roberfroid et al. 2010). The diversity of bacterial species in the gut is one of the most important factors for the establishment of a stable ecosystem in the intestinal tract. Young birds have fewer bacterial species in the intestinal tract than do adult birds, so their gut microflora are more susceptible to disturbances than that of the adults (Mead 1989). A stable flora is essential for an animal to resist infections, particularly in the gut.

Although the concept of prebiotics has been developed over time, to be considered as an effective prebiotic, it is proposed that a candidate prebiotic must fulfill the following criteria, which are to be proven by in vitro and in vivo studies: (1) nondigestibility (i.e., resistance to low pH gastric acid, enzymatic digestion, and intestinal absorption in the upper part of the GIT), (2) good fermentation by the large intestinal microbiota; this can be investigated by measuring breath hydrogen or fecal recovery of the administered carbohydrate after a single prebiotic meal, and (3) selective stimulation of growth and activity of intestinal bacteria (i.e., measuring bacterial counts in faecal samples, or intestinal content, before and during exposure to the test material in batch or multi-chamber fermentation systems) that has associated health-promoting effects (Macfarlane et al. 2006; De Vrese and Schrezenmeir 2008).

Prebiotics are nonabsorbable carbohydrates, such as inulin, galacto-oligosaccharides (GOS), and FOS that promote growth and metabolic activity of presumably beneficial gut bacteria, most notably species of *Bifidobacterium* and *Lactobacillus*. These products may confer health benefits through the production of SCFA, including lactate and butyrate, which may reduce cytokine production within the intestinal mucosa (Sartor 2004).

Prebiotics undergo fermentation by beneficial microflora in the large intestine, providing sources of energy for the microflora. Only the carbohydrates [i.e., inulin and oligofructose (OF), (*trans*-galacto-oligosaccharides (TOS or GOS), or lactulose], which are not digestible but can be fermented by the intestinal flora (Gibson 1999), fulfill this criterion.

The "probiotic approach" adds one or two species to a spectrum of hundreds of species in the gut flora, but the "prebiotic approach" aims at fertilization of the intestinal ecosystem. Functional imitations of the naturally occurring prebiotics GOS and FOS stimulate intestinal growth of bifidobacteria as a marker of probiotics in a dose-dependent manner (Patterson and Burkholder 2003; Gaggìa et al. 2010).

In common terms, prebiotics are "food components" for live microorganisms that are considered beneficial for health and well-being, and it is scientifically accepted that prebiotics are valuable dietary additions for modulating the growth and activity of specific bacterial species in the colon that are considered health supporting (Gibson et al. 2010). An example of feed ingredients for animals is the eubiotic lignocellulose, which influences microflora that it does not digest but traverses to the large intestine where bacteria ferment it (Metzler and Mosenthin 2008).

A different substrate in the large intestine contains a diverse gut microflora. In pig faecal and turkey excreta samples, the amount of volatile fatty acids (VFA) (i.e., acetic acid, propionic acid, butyric acid, total VFA) and lactate can be high when the animals receive lignocellulose A and B. Dry matter digestibility was found to be about 75%, 28%, and <5% for swine/turkey diet, and lignocellulose A and B, respectively. Lignocellulose A resulted in a greater amount of gases, VFA, and lactate compared to product B. Lignocellulose A can be used as an alternative source of fiber to maintain the health and function of the digestive tract (Youssef and Kamphues 2018). The inclusion of lignocellulose in the chicken diet, in particular at a dose of 0.5%, promotes the growth of *Lactobacillus* spp. and *Bifidobacterium* spp., and reduces the number of *Escherichia coli* and *Clostridium* spp. as well as enhancing the concentration of SCFAs (i.e., acetic acid and propionic acids) and lactic acid, which suggests the prebiotic effect of lignocellulose on the broiler chicken GIT, although lignocellulose does not have a substantial effect on the pH of ileal and caecal digesta (Bogusławska-Tryk et al. 2015; Choct 2009; Mancabelli et al. 2016).

Although prebiotics are defined as "a nonviable food component that confers a health benefit on the host associated with modulation of the microbiota" (Pineiro et al. 2008), there are some limitations as to which food components actually count as prebiotics. The compounds need to be resistant to hydrolysis and absorption by the upper GIT so that they can reach the target organisms in the lower GIT. It is desirable that these compounds be substrates more or less only for those microorganisms that one intends to support. It has been argued that only fructo-oligosaccharides and inulin meet these criteria (Roberfroid 2007); however, numerous other compounds have been included in lists of prebiotics such as galacto-oligosaccharides, soy oligosaccharides, xylo-oligosaccharides, pyrodextrins, isomalto-oligosaccharides, lactulose, pectin oligosaccharides, lactosucrose, sugar alcohols, gluco-oligosaccharides, levans, resistant starch, and xylosaccharides.

Prebiotics are nondigestible feed ingredients (i.e., carbohydrates) that selectively promote the development of one or more species of microorganisms in the GIT of humans or animals. Oligosaccharides are the main components: the range is diverse and may be based on any of the hexose monosaccharides, including glucose, fructose, galactose, and mannose (Durst 1996) with a degree of polymerization

between 2 and 20 monosaccharides. Grain legumes are the most common natural sources of oligosaccharides (e.g., raffinose, stachyose, verbascose). "Synthetic" oligosaccharides are derived from the direct polymerization of disaccharides or from the fractionation of both vegetable and microbial cells. Oligosaccharides such as arabinogalactose, arabinoxylan, and rhamnogalacturonan, which are derived from polysaccharides of soybean (with about 3–5% galacto-oligosaccharides), wheat, and fruit, respectively (Van Craeyveld et al. 2009), are generally referred to as nondigestible oligosaccharides.

Some of the nondigestible oligosaccharides currently added to animal feed across different animal species are mannose oligosaccharides (MOS), fructose oligosaccharides (FOS), lactulose and galacto-oligosaccharides (GOS), chito-oligosaccharides (COS), arabinoxylan oligosaccharides (AXOS), xylo-oligosaccharide (XOS), $trans$-galacto-oligosaccharide (TOS), glucan, yeast cell wall, inulin, inactivated yeast bacteria, galactomannan, galactoglucomannan-oligosaccharide-arabinoxylan complex (GGMO-AX), levan, polydextrose, peptidoglycan, chitin, galactomannan-oligosaccharides (GMOS), acidic oligosaccharides (AOS), arabinogalactan, phosphorylated mannans (MAN), arabinoxylan, and mannobiose (Anadón et al. 2016a). These substances influence the intestinal ecosystem by, for instance, improving lactic acid fermentation.

Prebiotics compounds can reduce risk for certain conditions and promote better health; they have a long history of safe use and are known for their health benefits for humans including an increase in the bioavailability of minerals, modulation of the immune system, prevention of GI infections, modification of inflammatory conditions, regulation of metabolic disorders, and reduction of the risk of cancer (Roberfroid et al. 2010). For a dietary substrate to be classed as a prebiotic, at least three criteria are required: (1) the substrate must not be hydrolysed or absorbed in the stomach or small intestine; (2) it must be selective for beneficial commensal bacteria in the large intestine such as the Bifidobacteria; and (3) fermentation of the substrate should introduce beneficial luminal (systemic) effects within the host (Manning and Gibson 2004). The oligosaccharide β-glucans are thought to stimulate performance because of their immunomodulatory effects. Their main action is to enhance phagocytosis and proliferation of monocytes and macrophages (Novak and Vetvicka 2008). As macrophages have a crucial role in immunomodulation, the interaction of glucans with macrophages can have very large effects on the host. Recent reviews have elaborated the action of glucans on immune stimulation (Novak and Vetvicka 2008). Studies with broiler chickens have documented significant health benefits from using immune-modulating β-1,3- or 1,6-glucans (from yeast cell walls obtained from *S. cerevisiae*) as a feed ingredient. However, changes in thymus and liver relative weights and villus morphology of broilers were observed (Morales-López et al. 2009).

Prebiotics are a special form of dietary fiber, are not affected by heat, cold, acid, or time, provide a wide range of health benefits, and beneficially affect the host by selectively stimulating growth, activity, or both, of one or a limited number of bacteria species already resident in the colon (Nagpal and Kaur 2011). Prebiotics are specialized plant fibers which beneficially nourish the good bacteria located in the large bowel or colon. Prebiotics are used to increase bifidobacteria or lactobacilli towards being the numerically predominant genus in the colon, properly improving colonization resistance.

Prebiotic compounds are able to modulate both the luminal and mucosal microbial composition and activities and beneficially regulate host–microbe interactions. Moreover, the changes of gut microbiota composition (especially the number of bifidobacteria) contribute to modulate human metabolic processes associated with obesity and diabetes type 2 (Roberfroid et al. 2010). The prebiotics induce not only changes in the intestinal microbiota and the mucosal surface of the colon but the trans-epithelial transport of the SCFA, stimulating shifts of fluid to and from the lumen; furthermore, the transport of cationic minerals is stimulated by the lowered pH of the lumen. The intraluminal colonic propionate induces the nonneuronal release of acetylcholine synthesized by the epithelial crypt cells to the serosal surface, especially in the distal colon, and this was associated with modifications of the electrical parameters of the mucosa and chloride excretion (Yajima et al. 2011).

The proposed mechanisms of action for prebiotics include blocking receptor sites for pathogen adhesion, immunomodulation, production of antimicrobial compounds on fermentation, and modifying gut morphology (Pourabedin and Zhao 2015). Immunomodulation by prebiotics is thought to result from activation of innate immunity by the interaction of the sugars with certain receptors present on the surface of dendritic cells and macrophages, which can then stimulate production of cytokines, proliferation of lymphocytes, and the activity of natural killer (NK) cells (Saad et al. 2013).

Most prebiotics for the gut require an oral dose of 3 g/day or more to elicit an effect (Roberfroid et al. 2010). Products containing dosages lower than this level should not be called prebiotics, unless such a low dose has been proven to elicit selective effects on the microbiota with concomitant health aspects (Gibson et al. 2017).

3.1.1 Application to Benefit Animals

Prebiotics have been studied and used for companion animals and animal husbandry, including livestock, poultry, and aquaculture. The inherent differences among animal species with regard to the living environment, anatomy and physiology,

dietary composition, and reliance on the gut microbiota for energy must be considered when evaluating the effect of prebiotics on animal health (Stevens and Hume 1998, Gibson et al. 2017). Most prebiotics appear to stimulate acid lactic and bifidogenic bacteria. The functions described for prebiotics are that they attach to pathogens, serve as substrates for fermentation, increase osmosis in the lumen of the intestine, and may also indirectly stimulate the response of macrophages and the production of SCFAs and modulate the immune system (Patel and Goyal 2012).

Poultry

Poultry, which are used primarily for the production of meat or eggs, include land fowl species (for example, chickens, turkeys, quail) and waterfowl species (for example, ducks, geese) which respond to prebiotics although most have a fairly short midgut and hindgut that includes a short, straight colon and twin ceca. Dietary prebiotics, including inulin, yeast cell wall extracts, lactulose, and GOS are usually fed at concentrations up to 0.2% (weight/volume) of diet (Bednarczyk et al. 2016). Prebiotics provide substrates for microbial fermentation in the gut, resulting in the production of SCFA, an energy source for enterocytes. Fermentation of dietary fibers by commensal bacteria in the gut leads, in general, to production of SCFA in the hindgut that can be easily absorbed and contribute to the energy sources for the animal host. Many prebiotics, including fructo-oligosaccharide and mannan-oligosaccharide, increase levels of beneficial bacteria such as *Lactobacillus* and *Bifidobacterium* spp. and decrease levels of pathogens such as *Escherichia coli* (Fukata et al. 1999; Xu et al. 2003; Baurhoo et al. 2007). Terminal restriction fragment length polymorphism (T-RFLP) and denaturing gradient gel electrophoresis analysis have been used to demonstrate that FOS and MOS affect the composition of the bacterial population and *Lactobacillus* species profiles in broiler chickens. These microbial changes did not affect performance, indicating that numerous microbial compositions may facilitate a high level of performance under these conditions (Geier et al. 2009).

Compared to probiotics, the risks of undesirable side effects in the host are lower. Prebiotics are macromolecules that are either derived from plants or synthesized by microorganisms. MOS, derived from the outer cell wall layer of *Saccharomyces cerevisiae*, has been studied extensively as a prebiotic supplement in poultry diets.

Two kinds of prebiotics have been described for use in aviculture. Most of those currently used are nondigestible synthetic oligosaccharides that contain one or more molecules of a sugar, or a combination of simple sugars such as glucose, fructose, xylose, galactose, and mannose. MOS found in the cell walls of yeasts have proved to be most important as they contain compound proteins and glucan (Rehman et al. 2009). The other kind of prebiotic described

corresponds to lactose and lactose derivatives such as lactulose and lacto-sucrose (van Immerseel et al. 2002). Lactulose is a nondigestible, synthetic disaccharide that shows prebiotic effect in broiler chickens diets, improving body weight and FCR, increasing villi height, goblet cell numbers, total SCFA concentrations, and *Lactobacillus* counts (Calik and Ergün 2015). Other prebiotics found to have beneficial effects in poultry include lignin, inulin, and palm kernel extract.

Several studies of prebiotics in chickens provide evidence of positive effects for oligosaccharides of mannose or fructose in inhibition of the pathogens *Salmonella* and *E. coli* (Chambers and Gong 2011; Stanley et al. 2014). Conflicting results obtained with or without oligosaccharides that occur naturally in feed ingredients (e.g., the raffinose series oligosaccharides) present an unclear scenario regarding the effect of their inclusion in diets for broiler chickens (Iji and Tivey 1998); however, their nutritional impact cannot be separated from other anti-nutritive components in the diet.

Prebiotics in poultry indicate their usefulness in controlling or reducing the growth of *Clostridium perfringens* implicated in necrotic enteritis. Fucosyllactose, a functional oligosaccharide present in human milk that protects against infection by enteric pathogens, seems to favour coaggragation with pathogens instead of pathogen contaminant being eliminated by the mucosal lining of the poultry intestine (Lee et al. 2012). The addition of various levels of MOS to the broiler chicken diet significantly increased their body weight and improved FCR with increased intestinal villi height, improved immuno-competence in the intestine, altered jejunal gene expression, and influenced intestinal microbiota. FOS, which is derived from plants, has also been shown to possess significant prebiotic effects and improve performance in broiler chickens. Another class of prebiotics includes IOS showing their efficacy in improving weight gain and FCR when fed to broiler chickens (Mookiah et al. 2014). In pigeons, dietary administration of MOS induced changes of gut morphology and lowered the pH of excreta, reflecting a reduced bacterial challenge in the intestine; thus, MOS has potential as a prebiotic strategy in birds (Abd El-Khalek et al. 2012).

A number of characteristics should be taken into consideration when selecting prebiotics for poultry, including resistance to gastric acidic environment, intestinal/pancreatic enzyme hydrolysis, and absorption across intestinal epithelium. The most important characteristic of a standard prebiotic is the ability to selectively enrich beneficial microorganisms associated with health and well-being. Thus, the majority of the beneficial effects of prebiotics are thought to be mediated predominantly through altering the intestinal microbiota (Pourabedin and Zhao 2015). Prebiotics also prevent pathogen colonization either by binding directly or by competitive exclusion by promoting the growth of

beneficial microbes or by stimulating them to produce bacteriocins and lactic acid (Spring et al. 2000). In particular, MOS acts by binding to type 1 fimbriae of enteric pathogens and prevents their adhesion to intestinal epithelial cells (Spring et al. 2000) and acts as adjuvants, and help boost the host immune responses (Ferket et al. 2005). Overall, prebiotics also act by beneficially altering luminal or systemic aspects of the host immune system.

The fermentation of prebiotics by microflora also leads to the production of SCFAs that act as energy sources for intestinal epithelial cells and thus maintain the integrity of the gut lining (Ferket et al. 2005). Several studies have revealed that synbiotic treatment was more efficacious than an individual prebiotic in reducing pathogen transmission and infections in poultry.

Pigs

Different types of chemically defined or undefined dietary compounds are added to the diet of pigs to test their influence on GI microbiota or on the health status improvement during challenge with pathogens. When added to the pig diet, fermentable and nonfermentable fiber appears to have a significant positive impact on growth and gut health: it seems to influence the total digestion and fermentation processes, contributing to a different equilibrium particularly in the large intestine of monogastric animals. Prebiotics such as oligosaccharides of fructose, mannose, and chitin protect piglets against high environmental stressors (for example, antibiotics) and pathogen loads, including faecal *Escherichia coli* shedding, and reduced infection-associated responses to *Salmonella enterica* serovar typhimurium infection or porcine reproductive and respiratory syndrome virus (Liu et al. 2008; Che et al. 2011). Symbiotic applications could be beneficial as significant improvement of growth performance parameters in suckling and in growing pigs was observed (Modesto et al. 2011).

Feeding fiber-rich diets to pregnant sows at the end of their lactation period shows a positive impact as these additives continuously stimulate the GI tract and have a positive influence on the duration of partus (i.e., a shorter duration of partus increases piglet survival rates).

Refined functional carbohydrates (RFCs) are the components harvested from yeast cells (*S. cerevisiae*) using specific enzymes during the manufacturing process to ensure a high level of bioavailability and uniformity. This proprietary enzymatic hydrolysis yields MOS, β-glucans (1,3/1,6), and D-mannose. These compounds are naturally present in all yeast cells but are not readily bioavailable. The method of processing used to refine the yeast cells influences the size and structure of these liberated components, which in turn affect bioavailability and functionality. Research shows that each RFC has a specific mode of action and outcome when fed to various livestock species, including dairy, beef, and poultry. RFCs also have been shown to positively influence the immune response of nursery pigs. RFCs act as a prebiotic by feeding the beneficial bacteria found in the intestine while blocking sites for attachment by pathogens.

Ruminants

Calves are born in a pre-ruminant state and function as non-ruminants until the rumen and other compartments of the stomach fully develop (Quigley et al. 1997). During the first few weeks of life, or longer in the case of veal calves maintained on low-roughage diets (that is, low in fibrous material), prebiotics can be used to increase growth, improve FCR, reduce the incidence and severity of scours (diarrhea), or reduce the incidence of respiratory diseases (Quigley et al. 1997; Ghosh and Mehla 2012; Roodposhti and Dabiri 2012).

The use of prebiotics in cattle has been limited by the ability of ruminants to degrade most prebiotics; however, enhancement in rumen-protective technologies may allow these feed substances to be used in feedlot and dairy cattle, considering also that several classes of nondigestible oligosaccharides are found in the plant cell wall in nature including plants normally used for livestock feeding (Callaway et al. 2008). However, the prebiotics used in pre-weaned calves are cello-oligosaccharides, galactosyl lactose, yeast cell wall extracts, and MOS.

Horses

Horses are large non-ruminant herbivores that rely heavily on microbial fermentation for energy, with more than half of their maintenance energy requirement coming from microbial fermentation occurring in their enlarged caecum and colon (Stevens and Hume 1998). As their typical diet is high in roughage and feedstuffs that are consumed throughout the day, prebiotic interventions might improve the effectiveness of fermentation (Morgan et al. 2007; Respondek et al. 2011). Commonly used prebiotics have stimulatory effects on lactic acid bacteria (i.e., *Lactobacillus*, *Bifidobacterium*, *Enterococcus*). Many of these bacterial strains have been used successfully as equine probiotics. These indigestible prebiotics serve as a substrate for lactic acid bacteria (LAB), potentially improving the microbiota of the large intestine. There have been a limited number of equine digestibility studies using prebiotics as digestive aids. Similar to studies using probiotics, the results are variable. When *S. cerevisiae* fermentation products were supplemented in conjunction with low-quality forage, the apparent digestibility of dry matter, crude protein, and neutral detergent fiber was greater,

indicating prebiotic supplementation is more effective when high-starch, high-fiber, or low-quality forage diets are fed.

Starch intake after supplementation the diet of the horse with short-chain fructo-oligosaccharides (scFOS) (Respondek et al. 2011) or MOS have beneficial effects in preventing digestive disorders associated with both prebiotics. Furthermore, use of scFOS in horses reduced disruptions in colonic microbial populations after an abrupt change in diet and altered faecal VFA concentrations towards propionate and butyrate (Coverdale 2016).

Dogs and Cats

Dogs and cats evolved as Carnivora, eating diets high in protein and fat but low in fibre. They are non-ruminants with short, simple GIT that have little capacity to ferment nondigestible substances, which action occurs predominantly in the colon (Stevens and Hume 1998). Nevertheless, some health benefits have been achieved with prebiotic administration such as reduced infections, improved insulin sensitivity, and better faecal consistency (Respondek et al. 2008; Verbrugghe et al. 2009). Most studies have investigated the effects of dietary supplementation with prebiotics on the bacterial flora in healthy dogs and cats. FOS supplemented at 0.75% dry matter produced qualitative and quantitative changes in the faecal flora of healthy cats (Sparkes et al. 1998a, b).

Compared with samples from cats fed a basal diet, increased numbers of lactobacilli and *Bacteroides* species and decreased numbers of *E. coli* were associated with the FOS diet. However, bacteriological examination of the duodenal juice in these same cats showed wide variation in the composition of the duodenal flora, across sampling periods, which was not affected by FOS supplementation. In a separate trial, healthy cats fed a diet containing short-chain GOS and FOS had greater faecal *Bifidobacterium* species populations and butyrate concentrations versus the control (Kanakupt et al. 2011). Further, healthy Beagle dogs fed a 1% FOS diet over a 3-month trial showed inconsistent faecal excretion of species of *Lactobacillus* and *Bifidobacterium* (Willard et al. 2000). There is a single report evaluating the effects of GOS on the faecal microbiota in healthy cats and cats with inflammatory bowel disease (IBD) (Biagi et al. 2013). Using a randomized, double-blinded, cross-over feeding trial, oligonucleotide probes targeting specific bacterial populations showed no significant differences in the faecal microbiota of IBD cats and healthy cats fed the same diet. Overall, interanimal variation was moderately high whereas a trend of increased *Bifidobacterium* species levels was observed with GOS supplementation.

Farmed Aquatic Species

Farmed aquatic species include finfish and shellfish. Although anatomy varies among carnivorous (e.g., turbot), omnivorous (e.g., catfish), and herbivorous (e.g., sturgeon) species, all fish have a fairly simple and short GIT. The short length and simple structure (lack of special adaptations) of the fish gut results in the rapid transit of digested material, limiting the time available for microbial or prebiotic activity. The prebiotics are indigestible fibers that increase beneficial gut resident commensal bacteria, resulting in improvement of the host health. Prebiotics are found to stimulate the growth of species of intestinal bacteria in aquatic species. Effective prebiotic doses in aquatic host species are in the range of 1% to 3% (weight/volume) of diet (Li et al. 2007; Hoseinifar et al. 2013, 2014). The beneficial effects of prebiotics result from the by-products generated from their fermentation by gut commensal bacteria. Prebiotics such as FOS, MOS, inulin, or β-glucan are called immunosaccharides. These additives directly enhance innate immune responses including phagocytic activation, neutrophil activation, activation of the alternative complement system, and increased lysozyme activity, among others, in farmed aquatic fish (Table 1). Immunosaccharides directly activate the innate immune system by interacting with pattern recognition receptors (PRR) expressed on innate immune cells (Kyu Song et al. 2014). They can also associate with microbe-associated molecular patterns (MAMPs) to activate innate immune cells. Proper immune responses are important not only for combating pathogens but also for appropriate weight gain. Many studies have indicated that immunosaccharides are beneficial to both finfish and shellfish (see Table 1).

The prebiotic mannan-oligosaccharide improves growth and enhances digestive enzymes such as protease and amylase (Xu et al. 2009).

4   Probiotics

Prebiotic and probiotic approaches both demand the use of microbial food supplements that beneficially affect the host by improving its intestinal microbial balance (Gibson and Roberfroid 1995). Probiotics are another feed additive that

Table 1 Prebiotics as immunostimulants in aquaculture

Oligosaccharides	Polysaccharides
• Fructo-oligosaccharides (FOS)	• Inulin
• Mannan-oligosaccharide (MOS)	• β-Glucan
• Mannan-oligosaccharide (MOS) + β-glucan	• Chitin/chitosan
• Galacto-oligosaccharide (GOS)	
• Arabinoxylan-oligosaccharide (AXOS)	

is gaining acceptance as a potential alternative to antibiotics to improve production efficacy in livestock, poultry and aquaculture. Probiotics have been defined as "mono- or mixed cultures of living microorganisms which beneficially affect the host by improving the properties of the indigenous microbiota" (Fuller 1992). Probiotics are defined as "live microbial feed additive that beneficially affects the host animal by improving its intestinal balance; probiotics, sometimes used interchangeably with the term direct-feed microbial (DFM), used in the US for products that are given to animals. Microorganisms used in animal feed in the European Union (EU) are mainly bacterial strains of gram-positive bacteria belonging to the types *B. licheniformis*, *B. subtilis*, *Enterococcus* (*E. faecium*), *Lactobacillus* (*L. acidophilus*, *L. casei*, *L. farciminis*, *L. plantarum*, *L. rhamnosus*), *Pediococcus* (*P. acidilactici*), and *Streptococcus* (*S. infantarius*); some other probiotics are microscopic fungi such as strains of yeast belonging to *Saccharomyces cerevisiae* and *Kluyveromyces* (Anadón et al. 2006). *Bacillus* and *Lactobacillus* bacteria differ in many characteristics, and *Bacillus* and the yeasts are not usual components of the gut microflora. Although most of the species and genera are apparently safe, particularly lactobacilli and bifidobacteria, certain microorganisms may be problematic, particularly the enterococci, which may harbour transmissible antibiotic-resistant determinants and bacilli, especially those belonging to the *Bacillus cereus* group that are known to produce enterotoxins and an emetic toxin (Anadón et al. 2006). For example **Bacillus cereus var. toyoi NCIMB 40112/CNCM I-1012) as a feed additive was withdrawn in the EU in 2015 for all animal species authorized previously**.

To date, reported performance enhancement in domestic animals has primarily been obtained through the application of one specific species or a mixture of probiotic strains within a species (Williams et al. 2001). For humans, specific microbial strains could have an important role in colonization resistance in the intestinal, respiratory, and urogenital tracts, cholesterol metabolism, inhibition of carcinogenesis by stimulating the immune system and lactose metabolism, absorption of calcium, and synthesis of vitamins (Anadón et al. 2016). For farm animals, the most important claims are growth promotion, improved FCR, health control such as prevention of intestinal disturbances (especially in young animals), pre-digestion of anti-nutritional factors (e.g., trypsin inhibitors, phytic acid, glucosinolates) (Havenaar et al. 1992), and welfare promotion.

Livestock probiotics commonly feature various strains of *Bacillus*, *Lactobacillus*, *Enterococcus*, and *Saccharomyces* yeast. There is still much to learn about their interactions with pathogens, but it is understood that certain strains of *Bacillus* have been proven to decrease growth of certain species of pathogenic bacteria including *E. coli*, *Clostridium*,

Streptococcus, and *Salmonella*. Probiotics help prevent and control GI pathogens or improve the performance and productivity of livestock animals through various mechanisms. The selection of suitable probiotic strains is absolutely essential because such strains must not carry antibiotic resistance genes. Other genes that should, of course, be absent in probiotic strains are those that code for the production of toxins or compounds that can interfere in any way with an animal's well-being and productivity (Anadón et al. 2016; Joerger and Ganguly 2017).

A few genetically modified strains have been tested with the aim of improving animal performance. For example, *Lactococcus lactis* was engineered to express the epidermal growth factor EGF-LL in an effort to boost the performance of early-weaned piglets (Bedford et al. 2012), and the yeast *Pichia pastoris* was modified by the introduction of the *Clostridium perfringens* alpha toxin gene in an attempt to induce immunity against *C. perfringens* in broiler chickens (Gil de los Santos et al. 2012).

A suitable probiotic organism should be able to resist processing and storage, survive in the gastric acidic environment, adhere to the epithelium or mucus in the intestines, produce antimicrobial compounds, and modulate immune responses. However, not all probiotic strains exhibit all these properties and the most suitable probiotic strains or their combinations that will achieve maximum beneficial effect should be selected. Protection of probiotic organisms during their passage through the upper alimentary tract, such as a microencapsulation, should be considered to ensure viability and colonization in the intestine (Anadón et al. 2016b).

Probiotics or active microbials can help modulate the microflora, and slow-release butyrates can have a positive effect on intestinal integrity. Butyrate seems to have a positive influence on epithelial cells, leading to better epithelial cell proliferation and differentiation. Apart from better water and nutrient absorption, this leads to an improved barrier and pathogen control and seems positively to influence the immune system (Eeckhaut et al. 2008).

Probiotics are live organisms that, when ingested in sufficient quantities, transfer a health benefit to the host. Common probiotics include members of the *Lactobacillus* and *Bifidobacterium* genera and organisms such as *Saccharomyces cerevisiae*. Probiotic activity could be related to genera, species, or strains. The efficacy of single-strain and multi-strain probiotics for livestock has been investigated: the beneficial properties include removal or competitive exclusion of pathogens, enhanced immune system development and responsiveness, and production of beneficial compounds and metabolic by-products (Patterson and Burkholder 2003).

For probiotics, two main mechanisms of action have been suggested, summarized as follows: (1) nutritional effect, characterized by reduction of metabolic reactions that

produce toxic substances, stimulation of indigenous enzymes, and production of vitamins and antimicrobial substances; and (2) health or sanitary effects, distinguished by increase in colonization resistance, competition for gut-surface adhesion, and stimulation of the immune response (Guillot 2003); the latter effect acting as 'bio-regulators of the gut microflora' and reinforcing the host natural defences. In this latter mechanism there is an increase in cell-mediated immune response, TLR signalling, antibody production, and decrease of cellular apoptosis, among others (Khan et al. 2016). Host intestinal epithelial cells and dendritic cells have certain receptors [e.g., TLRs, nucleotide-binding oligomerization domain (NOD) proteins] activated by probiotic MAMPs such as fimbriae, flagellae, lipopolysaccharide, lipoteichoic acid, and peptidoglycan. Activation of these receptors leads to induction of signal transduction pathways in the host cell for transcription of genes coding for chemokines and cytokines, which can subsequently stimulate host systemic and mucosal immunity (Hardy et al. 2013). Interleukin 12 (IL-12), a proinflammatory cytokine, and interleukin (IL-10), an antiinflammatory cytokine, are of particular interest with respect to probiotics. Immunostimulatory probiotics induce IL-12 proliferation, which in turn increases the potency of NK cells and induces T-helper pathways. Immunoregulatory probiotics induce IL-10 proliferation, which then induces the T-regulatory pathway (Yaqoob 2014). Probiotics have also been known to alter the gut epithelial architecture. The mucus layer, composed of a class of glycoproteins known as mucins, forms the first line of host defence along with the gut epithelium. Studies have shown that certain probiotic species increase the expression of mucin 2 (MUC-2) and mucin 3 (MUC-3) genes, which code for the synthesis of mucins by goblet cells. Increased mucus production in the gut prevents the adherence and subsequent colonization of the intestinal epithelium by pathogenic bacteria (Hardy et al. 2013). Probiotics can also maintain the integrity of epithelial tight junctions by upregulating genes that code for junction proteins, which are responsible for tight junction signalling, as well as the restoration of mucosal integrity (Syngai et al. 2016). It was reported that *Lactobacillus rhamnosus GG* produced soluble proteins which protect the intestinal epithelial tight junctions and the barrier function from hydrogen peroxide-induced disruption by the activation of protein kinase C isoforms and the mitogen-activated protein kinase (MAPK)-dependent mechanism (Seth et al. 2008).

The delivery of certain living microorganisms during food animal production, with a variety of microorganisms used being LAB, various *Bacillus* species, and the yeast *Saccharomyces cerevisiae*, have been particularly used in the pig industry. The establishment of a beneficial LAB population at birth may lead to healthier animals: this may be most readily achieved by treating sows, which provides an amplification step and floods the neonatal pig environment with desirable

bacterial strains (Kenny et al. 2011). Dietary supplementation with DFMs are used to promote health in livestock and poultry resulted in energy repartitioning to the immune system and an increase in antibody production independent of changes in whole-body metabolism or growth performance (Qiu et al. 2012).

Dietary feeding of probiotic-supplemented feed reduced intestinal inflammatory cytokine expression and enhanced growth performance in poultry (Higgins et al. 2011). Moreover, *Bacillus subtilis* strain PB6, provided as a powder preparation, may have preclinical antiinflammatory effects in an acute mice model, reducing symptoms of inflammatory bowel disease that are dependent on immunomodulatory responses (Foligné et al. 2012).

Probiotics would therefore have a role in the balance of gut microflora, increasing resistance to pathogenic agents, both through a strengthening of the intestinal barrier and by stimulating the immune system directly.

Competitive Exclusion Probiotics operate by "competitive exclusion," meaning that when adequate populations of probiotic bacteria are present, they reduce the ability of pathogenic bacteria to get out of control and overwhelm the host. The available probiotics can be classified into (1) 'colonising' species, such as *Lactobacillus* and *Enterococcus* spp., and (2) free-flowing 'noncolonizing' species, such as *Bacillus* spp. (spores) and *S. cerevisiae*. Competitive exclusion describes the treatment of day-old chicks with an undefined microbiota derived from adult animals, resulting in colonization resistance against pathogenic microorganisms (Huyghebaert et al. 2011).

Current feed additive products designed for microbiotal manipulation of food animals with live microorganisms (or with products directly derived from the culture of these organisms) fall into two categories. The predominant group (first category) attempts to improve or maintain animal health status under the conditions encountered in modern animal husbandry practices without making specific claims to target pathogens that are of concern to human health. The second category (smaller group) claims to establish or modify intestinal microbiota that have a direct measurable effect on pathogens of concern to humans, such as *Salmonella enterica* and *Clostridium jejuni*. The two categories are not mutually exclusive because healthier animals are expected to be less susceptible to colonization with certain human pathogens or to carry fewer of these pathogens. Similarly, the microbiota changes designed to inhibit food-borne pathogen colonization can also improve overall animal health and lead to gains in body weight. The number of probiotic products put on the market that claim to be directed against pathogens or are "competitive exclusion" products is exceedingly small compared to products that claim to improve FCR, growth, immune system function, or resistance to stressful events (Joerger and Ganguly 2017). The ecological definition of

"competitive exclusion" states that two species competing for the same resources cannot coexist stably. Therefore, one of the competitors will always dominate the other, leading to an evolutionary modification, a shift to another niche, or extinction. The intestinal microbiota competes with the colonizing pathogenic bacteria and can reduce the adhesion and colonization of pathogens in the intestine. This reduction might be a consequence of different mechanisms, perhaps the physical occupation of space, competition for resources in a given niche, or direct physical or chemical confrontation with the potential colonizer (Chaucheyras-Durand and Durand 2010; Clavijo and Vives Flórez 2018). In birds, competitive exclusion is the process by which favourable bacteria exclude bacteria that may be detrimental to the animal or that are of public health interest, such as *Salmonella* spp. The exclusion of *Salmonella* implies preventing the establishment of harmful bacteria in the gut. The aim is to provide, early in a bird's life, good bacteria having optimal ability to establish and maintain themselves in the gut environment. Thus, administering bacterial mixtures of *Salmonella* spp. from faecal or caecal sources of broiler chickens was more protective than administering single bacterial isolates or a combination of only a few isolates (Kerr et al. 2013). In practice, it is mainly used as a prophylactic measure aimed at increasing the resistance of chicks and poults to *Salmonella* infection. It does imply that the young birds being treated are *Salmonella* free, because the good bacteria are not likely to be able to displace *Salmonella* if it has had the opportunity to become established first in the gut. To achieve this, is imperative to administer the treatment immediately posthatch, before the chicks or poults can be exposed to *Salmonella* spp. The main mode of action of "competitive exclusion" is the establishment of a physical barrier (good bacteria culture attaching to the gut wall) between the intestinal wall and the lumen of the gut. Establishment of favourable bacteria increases the production of VFA and lactate, which lower the gut pH. The lower pH and high VFA concentration produces a hostile environment for unwanted bacteria, such as *Salmonella* spp. and *E. coli* (Lutful Kabir 2010).

4.1　Probiotic Application in Different Animal Species

The International Scientific Association for Probiotics and Prebiotics has defined probiotics "as a mixture of live microorganisms which when administered in adequate amounts confer a health benefit on the host" (Smith 2014). Before explaining the mechanisms and benefits provided by these microorganisms, it is important to specify why, if a microorganism is to be considered a probiotic, it should meet a range of requirements: (1) not pathogenic; (2) can adhere to epithelial cells; (3) can colonize and reproduce itself in the host; (4) able to survive passage through the GIT; (5) is resistant to gastric acidity and bile content; (6) produces metabolites that inhibit or kill pathogenic bacteria; and (7) has undergone trials in vitro and in vivo that demonstrate its benefits. Finally, a probiotic should remain viable under processing, production, and storage conditions (Kabir 2009). The following benefits are expected from administering probiotics (Syngai et al. 2016): (1) stimulation of the development of beneficial microbiota; (2) reduction and prevention of colonization by enteric pathogens; (3) modulation of immunological activity; (4) stimulation of epithelial health; (5) increased digestive capacity; and (6) aid in maturation of intestinal tissue. Probiotics can influence the immune system both directly and indirectly. Direct influence is exerted by different species of *Lactobacillus* that increase cytokine and antibody levels (Haghighi et al. 2006; Brisbin et al. 2011).

Poultry

Numerous probiotic strains have been tested for use for poultry to improve performance, prevent pathogenic colonization, and improve immunity. Probiotics such as *Lactobacillus johnsonii*, *Bacillus subtilis,* and a multi-strain *Lactobacillus johnsonii* FI9785 modified the intestinal microbiota by reducing levels of pathogenic bacteria such as *Salmonella enteritidis* and *Clostridium perfringens* in neonatal broiler chicks (La Ragione et al. 2004; Higgins et al. 2008). Also, a multi-strain probiotic significantly increased the numbers of lactobacilli and bifidobacteria in the caeca of broiler chickens (Mountzouris et al. 2007).

Probiotics for poultry may contain one or more strains of microorganisms and may be given either alone or in combination with other additives in feed or water. The use of probiotics in broiler chickens to control *Salmonella* spp. was effective when hatched chicks were fed a suspension of the intestinal contents of adult chickens (Nurmi and Rantala 1973). However, this first proposed use of "probiotic" proved to have serious limitations, principally the potential transfer of diseases along with the beneficial microorganisms. For this reason, subsequent research has focused on developing defined probiotics capable of being cultivated and administered as pure cultures (Smith 2014). A range of probiotics has been developed, obtained in various ways and for which dosage and time in the cycle in which they are administered also varies. Novel application strategies such as spraying the probiotic on chicks or embryonated eggs are also studied, and potential methods such as in ovo application are being evaluated (Cox and Dalloul 2015).

A variety of bacteria species (*Bacillus*, *Bifidobacterium*, *Enterococcus*, *Lactobacillus*, *Streptococcus*, and *Lactococcus* spp.) and yeast species (*Saccharomyces* spp.) have been tested and used as probiotics in poultry. The majority of the conducted research was specifically aimed at investigating the effects of probiotics in reducing the numbers of pathogenic microorganisms in the GIT. However,

a considerable amount of research also examined the effects of probiotics on improving growth and performance in poultry without apparent disease. Supplementation of diets with a single strain of *Lactobacillus* sp. (*L. casei*, *L. fermentum*, *L. bulgaricus*, *L. reuteri*) was shown to improve body weight and feed efficiency in broiler chickens. Similar results were shown when broiler chickens were given multiple strains of *Lactobacillus* sp. Probiotics based on *Bacillus* sp. (*B. coagulans*, *B. subtilis*, *B. licheniformis*, and *B. amyloliquefaciens*) were also successfully employed in poultry diets and shown to have growth-promoting effects.

Different trial studies have shown that chickens treated with probiotics produce a greater number of antibodies in response to a given antigen (Brisbin et al. 2010). Probiotics may also have indirect effects, promoting the growth of other bacteria. For example, *Lactobacillus agilis* and *Lactobacillus salivarus* can stimulate butyrate-producing microbiota and reestablish microbiota balance (Meimandipour et al. 2009). Another benefit of probiotics is competing with pathogenic microorganisms such as *Salmonella*, *Enterobacter sakasaki*, and *Clostridium difficile*, which have a high capacity of adhesion to the intestinal mucosa (Collado et al. 2005).

Strains of probiotics that help to reduce these levels of adhesion include bacteria of the genera *Bifidobacterium* (Collado et al. 2005) and *Lactobacillus* (Servin and Coconnier 2003). However, this ability is highly dependent on the source of the microorganism, as bacteria from the intestines of chickens show a greater capacity to adhere to the mucosa and, therefore, to displace pathogenic microorganisms (Collado et al. 2005).

The inhibitory effects of probiotic bacteria on undesirable microorganisms might result from the production of metabolites such as hydrogen peroxide (H_2O_2), diacetyl, bacteriocins, and organic acids. A purified bacteriocin produced by *Lactobacillus salivarius* NRRL B-30,514 was used to treat chickens, causing a clear reduction in the numbers of *C. jejuni* in their intestines (Stern et al. 2006). Other compounds that assist in the exclusion of human pathogenic microorganisms are organic acids such as lactic, acetic, or propionic acid, which diminish pH levels in the intestine and reduce the speed of pathogen multiplication (Blajman et al. 2015).

The effectiveness of probiotics depends on several factors, such as the composition of the mixed rations, the time when they are administered, and the origin of the microorganisms. It seems that the effectiveness of probiotic cultures is greater when they contain a larger number of genera (Chambers and Gong 2011). Similarly, origin affects effectiveness, as strains that come directly from chicken intestines are more effective than those from other sources. Additionally, the probiotic composition may be beneficial for one breed of chicken but not for others. Another factor affecting the effectiveness of probiotics is the time point at which they are administered.

When probiotics are administered at an early stage of the cycle they will have positive effects only up to week 6, showing greater diversity and abundance of *Lactobacillus* and a significant reduction in the presence of chicken pathogens compared to the control (Nakphaichit et al. 2011). It has also been suggested that the administration of probiotics has a greater effect on pathogenic microorganisms following a change in diet or after antibiotic therapy (Zulkifli et al. 2000).

Lactobacillus is the most commonly used probiotic; its reported benefits include increased weight gain, improved feed utilization effectiveness, and reduction in mortality (Zulkifli et al. 2000; Kalavathy et al. 2003; Timmerman et al. 2006). The probiotic model has been used widely in broiler chickens for the control of *Salmonella*, and it has been reported that employment of these cultures led to reductions in colonization by this pathogen, an effect that is also correlated with increased weight gain and improved conversion of feed into body mass (Chambers and Gong 2011).

Pigs

Weaning as currently practised is one of the most critical periods for pigs, being characterized by a drop in food consumption, leading to severe anorexia, increased susceptibility to digestive disorders, growth delays, and microbial infections. The change in food substrate also leads to significant changes in the functionality of the intestine. *Saccharomyces cerevisae* yeasts, their cell walls or extracted fractions (mannan-oligosaccharides, β-glucans), seem to constitute positive alternatives. Their use in porcine diets can contribute to improving growth performance, stimulating the immune system, maintaining the balance of digestive microflora, and preventing bacterial adhesion to intestinal epithelial cells. Yeasts or yeast products might be potential alternatives to antibiotic growth promoters for swine.

The effect of including the yeast *S. cerevisiae* or its cell wall fraction in diets for weanling piglets for growth performance, nutrient utilization, and some morphological and immunological parameters has been evaluated. Two diets were supplemented with 1 g/kg of live yeast or yeast cell walls for an experiment lasting 5 weeks. Overall, increases in weight gain and in final body weight were observed, and the feed:gain ratio tended to improve with yeast diets. The inclusion of yeasts or yeast cell walls reduced the number of intraepithelial lymphocytes, and increased VFA production and the percentage of acetate, having beneficial effects on the productive performance of piglets after weaning (Lizardo et al. 2008).

Effects of *S. cerevisiae* [strain CNCM I-4407, 10(10) cfu/g] has been studied on postweaning diarrhea, immune response, and growth performance in weaned piglets orally challenged with enterotoxigenic *Escherichia coli* strain O149:K88. The live yeast was fed to sows and their piglets

in the late gestation, suckling, and postweaning periods. Sows were fed a basal diet without or with supplementation (i.e., 1 g/kg of live yeast) from day 94 of gestation and during lactation until weaning of piglets (day 28). Suckling piglets of the supplemented sows were orally treated with 1 g live yeast in porridge carrier three times a week until weaning. Weaned piglets were fed a basal starter diet without or with supplementation (i.e., 5 g of live yeast/kg feed for 2 weeks). Significantly lower daily diarrhea scores, duration of diarrhea, and shedding of pathogenic *E. coli* bacteria in faeces were detected in the supplemented piglets. Administration of live yeast significantly increased IgA levels in piglet serum. Evidence indicates that decreased infection-related stress and decreased severity of diarrhea in yeast-fed weaned piglets positively affected their growth capacity in the postweaning period. Thus, dietary supplementation with live yeast *S. cerevisiae* to sows and piglets in late gestation, suckling, and postweaning periods can be useful in the reduction of the duration and severity of postweaning diarrhea caused by *E. coli*. Decreased infection-related stress and severity of diarrhea in yeast-fed weaned piglets can positively affect growth performance in the pre-weaning period. The results from this study suggest that live yeast *S. cerevisiae* (strain CNCM I-4407) could be an alternative for prevention and treatment of postweaning diarrhea. In addition, *S. cerevisiae* can decrease inflammatory responses induced by F4+ enterotoxigenic *E. coli* in porcine intestinal epithelial cells.

Enterotoxigenic *E. coli* are pathogenic gram-negative bacteria that infect several species of farm animals, including pigs. Enterotoxigenic *E. coli* infection and enterotoxic secretion can induce intestinal inflammation and diarrhea, resulting in reduced growth rate, increased mortality, and economic loss (Fairbrother et al. 2005). Probiotic yeasts may provide protection against intestinal inflammation induced by enteric pathogens. In piglets, infection with F4+ enterotoxigenic *E. coli* causes inflammation, diarrhea, and intestinal damage. The yeast strains *S. cerevisiae* (strain CNCM I-3856) and *S. cerevisiae* var. *boulardii* (strain CNCM I-3799) were investigated for decreased expression of pro-inflammatory cytokines and chemokines in intestinal epithelial IPI-2I cells cultured with F4+ enterotoxigenic *E. coli*. Results showed that viable *S. cerevisiae* inhibited ETEC-induced TNF-α gene expression whereas *Saccharomyces boulardii* did not. In contrast, killed *S. cerevisiae* failed to inhibit the expression of pro-inflammatory genes: this inhibition was dependent on secreted soluble factors. *S. cerevisiae* culture supernatant decreased the TNF-α, IL-1α, IL-6, IL-8, CXCL2, and CCL20 enterotoxigenic *E. coli*-induced mRNA. Furthermore, the *S. cerevisiae* culture supernatant filtrated fraction at 10 kDa displayed the same effects, except for TNF-α.

Inclusion of *Lactobacillus sobrius* in pig diets may be significantly effective in the reduction of *E. coli* F4 colonization and may improve the weight gain of infected piglets (Konstantinov et al. 2008). Also, a multi-strain probiotic containing *L. acidophilus*, *L. bulgaricus, B. subtilis*, and *S. cerevisiae* significantly increased ileal and colonic bifidobacteria levels and decreased the levels of colonic coliforms.

Ruminants

The yeast *S. cerevisiae* has been used as an alternative to antimicrobial feed additives in ruminants for more than 15 years. Production responses showed improved live weight gain in beef cattle and increased milk yield and fat production in dairy cows. However, responses were highly variable and apparently influenced by diet composition and animal physiological stage. The yeast is generally available in two different DFM forms: yeast culture products and live yeast cell products. Ruminant animals, including cattle, sheep, and goats, principally depend on microbial degradation of their feed rather than on direct enzyme degradation, as in most non-ruminants. The enlarged foregut of ruminant livestock (reticulorumen) allows a large and diverse microbial population to gain access to feedstuff before the products of this fermentation and the microbial cells enter the absorptive regions of the GIT (Russell 2002).

The rumen is a symbiosis pathway between the ruminant host and microbes. It is known that microorganisms are involved in the animal host in supplying protein, vitamins, and short-chain organic acids. In the cattle rumen, for example, live yeast can improve milk yield and weight gain by microbial activity stimulation, although this stimulation might depend on certain microbial species. Cattle feed supplemented by live yeast *S. cerevisiae* Sc47 (0.5 or 5 g/day) modifies bacterial diversity and population and changes in the fermentation pattern and physicochemical parameters in the rumen which can modify microbiota composition. In this study the improvement of zootechnical parameters goes together with a shift in the mannin fibrolytic group (i.e., *Fibrobacter* and *Ruminococcus*) (Pinloche et al. 2013).

Early-lactation high-producing dairy cows have a nutrition strategy with the objective to provide adequate energy and rumen-undegraded protein to support high requirements in regard to milk production increase; most of the time, cows are in negative energy balance (Julien et al. 2015). Therefore, it should be important to use live yeast as a feed additive for the dietary rumen-degradable protein level, increasing the feed energy with a certain amount of grain (Julien et al. 2015).

Inclusion of live yeast stabilizes the rumen environment through higher pH values and enhances fibre digestion (Campanile et al. 2008). These key results certainly explain why the impact of live yeast supplementation has been studied primarily on energetic metabolism, in relationship to the dietary forage:concentrates ratio (Lascano et al. 2009). The

consensus was that the effects of live yeast were enhanced when animals consumed a highly concentrated diet or during an abrupt dietary transition (Chaucheyras-Durand et al. 2008). However, some studies have considered the potential effect of live yeast on ruminal ammonia nitrogen metabolism, more specifically on protein degradation or microbial proteosynthesis. In studying the interaction between live yeast and dietary rumen-degradable protein level, Julien et al. (2015) concluded that the rumen-degradable protein content of diet-fed lactating dairy cows could directly impact the acidogenic capacity of the diet: tanned soybean meal was less acidogenic than soybean meal when used as the main protein source. The positive effect of live yeast on ruminal pH in cows receiving a highly acidogenic diet and therefore suffering from subacute ruminal acidosis (SARA) is already known, as live yeast could modulate dietary N digestion in early-lactating dairy cows whose diet had an inadequate rumen-degradable protein content. In fact, live yeast seemed to have a post-ruminal effect on N digestibility even more pronounced than the quantity of bypass N, that is, with sources of protected dietary protein. In both cases, live yeast used as a dietary feed additive permits a better utilization of diet in dairy cows (Julien et al. 2015), and, moreover, increased ruminal total VFA.

Heat stress negatively affects the productivity and longevity of dairy cows. Heat stress has reduced intake and increased reliance on glucose, so feeding strategies capable of improving diet digestibility are plausible for improving post-rumen nutrient flow and performance. Advances in management such as cooling systems and nutrition strategies may attenuate the negative effects of heat stress, but the economic loss from reduced milk production, reproductive efficiency, and animal health during warm seasons is a major issue for the dairy industry worldwide (St. Pierre et al. 2003). The effect of live yeast (S. cerevisiae) on digestion and performance of lactating cows during the warm summer months of southeastern Brazil was evaluated (Salvati et al. 2015) using treatments with S. cerevisiae equivalent to 25×10^{10} cfu of live cells and 5×10^{10} cfu of dead cells top-dressed to the diet in the morning. A trend was observed for increased plasma glucose with yeast (62.9 vs. 57.3 mg/dl), lowered respiratory frequency (48 vs. 56 breaths/min), and increased plasma niacin content (1.31 vs. 1.22 μg/ml), although the cows had similar rectal temperature. Ruminal lactate and butyrate as proportions of ruminal organic acids were reduced by yeast. Plasma urea nitrogen was increased by yeast over 24 h. Yeast treatment produced a higher blood pH compared with the control, 7.34, and 7.31, respectively. Yeast supplementation improved the lactation performance of dairy cows under heat stress, although this improvement apparently involved regulation of body homeothermia rather than improved digestibility (Salvati et al. 2015).

In high-yielding dairy cows, live yeast S. cerevisiae differs from sodium bicarbonate to stabilize ruminal pH (Marden et al. 2008). Early-lactating Holstein cows were supplemented with 150 g/day of sodium bicarbonate or 5 g/day of live yeast during a 21-day experimental period. Total VFA, acetate, and propionate were greater with both additives, but butyrate remained constant; and mean total lactate concentrations decreased 67% with S. cerevisiae. The conclusion was S. cerevisiae prevented accumulation of lactate and allowed better fiber digestion, whereas sodium bicarbonate seemed to act only as an exogenous buffer.

Moreover, a bio-energetic–redox approach to the effect of S. cerevisiae on ruminal pH during induced SARA in dairy cows was described by Marden et al. (2013). The capacity of S. cerevisiae at 4 g/cow/day in optimizing ruminal pH was evaluated to understand its mode of action during induced acidosis in the cows. The beneficial effects of live yeast on concentrations of VFA and proportion of propionate in ruminal fluid were higher compared to the control diet. The proportion of butyrate decreased, from 15.8% to 14.2% total VFA, and lactate concentration decreased by 55% on average. Stabilization of ruminal pH (>6) is the outcome of S. cerevisiae ability to scavenge oxygen after feeding a high-starch diet. Live yeast seems to act on the reducing power of the ruminal milieu by decreasing oxygen partial pressure and thereby enhancing the activity of anaerobic bacteria.

Horses

Intensive management practices in the horse industry present a unique challenge to the microbiome of the large intestine. Common management practices such as high-concentrate diets, low forage quality, meal feeding, and confinement housing have an impact on intestinal function, specifically large intestinal fermentation. The microbiome of the equine large intestine is a complex and diverse ecosystem, and disruption of microbiota and their environment can lead to increased incidence of GI disorder. In horses, whose targeted digestive compartment is the caecum-colon, probiotic distribution appears particularly relevant in case of stress (e.g., transportation) or during distribution of a high-concentrate diet (Coverdale 2016). Research concerning the use of probiotics in horses to improve hindgut fermentation and diet digestibility has been limited. Most studies used live yeast culture (S. cerevisiae) supplemented to a variety of diets. Despite a lack of evidence for colonization with the supplemented strain of S. cerevisiae, improvements in cell wall digestibility were evident regardless of diet. In particular, when added to high-starch diets, S. cerevisiae supplementation appears to mitigate some of the disruptions, such as reduced fiber digestibility, that occur in the hindgut. Maintenance of fiber digestion is of particular interest when horses consume high-concentrate diets for the purpose of athletic

performance or maximum production (lactation, growth, etc.).

Probiotics, or direct-fed microbials, have been widely used in horses for treatment and prevention of GI disease. Introduction of these live, beneficial microorganisms orally into the intestinal tract has yielded variable results. However, it is difficult to compare data because of variations in choice of organism, dosage, and basal diet. Although there are still many unanswered questions about the mode of action of successful probiotics, evidence indicates competitive inhibition and enhanced immunity. A variety of microbial species have been tested in the horse as probiotics, such as *Lactobacillus* spp., *Enterococcus* spp., *Bifidobacterium* spp., and *Saccharomyces* spp. (Coverdale 2016). Diets containing *Lactobacillus acidophilus* had limited effects either on reducing the risk of acidosis associated with feeding high-starch concentrates to horses or on nutrient digestibility. Live yeasts have been demonstrated to elicit an increase in fibre digestibility in the colon and to modulate the balance of hindgut bacterial communities, with a decreased risk of lactic acidosis (Jouany et al. 2008). Use of these products has resulted in improved fibre digestibility in horses offered both high-starch and high-fibre diets.

Rabbits

Intensive breeding of rabbits can alter the environment, causing physiological stress, and increasing the frequency of enteric diseases, subsequently causing high mortality and decreased reproductive and productive performance of rabbit does (Combes et al. 2013). Application of probiotics as dietary supplements could control enteric diseases. Thus, some probiotics exert a barrier effect against pathogenic microorganisms by preventing their development and colonization within the digestive tract (Vanderpool et al. 2008). The most frequently examined microorganism related to probiotics has been *S. cerevisiae* yeast, known to improve growth performance in cross-breed rabbits. The effect of live yeast supplementation in the diet of rabbit does on their mortality and reproductive performance and the performance of their progeny was studied in two groups differing in diet during two reproductive cycles. Natural mating was performed 11 days after kindling and kits were weaned at 28 days of age. The addition of 1 g *S. cerevisiae*/kg of diet enhanced fertility and reduced mortality of rabbit does, while improving the viability rate of kits at birth; no difference was observed during the second lactation. However, diet supplemented with the tested probiotic had no effect on other reproductive performance traits in rabbit does (Belhassen et al. 2016).

Dogs and Cats

Lactobacillus spp. and *Enterococcus* spp. were studied as probiotics for dogs and *Bifidobacterium* spp. for cats.

Aquaculture

Probiotics intended for aquatic usage must take into account the relationship an aquatic organism has with its direct environment. Gram-negative facultative anaerobic bacteria are dominant in fish and shellfish digestive tracts; however, the intestinal microbiota may often change with the intrusion of microbes from water and food (Chaucheyras-Durand and Durand 2010). Thus, a large number of probiotics developed in aquaculture probably are bacteria directly originating from the aquatic environment. However, most probiotics commonly used in aquaculture are prokaryotic bacteria or yeast such as *Lactobacillus* spp., *Pediococcus* spp., *Bacillus* spp., *Vibrio* spp., and *S. cerevisiae*. Yeast species have also been used as probiotics and for delivery of enzymes in animal feeds. A number of eukaryotic microorganisms are able to survive passage through the acidic conditions and bile salts of the GIT to the intestine. Because these microorganisms may be beneficial for host health, feed utilization, and growth performance, they could also be used as alternative probiotics. Probiotics can target fish eggs and larvae, fish juveniles and adults, crustaceans, bivalve mollusks, and also live food such as rotifers, *Artemia*, or unicellular algae (Verschuere et al. 2000). Growth-promoting effects through better feed utilization and digestion, as well as biological control of pathogen colonization, are the most important expected benefits of probiotic applications (Chaucheyras-Durand and Durand 2010). Disease outbreaks caused by *Vibrio* spp. or *Aeromonas* spp. have been recognized as a significant constraint on aquaculture production (Verschuere et al. 2000), particularly in the shrimp subsector, where vibriosis is currently one of the main diseases identified (Castex et al. 2008). Some probiotics have been shown to protect rainbow trout against skin infections caused by *Aeromonas bestiarum* and a eukaryotic pathogen, *Ichthyophthirius multifiliis* (Pieters et al. 2008).

The effects of dietary probiotic *S. cerevisiae* microencapsulated with guar gum in the striped catfish (*Pangasianodon hypophthalmus*) for a 120-day culture period demonstrated that *S. cerevisiae*-supplemented diets significantly improved growth performance, including growth rate and FCR. *S. cerevisiae* had no effects on hematological parameters and blood chemistry but increased the humoral immune parameters including total immunoglobulin, lysozyme, and alternative complement activities (Boonanuntanasarn et al. 2018).

In Asian sea bass, the mixture of *L. casei* M15, *L. plantarum* D8, *L. pentosus* BD6, *L. fermentum* LW2, *Enterococcus faecium* 10–10, *B. subtilis* E20, and *S. cerevisiae* P13 improved either growth performance or disease resistance. A diet containing 10^9 cfu (kg diet)$^{-1}$ probiotic mixture is recommended to improve the growth and health status of Asian sea bass (Lin et al. 2017).

The effects of dietary substitution of fishmeal with live yeast, *S. cerevisiae*, and increasing water temperature on the

diversity and composition of gut microbiota of rainbow trout were described. The trout were reared in water temperatures of either 11 °C (cold) or 18 °C (warm) for 6 weeks. Feeding live yeast mainly increased yeast load in the trout gut, whereas increased water temperature significantly altered the bacterial diversity and abundance of the gut microbiota. Live yeast can replace 40% of fishmeal without disrupting bacteria communities in the gut of rainbow trout, although increased water temperature from seasonal fluctuations or climate change may cause a gut dysbiosis that jeopardizes farmed fish health (Huyben et al. 2018).

Honey Bee

Honey bees (*Apis millifera*), as pollinators in agriculture, have a critical role in global food production. Worldwide, 75% of the crops traded on the global market depend on pollinators to some degree. Bees are often the most important crop pollinators and honey bees are the pollinators most widely used. Studies show that a diversity of pollinators can improve crop yield or fruit quality. Restoring and maintaining pollinator diversity is thus very important for agriculture as well as for natural vegetation. Recently, honey bee populations in the US, Canada, and Europe have suffered unexplained annual losses from a phenomenon known as "colony collapse disorder." Several members of the *Apis mellifera* microbiota (*Acetobacteriaceae*, *Bifidobacterium*, *Lactobacillus*, *Simunsiella*) produce SCFA such as lactic and acetic acids as waste products during the metabolism of carbohydrates (Vasquez et al. 2012). Honey bees possess an abundant, diverse, and ancient LAB microbiota in their honey crop with beneficial effects for bee health, defending them against microbial threats. This microbiota will become central to studies on honey bee health, including colony collapse disorder, and act as an exemplar case of insect–microbe symbiosis. Honey bee species plus related apid bees show one of the largest collections of novel species from the genera *Lactobacillus* and *Bifidobacterium* ever discovered within a single insect, suggesting a long (>80 million years) history of association. Bee-associated microbiota highlight *Lactobacillus kunkeei* as the dominant LAB member. Prophylactic practices that enhance LAB, or supplementary feeding of LAB, may serve in integrated approaches to sustainable pollinator service provision (Vasquez et al. 2012).

SCFA can be absorbed through the rectal wall in insects, and the majority of the pollen and bacterial biomass within an adult *A. mellifera* is contained inside the rectum (Bradley 2008). Overwintering *Apis* may obtain additional nutrition from these rectal bacteria, as consumed food is stored for longer periods of time within the rectum during winter months (Lindström et al. 2008). The probiotics *Lactobacillus* and *Bifidobacterium* have evolved in synergy with bees and are important in defending their host.

5 Synbiotics

A synbiotic is a combination of one or more probiotics and prebiotics. Prebiotics may enhance the survival of probiotic strains, as well as stimulating activity of the host endogenous bacteria. Synbiotics are additives that combine the use of probiotics and prebiotics such that they act synergistically (Alloui et al. 2013). The use of synbiotics was based on the concept that a mixture of probiotics and prebiotics beneficially affect the host by improving the survival and implantation of probiotic organisms and by selectively promoting the growth or metabolism of beneficial bacteria in the intestinal tract (Gibson and Roberfroid 1995). It was suggested that clinical effects vary from modest to significant from a single strain of probiotics < multi-strain probiotics < or < single-strain/single-fiber synbiotics < multi-strain/multi-fiber synbiotics. A combination of a prebiotic and probiotic, termed a synbiotic, is thought to exert synergistic effects to maintain gut health. Specifically, the probiotic fraction is thought to promote the growth of pathogenic bacteria. Moreover, synbiotics help to reduce the concentration of undesirable metabolites, including nitrosamines, to inactivate carcinogens, and to prevent constipation and diarrhoea of diverse aetiology in human beings (Bengmark and Martindale 2005). Compared with the use of individual components, synbiotics seem to modulate beneficially the composition of the gut microbiota by increasing beneficial bacteria (i.e., lactobacilli and bifidobacteria) and reducing other less desirable bacteria (i.e., coliforms, enterococci) (Modesto et al. 2011).

Poultry

Few research trials have been conducted to demonstrate the effects of synbiotics on broiler chicken performance. Supplementation of diets with a synbiotic product compared with basal diets supplemented with probiotic (homofermentative and a heterofermentative *Lactobacillus* sp.) was shown to significantly improve body weight, average daily gain, feed efficiency, and carcass yield percentage of synbiotic products compared with controls or probiotic-fed broiler chickens (Awad et al. 2009). Synbiotics were also shown to beneficially alter their intestinal microbiota composition and increase villi height and crypt depth in the intestinal mucosa. The increase in the villus height and villus height:crypt depth ratio was associated with improvement of growth performance for both synbiotics and probiotics (Awad et al. 2009). Significant increase in weight gain and a decrease in the FCR was reported when birds were fed diets with a combination of IOS and a multi-strain probiotic (consisting of 11 strains of *Lactobacillus* spp.). A combination of these dietary additives as a synbiotic on caecal bacterial populations and concentrations of caecal volatile fatty acids

and nonvolatile fatty acids of broiler chickens were also evaluated (Mookiah et al. 2014).

Dogs and Cats

There are sparse data on the use of synbiotics in dogs and cats. In one study, the effect of a multispecies symbiotic on the faecal microbiota was investigated in healthy dogs and cats (Garcia-Mazcorro et al. 2011). The symbiotic (containing 5×10^9 colony-forming units of a mixture of seven probiotic strains and a blend of FOS + arabinogalactans) was fed daily for 21 days, with changes in faecal microbiota analysed by culture-independent analyses targeting 16S rRNA bacterial genes (e.g., 454-pyrosequencing). Synbiotic ingestion led to increased abundance of some probiotic species in the faeces; however, no significant changes in bacterial species composition were identified.

Aquaculture

One example of synbiotics in aquaculture is the combination of prebiotic oligosaccharides and probiotic bacteria. An evaluation of the acute-phase response in rainbow trout (*Oncorhynchus mykiss*) fed functional diets supplemented with pre- and probiotics (i.e., mannan-oligosaccharides and *S. cerevisiae*, respectively) and challenged by either *Vibrio anguillarum* or chronic stress via maintenance under high stocking densities suggests that both supplements have high immunostimulatory potentials for farmed fish. In juvenile rainbow trout (*Oncorhynchus mykiss*) fed functional diets supplemented with either pre- or probiotics (0.6% mannan-oligosaccharides and 0.5% *S. cerevisiae*, respectively) or the mixture of both shows a dynamic shift of the microbiome composition and the microbiome modulation dynamics by functional diets based on mannan-oligosaccharides (Goncalves and Gallardo-Escarate 2017).

In Nile tilapia (*Oreochromis niloticus*), encapsulated and freeze-dried *S. cerevisiae* JCM 7255 improved intestinal structure and growth performance. Intraepithelial lymphocytes in the proximal intestine were significantly greater than in the control, and reduced cumulative mortality after the oral streptococcal challenge was also seen (Pinpimai et al. 2015).

In juvenile pacu (*Piaractus mesopotamicus*) stressed and experimentally infected with *Aeromonas hydrophila*, the efficacy of a commercial product (Glucan-MOS®) derived from the yeast *S. cerevisiae*, containing two combined products, β-1,3- or 1,6-glucans and mannans, fed during 30 days, in periods before intensive management, improved growth and innate immunity. The supplementation of 0.1% Glucan-MOS® improved weight gain, feed conversion, and the protein efficiency ratio compared to a control diet. The 0.2% and 0.4% Glucan-MOS® diets were sufficient to increase the respiratory burst of leukocytes and lysozyme activity, the number of thrombocytes, neutrophils, and monocytes in the blood after stressful handling and bacterial challenge, and minimized stress response as shown by decreased cortisol and glucose levels when compared to the control. The 30-day period was sufficient to stimulate growth performance, improve nutrient utilization, minimize stress response, and modulate innate immunity responses (Pereira Soares et al. 2018).

6 Concluding Remarks and Future Directions

There is great potential for the use of prebiotics and probiotics as alternatives to antibiotics to improve performance and reduce pathogenic load in the intestines of animals. The intestinal microbiota is complex and it is not clear how bacteria provide benefit to the host. Modulation of the intestinal bacteria towards a "healthy" community by specialty carbohydrates supporting beneficial bacteria (so-called prebiotics) or by feeding live bacteria (so-called probiotics) is currently undergoing active research. Microbial community analysis has become more accurate, providing reliable data that bacteria in the GIT can be modulated in a number of ways. Although the mechanisms by which antibiotics enhance health and productivity have not been fully elucidated, new research tools, for example, metagenomics and other genome-enabled technologies, may provide new ways to elucidate the ecology of the gut microbiome, host–pathogen interactions, immune development, nutrition, and health. Careful consideration must be given when selecting combinations of prebiotics and probiotics to be used as synbiotics, and research trials should be conducted according to the guidance approved by the regulatory authorities to demonstrate their synergistic effect compared with the use of either product alone and depending on the intended use, and finally according to the quality, efficacy, and safety requirements. The inclusion of specific prebiotics will not be of any benefit without the presence of the targeted, beneficial bacteria products and will not succeed if the environment into which they are introduced is unfavourable. The growth enhancement and health improvement of domestic animals achieved by promoting the growth of certain microbes in the GIT with prebiotics or probiotics is a beneficial and rational strategy, but their use in some production systems such as aquaculture is just beginning. Limited research concerning the use of probiotics in horses to improve hindgut fermentation and diet digestibility also produced contrasting results. Probiotic bacteria have a positive effect on GI function on different species. Yeast species have also been used as probiotics, and for delivery of enzymes in animal feeds; development of genetically engineered yeast and bacterial cells expressing new

substances as antibacterials may have potential as probiotics (Biliouris et al. 2012). In many of these studies it is unclear how much of the positive response obtained with probiotics can or should be considered in the context of their effect on preventing health problems of improving health and welfare and how much to their direct effect on diet utilization. These approaches have been utilized in production systems of food animals for promoting health, but assessing their effectiveness and mechanisms of action is needed. The target of such nutraceutical products is to improve GI health by selecting for beneficial microflora and suppressing known intestinal and food-borne pathogens. If the growth requirements of the bacteria differ, it is possible in theory to shift the microbial community from harmful to nonharmful bacteria by changing the diet and consequently the gut dynamics. Specific species can be selected for resistant feed components, which escape digestion by the host but are readily available for the metabolic machinery of the target microbes. Direct-fed microbials (probiotics) are targeted to improve GI health, but these are likely to be effective only if their growth requirements are fulfilled. In fact, a synbiotic product, which contains both a probiotic strain and a prebiotic favouring the growth of that strain, may be a good solution in many cases.

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