

Non-Invited Review

The gastrointestinal microbiome and its association with the control of pathogens in broiler chicken production: A review

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ABSTRACT The microbiome of the broiler chicken gastrointestinal tract (GIT) has been extensively studied, and it has been amply demonstrated that it plays an important role in the health of the host, as it has a positive impact on the immune system, the physiology of the GIT, and productivity. Also, the microbiota is involved in reducing and preventing colonization by enteric pathogens through the process of competitive exclusion and the production of bacteriostatic and bactericidal substances. The taxonomic composition of the microbiota is affected by different factors, such as the organ, the age of the animal, diet and the use of antimicrobials.

Different kinds of additives that regulate the microbial community in feed include probiotics (live microorganisms that when administered in adequate amounts confer a health benefit on the host), prebiotics (ingredients that stimulate increased beneficial microbial activity in the digestive system in order to improve the health of the host) and phytobiotics

(primary or secondary components of plants that contain bioactive compounds that exert a positive effect on the growth and health of animals). Phages may potentially provide an integrated solution to modulate the intestinal microbiome of chicken intestines, as they reduce specific pathogenic microbial populations, permitting the proliferation of beneficial microbiota. Studies have shown that the use of cocktails of phages, especially in high concentrations and with short lapses of time between exposure to the bacteria and treatment with phages, optimize the reduction of *Salmonella* in chickens. Each of these technologies has demonstrable positive effects on the health of the host and the reduction of the pathogen load in controlled assays.

This paper presents a comprehensive summary of the role of the microbiota in the broiler chicken gastrointestinal tract, and discusses the usefulness of different strategies for its modulation to control pathogens, with a particular emphasis on bacteriophages.

Key words: broiler microbiota, bacteriophage, pathogen control, phage-therapy, *Salmonella*

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INTRODUCTION

Aviculture is currently the most efficient animal productive system, and forms the basis of global protein production. An intensive selection production process carried out over the last 6 or 7 decades has produced chickens that convert feed into muscle mass efficiently, making them an effective system for high-quality protein production. The extraction of energy and nutrients from food requires interaction between the biochemical functions of the chicken and the microbiota present in

the gastrointestinal tract (**GIT**). Thus, the selection of beneficial microbiota plays an important role in the production, health, protection from pathogens, detoxification, and modulation of the immune system (Mead, 1989; Brisbin et al., 2008).

The microbiota is defined as the microbial community, including commensal, symbiotic and pathogenic microorganisms, which usually colonize an area of human and animal organisms, and are around 2 times more plentiful than somatic and germinal cells of the host (Sender et al., 2016). The collective genome of these symbionts is known as the microbiome. The microbiota exerts an important influence on the health and development of hosts, leading to any organism made up of host and microbial components to be considered “supraorganisms” (Turnbaugh et al., 2007).

This review focuses on the modulatory role that bacteriophages, probiotics, prebiotics, and phytobiotics exert on the chicken GIT. Also, presents an overview of the microbiome impact on the chicken’s health, and the main factors influencing microbiota composition. For

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deeper information on these subjects, readers will be directed to the appropriate previous review.

METHODS USED TO STUDY THE MICROBIOTA

The principal difficulties faced when reproducing the environmental conditions of the bacteria in the GIT include the requirement for strict anaerobic conditions, the need to co-culture with other bacteria with which they co-metabolize, and an extreme sensitivity to freezing. Bearing these factors in mind, it is important to profile and investigate the community, in order to study and better understand the behavior and significance of the complex interaction between host and microbiota within the GIT.

Our knowledge of the microbiota was limited to microorganisms that could be recovered using culture media, however, fewer than 20% of the microorganisms found in the GIT have been cultured due to the fact that most intestinal bacteria are fastidious and often demand unknown requirements (Gaskins et al., 2002).

Culture-independent methods used to characterize the chicken microbiota can be divided into the ones that determine the genetic fingerprint of the communities and those based on sequencing methods (Zoetendal et al., 2004). Genetic fingerprint techniques determine the microbial composition of a community using genomic DNA. These techniques are useful for comparing and identifying changes in communities and include: denaturing gradient gel electrophoresis (DGGE) (Van Der Wielen et al., 2002), single-strand conformation polymorphism (SSCP), and terminal restriction fragment length polymorphism (T-RFLP) (Torok et al., 2008; Geier et al., 2009). Although these are cheap technologies that can be used rapidly in the laboratory, their principal limitations include low sensitivity (they can only detect taxa with abundance levels > 1%), inexactitude in their calculations of abundance, and low data reproducibility. An alternative to these techniques is the employment of 16S ribosomal RNA gene microarrays. However, the principal limitation of this technique is the difficulty of testing for the entire diversity of the prokaryotes in the microbiome (Zoetendal et al., 2004).

Sequencing methods have rapidly replaced these techniques, as they resolve several of the difficulties presented by genetic fingerprinting, i.e., sequencing methods may be used to detect taxa with abundance levels below 1% (between 0.01% and 0.1%); in addition, the precision and abundance of taxonomic profiles are improved. However, sequencing is still limited by the bias generated by the polymerase chain reaction (PCR) and by the depth of the sequencing involved, on which the exactitude and precision of the data depend (Zoetendal et al., 2004; Stanley et al., 2014).

The most commonly-used sequencing technique amplifies and sequences the 16S rRNA gene of the total DNA in a sample, a method that makes it possible to

determine taxonomic composition and abundance. Another approach that is having increasing impact is the direct shotgun sequencing of samples of the DNA of the entire community. This kind of approach permits functional metabolic profiles within bacterial communities to be determined, and thereby, the metabolic pathways present in a given environment to be elucidated with more precision (Deusch et al., 2015).

THE GASTROINTESTINAL TRACT IN BIRDS

The digestive system in chickens breaks foods down mechanically and chemically, permitting nutrients to be absorbed. An understanding of the chicken GIT makes possible to determine how foods are transported, stored and broken down, aspects of a process that ensures efficient digestion, absorption and excretion.

The digestive system of the chicken and its function is presented in a schematic form in Figure 1.

COMPOSITION OF THE MICROBIOTA

Overall, the microbiota in chickens varies according to diverse factors that will be discussed below, such as diet, location, and age. For this reason, profiles of taxonomic composition differ greatly in reported studies. A study by Wei et al. (2013) used all the available data on the GIT (both published and unpublished) to analyze the intestinal microbiome of broiler chickens. This article remains the most authoritative study available on the diversity of the chicken microbiome.

Wei et al. (2013) established the presence of 915 operational taxonomic units (OTUs), equivalent to species (defined as having a phylogenetic distance of 3%), classified in 13 phyla, of which Firmicutes (70%), Bacteroidetes (12.3%) and Proteobacteria (9.3%) accounted for >90% of all the sequences. Overall, 117 genera were described, among which *Clostridium*, *Ruminococcus*, *Lactobacillus* and *Bacteroides* predominated. It was shown a high prevalence of the genus *Ethanoligenes* (Firmicutes), which contains ethanol-producing bacteria. *Desulfohalobium* was the most frequent Proteobacteria. Among phyla Actinobacteria, the genus *Bifidobacterium* was represented by 1% of the sequences. Other phyla, found in small proportions, included Cyanobacteria, Spirochaetes, Synergistetes, Fusobacteria, Tenericutes, and Verrucomicrobia. The Archaea were represented only by the phylum Euryarchaeota, with a very small number of sequences (11 out of a total of 3,184), corroborating the scarcity of methanogens in the chicken GIT. These studies have shown that 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 instance, a typical retention time for a 29-day-old broiler chickens is between 4 and 5 h, compared to humans, where the average is 20 h (Rougière and Carré, 2010).

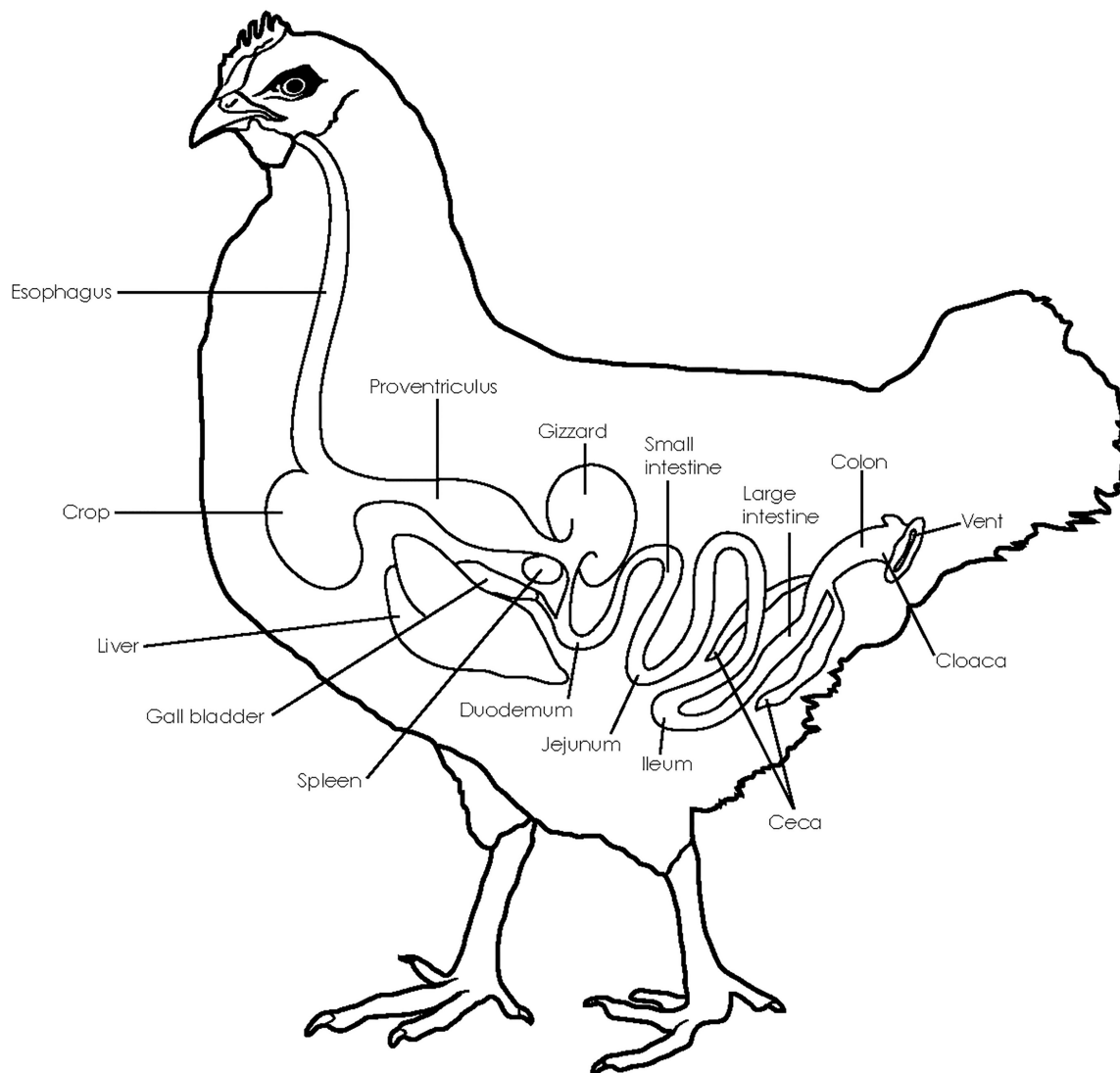


Figure 1. Gastrointestinal tract in chickens and function. The beak gathers food; the bifurcated tongue, located in the posterior part of the beak, is used to drink and to moisten the material that has been taken up. Subsequently, the food passes to the esophagus, which transports the food and water to the crop. The esophagus contains mucus glands that help to lubricate the passage of the food to the crop where it is stored temporarily. In its passage through the esophagus, the food is softened and undergoes pre-digestion by enzymes such as ptyalin, present in saliva, and enzymes from other organs, such as amylase-types from the duodenum and the proventriculus. The crop fills up when the chicken has eaten enough, and the food passes slowly to the proventriculus, or glandular stomach. Here, foodstuffs are bathed in gastric juices, hydrochloric acid, and digestive enzymes, beginning the process of nutrient breakdown and the construction of the food bolus, which then passes to the gizzard. The enzyme pepsin, which performs its proteolytic activities in the proventriculus, is also produced in the gizzard, as acid levels in the stomach are below the optimum levels required for it to function. The gizzard, also known as the masticatory organ in chickens, accumulates insoluble grains, which are ground by frequent and repeated contractions that exert enormous pressure, breaking the grains down into small particles and mixing them with juices from the proventriculus. From the gizzard, the food passes to the small intestine, an organ that is distinguished histologically by the presence of villi, which complete the digestion of proteins through the secretion of intestinal juices and digestive enzymes such as aminopeptidase, amylase, maltase, and invertase; another function is to absorb the nutrients in the digested foodstuffs so that they can enter the bloodstream; finally, the small intestine provides peristaltic action that passes undigested materials to the ceca. The small intestine has 3 sections: the duodenum, the jejunum, and the ileum. The pancreas is the organ that secretes juices enriched with amylases, trypsin, lipases and carboxypeptidases. The liver secretes bile into the duodenum, which helps break down fats; the bile, though produced in the liver, is stored in the gallbladder. The ileum opens into the ceca, a pair of tubes where undigested foodstuffs are fermented, and which is emptied every 24 h. The water and the foodstuffs that are not digested in the small intestine, such as non-starch polysaccharides, are absorbed in the large intestine, a section of the digestive tract that leads from the junction with the ceca, through the colon, and ends in the external opening of the cloaca (Noy and Sklan, 1995; Uni et al., 1999; Rebollar Serrano and Serrano, 2002).

When sequences drawn from the ceca were analyzed (Wei et al., 2013), the predominant phyla found were Firmicutes and Bacteroidetes, followed by Proteobacteria and Actinobacteria. Thirty-one genera from the Firmicutes phylum were found, of which just 3, *Ruminococcus*, *Clostridium*, and *Eubacteria* represented >5% of the sequences. The data on the predominance

of Firmicutes and Bacteroidetes in the ceca suggests that the microbiota present plays an important role in the recycling of nitrogen using uric acid, in the production of essential amino acids and in the digestion of non-starch polysaccharides, which stimulate the production of short-chain fatty acids (SCFAs) (Józefiak et al., 2004). Other genera that accounted for more than

1% of the total number of sequences found in the ceca include *Faecalibacterium*, *Blautia*, *Butyrivibrio*, *Lactobacillus*, *Megamonas*, *Roseburia*, *Ethanoligenes*, *Hespelia*, *Veillonella*, and *Anaerostipes* (for phylum affiliation of these and other genera, see Table S1 in Supplementary Data). The principal representative of the Bacteroidetes phylum was *Bacteroides* (40%). Other genera belonging to this phylum found were *Prevotella*, *Paraprevotella*, *Tannerella*, and *Riemerella*. Among the Proteobacteria, the predominant genera were *Desulfohalobium*, *Escherichia*, *Shigella*, and *Neisseria* (Wei et al., 2013).

Composition of the Microbiota According to GIT Location

Each organ of the digestive system performs functions that are important to the digestive process and the absorption of nutrients. Microorganisms perform independent functions in each of the organs, and it has been suggested that there is a significant difference in the taxonomic composition of the different organs of the digestive tract, so they could be considered separate ecosystems, despite the fact that they are strongly interconnected (Van Der Wielen et al., 2002). It is important to note that the taxonomic profiles described for each section of the GIT differ considerably between studies and are influenced by factors including sex, individual genetics, diet, the use of antimicrobials and the technique employed. This makes it difficult to define a typical profile for each section. A literature summary with the profiles of the most abundant bacteria in each section of the GIT, was provided by Stanley et al. (2014).

Briefly, different species of *Lactobacillus* predominate in the crop; these are believed to be responsible for the decomposition of starch and the fermentation of lactate. This organ also hosts several species of the Clostridiaceae family. Similarly, the gizzard is dominated by the same 2 genera. However, the principal difference between the 2 organs is the presence of gastric juices, pepsin, and hydrochloric acid in the gizzard, which acidifies the medium, resulting in lower bacterial and less fermentation activity. The small intestine has the highest concentration of bacterial cells, principally *Lactobacillus*, *Enterococcus*, and various Clostridiaceae; *Lactobacillus* was the dominant genus accounting for almost 70% of the total (Han et al., 2016). The cecum (made up in chickens of 2 loops, the ceca) is described as the organ with greatest taxonomic diversity and abundance, doubtless because it is the organ in the digestive tract that retains food for the longest period (12 to 20 h). Other characteristics that make this organ an important niche for the microbiota are that it is the site of greatest water absorption, it is responsible for regulating urea, and it carries out the fermentation of carbohydrates. The microbiota of the ceca is associated with the digestion of foodstuffs rich in cellulose, starch,

and polysaccharides, which are resistant to bacterial digestion in the small intestine (Goldstein, 1989; Obst and Diamond, 1989; Clench and Mathias, 1995). This organ principally hosts Firmicutes, Bacteroides, Proteobacteria and Clostridiaceae. Of particular interest is that the most abundant microorganisms are grouped in unknown Firmicutes phylotypes (Gong et al., 2002). Additionally, functional metabolic profiles have begun to be described within the community, elucidating with more precision the metabolic pathways present in a given environment. For example, the presence of hydrogenase, which seems to stimulate the production of SCFAs and is attributed to microorganisms abundant in the ceca (*Megamonas*, *Helicobacter*, and *Campylobacter*) (Oakley et al., 2014b). Accordingly, the study carried out by Sergeant et al. (2014) achieved a deep metagenomic analysis of a single cecal microbiota from Ross broilers at 42 d, housed indoors under standard commercial conditions. They found numerous polysaccharide and oligosaccharide-degrading enzymes with genetic evidence for the coordination of polysaccharide degradation with sugar transport and utilization. As was expected, they found in the cecal metagenome several fermentation pathways leading to the production of SCFAs (Sergeant et al., 2014).

The Presence of Pathogens

The presence of pathogenic bacteria in the broiler chicken microbiota is important to animal and human health alike. Among the taxa that can cause illness in humans and that have been reported in the chicken microbiota are *Campylobacter* (principally *Campylobacter jejuni* and *Campylobacter coli*), *Salmonella enterica*, *Escherichia coli*, and *Clostridium perfringens* (Oakley et al., 2014b).

Gastrointestinal infections caused by *Campylobacter* and *Salmonella* are principally associated with the consumption of products from the poultry chain and for this reason the control of pathogens from the farm is of great importance (Wegener et al., 2003). *Campylobacter* is found in high concentrations in the intestinal microbiota (10^7 UFC/g). However, it is generally accepted not to be pathogenic in birds. While *Salmonella enterica* is considered to be a low-prevalence taxon of sporadic distribution and transitional colonization, it can cause disease in chickens, depending on age, immune status, and type of serovar (Stern et al., 1995; Lee and Newell, 2006).

On the other hand, *E. coli* is a gammaproteobacterium present in the intestine, which is found in low abundance during the entire life cycle of healthy chickens. However, only certain strains have specific virulence factors that may cause disease in chickens; these strains are known as avian pathogenic *E. coli* (APEC). APEC is principally associated with extra intestinal infections, most of which affect the respiratory tract. APEC respiratory tract infections are secondary to the

initial infection by one or more infectious agents of the tract, such as viruses and *Mycoplasma gallisepticum*. The pathogenicity of *E. coli* has been observed to be stimulated by high levels of ammonia in battery sheds and by physiological changes in the host chicken, such as egg peritonitis (Dho-Moulin and Fairbrother, 1999). *E. coli* is also considered to be a zoonotic bacterium that is potentially pathogenic in humans. However, it has not been demonstrated a relation between the isolates from the poultry chain and those causing extra-intestinal disorders. It is, however, clear that the intestinal microbiota, including *E. coli*, can act as a reservoir for the dissemination of resistance to antibiotics in other pathogenic bacteria such as *Salmonella* (Nandi et al., 2004; Fricke et al., 2009; Castellanos et al., 2017).

C. perfringens is found in the population of commensal bacteria in the intestines of healthy chickens at very low levels of abundance. However, *C. perfringens* is recognized as a pathogen in birds that causes necrotic enteritis, though the colonization mechanism of the bacterium and the factors implicated in toxin production are still to be fully described. It is known that certain factors predispose chickens to contract the disease; these include damage to the mucosa and diets with high levels of non-starch soluble polysaccharides. Additionally, *C. perfringens* is a human pathogen that is transmitted through food and has been traced to different origins, including foodstuffs of avian origin (Van Immerseel et al., 2004, 2009).

FUNCTIONS OF THE MICROBIOTA

The digestive system is the most important reservoir of microorganisms. Therefore, various kinds of interaction have been found among broilers and in their intestinal microbiota, focused principally on 1) nutrient exchange, 2) modulation of the immune system, 3) the physiology of the digestive system, and 4) the exclusion of pathogens. These interactions are reviewed by Vispo and Karasov (1997); Chambers and Gong (2011); Pan and Yu (2014); Stanley et al. (2014); Oakley et al. (2014b). The following sections briefly summarize these functions.

Nutrient Exchange

The commensal bacteria of the digestive system contribute nutrients that are both directly and indirectly important to the metabolism of chickens. These include SCFAs, ammonium, amino acids, and vitamins (Pan and Yu, 2014).

Most intestinal bacteria are capable of hydrolyzing polysaccharides, oligosaccharides, and disaccharides into primary sugars. Intestinal bacteria ferment these sugars, producing SCFAs such as acetate, propionate, and butyrate (Hooper et al., 2002; Tellez et al., 2006). In the ceca, SCFAs are absorbed through the epithelium by passive diffusion, entering a variety of metabolic pathways (Tellez et al., 2006). Chickens employ SCFAs

as a source of energy and carbon. In addition, they regulate blood flow, stimulate the growth and proliferation of enterocytes, and regulate the production of mucin, affecting the immune response of the intestine (Pryde et al., 2002; Sanderson, 2004; Tellez et al., 2006). There are several studies that support the argument that these compounds fulfill an immunological role (Chambers and Gong, 2011).

Intestinal bacteria also contribute to the metabolism of nitrogen. For example, bacteria from the urogenital tract capable of catabolizing uric acid into ammonium can travel from the cloaca to the ceca, affecting the metabolism in the latter and permitting the host to absorb ammonium, which is then able to use it for synthesizing amino acids (Vispo and Karasov, 1997; Denbow, 2014). On the other hand, the same intestinal bacteria can themselves be a source of amino acids (Metges, 2000) and vitamins (LeBlanc et al., 2013), though most of the proteins and vitamins produced by these bacteria are lost during excretion, as most intestinal bacteria are found in the cecum and this organ is unable to digest or absorb proteins (Vispo and Karasov, 1997).

Chickens may also, in a reciprocal manner, provide nutrients to intestinal bacteria. For example, the mucin produced by calceiform cells in the intestine is an important source of carbon, nitrogen, and energy for commensal bacteria and pathogens alike (Tellez et al., 2006). The presence of mucin-degrading bacteria is associated with intestinal health, as they exert selection pressure on bacteria that cannot adhere to the mucosal surface (Pan and Yu, 2014).

Immunological Modulation

The immunological system of chickens includes both the innate and the acquired immune response. The microbiota plays an important role modulating the regulation and activation of both elements.

Regarding the innate immune response, the intestinal mucosa is considered the first line of defense against infection and a barrier that prevents commensal bacteria from penetrating the intestinal epithelium (Carter et al., 2009). The interior surface of the avian intestine is covered in a mucous layer made up of the glycoprotein mucin, secreted by calceiform epithelial cells (Brisbin et al., 2008). It has been found that mucins with sialic acid are more abundant in conventionally reared chickens (that is, living in sheds and able to feed on demand) when compared to mucins with sulfate, which are common in birds with low bacterial loads. These differences are observable from d 4 after birth; this suggests that the intestinal microbiota is involved in regulating the establishment of the mucous layer (Forder et al., 2007). The intestinal microbiota also regulates the production of antimicrobial peptides present on the surface of the intestinal epithelium, which are capable of rapidly killing or suppressing the activity. Some of these peptides are expressed constitutionally, while others are induced in host cells by bacteria. These aspects

have been reviewed by Pan and Yu (2014), where some examples of peptides and its action are presented.

Regarding the acquired immune system, it would appear that the commensal bacteria provide protection to the mucosa membrane by modulating the immune response, by controlling the quantity of mediators secreted by the cells of the acquired immune system, and stimulating the helper T cells. This issue is reviewed by Oakley et al. (2014b) and several other papers have shown these effects. However, the mechanisms have not yet been completely clarified (Brisbin et al., 2008; Haghighi et al., 2008; Mwangi et al., 2010). Using germ-free chickens, it was demonstrated that microbiota has a dramatic effect on the repertoire of intestinal T cells and their expression of cytokines (Mwangi et al., 2010; Ren et al., 2014; Oakley et al., 2014b).

The Physiology of the Digestive System

The period following eclosion from the egg is critical to the growth and health of chicks because it is when they change their source of nourishment from the yolk to a diet of carbohydrates and proteins. This is why the organs of the digestive system undergo anatomical and physiological changes during this early stage. The rapid development of the intestinal tract offers an ideal niche for colonization by microorganisms, and the microbiota also plays an important role in the development of the digestive tract (Uni et al., 1999). This process has been demonstrated in studies of germ-free chickens, which develop smaller intestines and ceca that weigh less and have thinner walls compared to conventionally reared counterparts. It has been suggested that SCFAs increase the proliferation and growth of enterocytes, which would partially explain the difference (Mitsuhiro and Jun-ichi, 1994). Another studies that supports this hypothesis are summarized by Chambers and Gong (2011).

The activity of the digestive enzymes in chicken intestines may also be affected by the intestinal microbiota. When the activity of the alkaline phosphatase enzyme in germ-free chickens and conventionally reared chickens is compared, the latter display greater enzymatic activity. Diet can also stimulate the growth of certain bacteria such as *Bifidobacterium* and *Lactobacillus*, which help to increase the enzymatic activity of proteases, trypsin, and lipases (Palmer and Rolls, 1983).

Pathogenic bacteria can also cause morphological changes. For example, chickens that are co-infected with *Eimeria* sp. and *C. perfringens* have significantly reduced length of intestinal villi. This was also shown in chickens infected with *Salmonella* Typhimurium (Golder et al., 2011).

Competitive Exclusion

The ecological definition of competitive exclusion states that 2 species competing for the same resources

cannot coexist stably. Therefore, one of the competitors will always dominate the other, leading to an evolutionary modification, shift to another niche, or extinction. The intestinal microbiota competes with the colonizing pathogenic bacteria and is able to reduce the adhesion and colonization of pathogens in the intestine. This reduction might be the result 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). For example, the production of bacteriocins is associated specifically with interference in the process of colonization by pathogens (Stern et al., 2006; Messaoudi et al., 2012; Razmyar et al., 2017). Other competitive exclusion mechanisms are reviewed in detail by Oakley et al. (2014b) and Pan and Yu (2014).

In spite of the fact that the mechanism that leads to this protection has not been decoded, the competitive exclusion process remains one of the most effective approaches to prevent intestinal colonization by *Salmonella* in broiler chickens. Armed with this understanding, different products have been developed to control this pathogen, which range from the use of probiotics to the inoculation of bedding with cultures drawn from the fecal material produced in more productive sheds with better intestinal health (Chambers and Gong, 2011). These protection mechanisms are explained in more detail below in the probiotics section.

IMPACT OF THE MICROBIOTA ON PRODUCTIVITY AND DEVELOPMENT

Recent studies using mouse and human models have demonstrated that the intestinal microbiota plays a very important role in the absorption of nutrients (Turnbaugh et al., 2007). Thus, an understanding of the variations in the intestinal microbiota might help to clarify how changes in its composition might alter energy efficiency in the host. The principal objective of the poultry industry is to increase the productivity of broiler chickens by producing birds that gain weight more efficiently. Modern chickens require increasingly less feed to achieve their desired weight. In 1950, 3 times more feed was required than is needed today. Despite this major advance, there is still a high degree of variation in the indices of weight gain and feed conversion across and within battery sheds, which implies significant losses for the poultry industry (Mead, 1989; Brisbin et al., 2008). From this perspective, the study of the GIT microbiota in chickens has nowadays an enormous potential and importance.

Some studies have compared the bacterial taxonomic composition of chickens with high and low development in terms of conversion efficiency. Conversion efficiency (CE) is defined as the quantity of feed consumed by unit of weight of animal produced. For example, if 4 kg of feed are used to produce a 2 kg animal, CE

is 2 (4 kg/2 Kg). Thus, the lower the conversion value, more efficient and better performance is achieved. Several studies have correlated changes in the microbiota with the performance of chickens. For example, in 2013 Stanley et al. (2013), used pyrosequencing of the V3 region of the 16S rRNA gene and found that butyrate-producing and cellulose- and starch-degrading bacterial communities in the ceca are associated with high performing chickens (CE = 1.32). This group of beneficial bacteria included *Clostridium islandicum*, *Ruminococcus* sp., *Bacteroides fragilis*, and *Lactobacillus coleohominis*. This study determined that the bacteria that had a negative effect on development were undescribed genera of the Firmicutes phylum.

In another, more recent study, Mancabelli et al. (2016) amplified the V3 region of the 16S rRNA gene and sequenced it using an Illumina MiSeq sequencer. This study identified 4 OTUs that were more abundant in high performance chickens. It was only possible to define these OTUs with confidence up to the level of class, and they might be different species of a new family and genus. Three of these potential OTUs were linked with microorganisms from the rumen, with superior capacity to degrade cellulose. It was possible to associate the other OTU with *B. fragilis*, a bacterium that displays high levels of hydrolytic activity and is considered to be among the most effective degraders of digestible carbohydrates. Similarly, the study identified a greater abundance of 20 OTUs in low performing chickens, confirming that these animals contain bacteria that exert a negative influence on the effective uptake of nutrients (Mancabelli et al., 2016).

Recently, Han et al. (2016) amplified and sequenced the V4 region of the 16S rRNA gene using an Illumina MiSeq sequencer to correlate some bacterial groups with the weight of chickens. In the ceca, *Akkermansia*, *Prevotella*, and *Anaerovibrio* affect weight negatively, while *Lactococcus* showed a positive correlation. *Akkermansia muciniphila* is recognized as a mucin-degrading bacterium, which in other studies of humans and mice has been correlated negatively with weight gain (Everard et al., 2013).

FACTORS AFFECTING THE MICROBIOTA

Age

Dramatic changes have been described in the microbial community as chickens grow older. However, most of these studies have used methods based on traditional culture-dependent microbiology, or on low-resolution molecular methods such as denaturing gradient gel electrophoresis (DGGE), terminal restriction fragment length polymorphism (T-RFLP), or sequencing using the Sanger methodology, which offer little depth. Few have used new mass sequencing technologies to determine these changes. Most of the studies that examined the effect of time on the chicken microbiota have looked at the cecum, since this is the organ with the greatest diversity and abundance in the entire

intestinal tract (Clench and Mathias, 1995; Sergeant et al., 2014). Studies have described a significant successional change in taxonomic composition, which becomes more abundant and taxonomically diverse as the life cycle advances (Van Der Wielen et al., 2002).

Bacterial concentrations increase rapidly immediately after eclosion. A concentration of 10^8 to 10^{10} cells/g of digesta has been reported in day-old chick ceca, which increases and establishes in less than 1 wk, reaching a maximum of 10^9 to 10^{11} cells/g (Rinttilä and Apajalahti, 2013).

Based on several studies using culture-dependent methods, a successional change in the broiler chicken microbiota during the life cycle has been proposed, and was explained by Rinttilä and Apajalahti (2013). A recent study, using mass sequencing tools, determined the composition of the microbiota at 7, 14 and 42 d (Oakley et al., 2014a). Briefly, on d 7 the microbiota is dominated principally by 3 genera belonging to the order Clostridiales (*Flavonifractor*, *Pseudoflavonifractor*, and *Lachnospiraceae*). These 3 genera are considered as responsible for converting polysaccharides into SCFAs, whose positive effects were mentioned above. On d 21, the genus *Faecalibacterium* apparently predominates. This genus has been described as having anti-inflammatory properties. By d 42 *Faecalibacterium* remains predominant, although the proportion of the genus *Roseburia* increases. This genus is described as a saccharolytic bacterium that produces butyrate. In addition, by d 42 the abundance of other SCFA-producing bacteria, such as members of the family Lachnospiraceae incertae sedis and the genus *Oscilibacter*, increases.

Location

See the section *Composition of the Microbiota According to GIT Location*.

Diet

The nutrients contained in the diet provided to chickens are also the nutrients that modulate the growth and establishment of the microbiota, thus diet is the factor that has the major impact. The principal characteristics of feed that may affect the microbiota are: the form of cereal (whole or milled grains, or pellets); the kind of cereal; the quantity of water-soluble non-starch polysaccharides; and the sources of fat, starch and proteins (Gabriel et al., 2006). Groups of bacteria that are favored by a particular type of diet are summarized by Chambers and Gong (2011). For instance, it has been reported that chickens fed with diets containing soya oil have a lower abundance of *C. perfringens* than birds fed with fats of animal origin (Luo et al., 2016). Several food supplements have been designed in an attempt to modulate the GIT microbiota, including probiotics and prebiotics, which will be discussed below.

Antibiotics

The effect of antibiotics on the microbiota of the digestive tract of chickens has been reported in several studies, denoting a reduction in the stability of the microbiota and also leading to the reduction of the population of *Lactobacillus* in the intestine (Lan et al., 2005; Danzeisen et al., 2011; Allen and Stanton, 2014; Mancabelli et al., 2016).

It has been reported that the Firmicutes/Bacteroidetes ratio increases when antibiotic supplements are provided. A study by Mancabelli et al. (2016) demonstrated that the metagenome of chickens fed with antimicrobial supplements presents a greater abundance of genes associated with antimicrobial resistance. Among these, of particular importance are the vancomycin- and chloramphenicol-resistance genes. This study also functionally characterized the chicken microbiome, showing that chickens treated with antibiotics present fewer functions associated with carbohydrate transport and metabolism. Specifically, chickens that do not received antibiotic supplements had a greater arsenal of families involved in the degradation of starch, cellulose, and hemicellulose, when compared with their counterparts who are fed these supplements.

Danzeisen et al. (2011) evidenced the effect of the coccidiostat monensin and the growth promoters virginiamycin and tylosin on the cecal microbiome and metagenome of broiler chickens, 16S rRNA and total DNA shotgun metagenomic pyrosequencing. In this study, *Roseburia*, *Lactobacillus*, and *Enterococcus* showed reductions, and *Coproccoccus* and *Anaeroflum* were enriched in response to monensin alone, or monensin in combination with virginiamycin or tylosin. Another important result was the enrichment in *E. coli* in the monensin/virginiamycin and monensin/tylosin treatments, but not in the monensin-alone treatment. Metagenome analysis identified enrichment in transport systems genes, including those for the transport of amino acids, iron and manganese, potassium and sodium, sugars, heavy metals, and calcium, which are associated to a reduction of acetate production. Regarding the antimicrobial resistance gene counts, no significant differences were observed (Danzeisen et al., 2011). These studies provide evidence that some effects are similar while others are different when antibiotics are used either as growth promoters or treatment; for example, *Lactobacillus* diminishment was established for both uses, but only when antibiotics are applied as a treatment was a higher presence of antibiotic-resistance genes detected.

METHODS USED TO MODULATE THE MICROBIOTA

Probiotics

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, namely: not pathogenic; ability to adhere to epithelial cells; ability to colonize and reproduce itself in the host; able to survive the passage through the GIT; resistant to gastric acidity and the contents of bile; produce metabolites that inhibit or kill pathogenic bacteria; characterized *in vitro* and have undergone trials *in vitro* and *in vivo* that demonstrate its benefits. Finally, a probiotic should remain viable under process, production, and storage conditions (Kabir, 2009). The following benefits are expected from administering probiotics (Syngai et al., 2016): stimulation of the development of beneficial microbiota; reduction and prevention of colonization by enteric pathogens; modulation of immunological activity; stimulation of epithelial health; increased digestive capacity; and help in the 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). Similarly, various 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 salivarius* have the ability to stimulate the butyrate-producing microbiota and to reestablish the balance of the microbiota (Meimandipour et al., 2009).

Another benefit of probiotics is the reduction and prevention of colonization by enteric pathogens, achieved through competitive exclusion mechanisms and the production of bacteriostatic and bactericidal substances (Wei et al., 2013; Pan and Yu, 2014). Probiotic cultures seek to compete 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 Connier, 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 be the result of the production of different metabolites such as hydrogen peroxide (H₂O₂), diacetyl, bacteriocins and organic acids. Stern et al. (2006) purified a bacteriocin produced by *L. salivarius* NRRL B-30,514 and treated chickens with it; there was a clear reduction in the presence 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 the pathogens multiplication (Blajman et al., 2015).

The effectiveness of probiotics depends on several factors, such as the composition of the mix, 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 composition of the probiotic 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. In 2011, Nakphaichit et al. administered *Lactobacillus reuteri* to broiler chickens only during the first wk of the life cycle and then proceeded to monitor the microbial composition of the ileum for a period of 6 wk by 16S rRNA gene pyrosequencing. This study concluded that if probiotics are administered at an early stage of the cycle they will have positive effects only up to wk 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).

The use of probiotics in the poultry chain has been reported since 1973, when Nurmi and Rantala pioneered their use in the control of *Salmonella* in broiler chickens (Nurmi and Rantala, 1973). They described feeding recently hatched chicks with a suspension of the intestinal contents of adult chickens, finding that the treatment protected chickens against *Salmonella* spp. However, this first proposed use of “probiotic” proved to have serious limitations, principally due to 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, which are obtained in different ways and for which dosage and the time in the cycle in which they are administered also varies. The results and effectiveness of these products has also been a controversial topic, as some studies report high levels of effectiveness, while in others the results are less clear. Consequently, more studies are required in order to achieve reproducible results. *Lactobacillus* is the most commonly used probiotic; its reported benefits include increased weight gain, improved feed utilization effectiveness, and reductions 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 the employment of these cultures led to reductions in colonization by this pathogen, an effect that is also correlated with an increase in weight gain and improved conversion of feed into body mass. Currently, various commercial probiotics are available on the market, including Aviguard[®], Primalac[®] and Interbac[®]. These 3 products are made up of different species of *Lactobacillus* and *Bacillus*. There are several areas that still need to be improved in the development and manufacture of these products if their effectiveness and quality is to be guaranteed. A deeper analysis of this topic with specially interest in *Salmonella* control is presented by Chambers and Gong (2011).

Prebiotics

Prebiotics are defined as ingredients that stimulate increased beneficial microbial activity in the digestive system in order to improve the health of the host. Compared to probiotics, they are cheaper to produce, the risks of undesirable side effects in the host are lower and the production process and administration are easier to manage. Most prebiotics seek to stimulate acidolactic 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).

Two kinds of prebiotics have been described for aviculture. Most of those currently used are non-digestible 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. Mannose oligosaccharides 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 in the literature corresponds to lactose and lactose derivatives such as lactulose and lactosucrose (van Immerseel et al., 2002).

Several studies of prebiotics in chickens provide evidence of positive effects for oligosaccharides of mannose or fructose in the inhibition of the pathogens *Salmonella* and *E. coli* (Chambers and Gong, 2011; Stanley et al., 2014).

Despite the positive effects observed, responses to supplements containing prebiotics have been inconsistent when applied in mass production systems. Explanations for this incongruousness include variation in the quality and dose of the compounds employed. It has also been proposed that the effectiveness of prebiotics is strongly dependent on the particular conditions found in each farm.

Phytobiotics

Phytobiotics are described as primary or secondary components of plants that contain bioactive compounds that exert a positive effect on the growth and health of animals. Primary components include the base nutrients, such as protein, fat, and carbohydrates, while secondary compounds include essential and/or volatile oils, bitters, colorants, and phenolic compounds (Grashorn, 2010). They may be classified into 4 groups: 1) herbs (products from flowering, non-woody, and non-persistent plants); 2) botanicals (whole plants or processed parts); 3) essential oils (hydro-distilled extracts of volatile plant compounds); and 4) oleoresins (extracts based on non-aqueous solvents). Properties such as the promotion of growth and health have been attributed to phytobiotics. These benefits are derived from improved intestinal health in the animal, including improved digestion, modification of digestive secretions and support to the histology of the intestine (Diaz-Sanchez et al., 2015).

The principal use of phytobiotics in aviculture has been the administration of essential oils, which have been used for a long time in the preparation of feed as artificial flavors and preservatives. Most essential oils have been classified as Generally Recognized as Safe (**GRAS**), by the US Food and Drug Administration (**FDA**). These oils are characterized as engaging in antimicrobial activities and having growth promoting properties. Several oils, including carvacrol and thymol obtained from oregano and eugenol from the clove plant, have been shown to inhibit a wide range of pathogenic bacteria (Dorman and Deans, 2000). Several studies have reported controlled experiments in which oils have been used as feed additives to reduce the presence of different pathogens in the intestine, including *Salmonella* (Tellez et al., 1993; Vicente et al., 2007); *E. coli* (Jamroz et al., 2005); *Campylobacter* (Ali, 2014) and *C. perfringens* (Mitsch et al., 2004). However, other studies have reported no effect on these pathogens (Cross et al., 2007; Gonzalez-Gil et al., 2014).

These results suggest that the effectiveness of essential oils varies, principally because their active components can differ depending on the method of extraction, geographical origin, plant genotype, and storage time. To summarize, essential oils have been extensively studied and have been used in aviculture to improve feed safety, but further research is required to confirm if they can improve the productive parameters and animal health (Diaz-Sanchez et al., 2015).

Bacteriophages

Bacteriophages (phages) are defined as specific intracellular parasites of bacteria that multiply using the metabolic machinery of their hosts. There are 2 large kinds of phages: virulent phages, with a lytic life cycle; and temperate phages, with a lysogenic life cycle. In a lytic life cycle, the phage recognizes specific bacteria,

injects its genetic material and then uses the metabolic machinery of the host to replicate and assemble copies of itself. A process of cellular lysis mediated by the phage then frees the virions assembled within the interior of the cell. Once freed, these new virions can infect another cell, reinitiating the cycle. By contrast, in the lysogenic life cycle the phage recognizes the host cell, the injected DNA is incorporated into the bacterium's genome and replicates with it. Under certain conditions, this DNA can detach itself from the genome and initiate a lytic life cycle. As it leaves the bacterial genome, the phage's DNA can take information with it that can be transferred to its next host. This process might impart undesirable characteristics to the new host, such as virulence factors or antibiotic resistance genes.

Phage therapy is defined as the use of phages to treat bacterial infections; the term is restricted to the employment of virulent phages. Its application to humans was described almost as soon as these viruses were discovered in 1915 (Abedon et al., 2011). However, its use was displaced by the discovery of penicillin and continued only in some countries of the former Eastern Block (Summers, 2012). Today, the problematic emergence of multi drug resistant bacteria has provided a new focus on bacteriophages as a natural, non-toxic alternative treatment of bacterial infections. The advantages of the technology have been described in detail in different review articles such as in Loc-Carrillo and Abedon (2011). The advantages of phage therapy include that treatment with phages can target a specific group of bacteria, with the result that the normal microbiota is not affected, reducing, thereby, the risk of secondary infections associated with antibiotic therapies. Phages are considered to be more effective than antibiotics as they only multiply when their specific host is present. This implies that phages have the ability to increase their density *in situ*. Equally, following infection, once the concentration of the host has been reduced, the population of phages diminishes as well. Another important advantage is that phages can be effective against sensitive bacteria as well as strains that are resistant to antibiotics (Loc-Carrillo and Abedon, 2011; Nilsson, 2014).

As mentioned, specificity plays an important role in phage therapy, as phages are able to target only certain groups of pathogenic bacteria without having any negative effect on the normal microbiota of a given niche (Sulakvelidze, 2011), which, as has been shown throughout, fulfills important functions in the host. The application of phages has been described for humans (Abedon et al., 2011), different models in animals, plants, and food (Cooper, 2016). In 2006 the US FDA approved a cocktail of phages formulated to control *Listeria monocitogenes* in food for human consumption (Sulakvelidze, 2013). This approval recognized the employment of phages in foodstuffs as a safe and effective practice; however, the use of such products is yet to be approved for use in live animals. In spite of this, several studies have used bacteriophages

in animals in order to control bacteria transmitted by foodstuffs. These models include the use of phages to control *Salmonella* and *Campylobacter* in broiler chickens (Grant et al., 2016; Wernicki et al., 2017). Most of the published studies on the control of *Salmonella* have been conducted using germ-free chickens that have been reared in batteries under tightly controlled conditions. In all cases, phages have been administered orally, either as a feed supplement, in water, or using a gavage after the birds have been challenged with a given concentration of the pathogen (Grant et al., 2016). Table 1 presents a summary of the *in vivo* studies carried out to date on broiler chickens to control *Salmonella*; the table provides a brief description of each study with the most relevant results. For information on *Listeria* and *E. coli*, readers are referred to Wernicki et al. (2017).

The results of some of these studies have been very promising, while in others there has been no observable effect. Reduction ranges from 0 to 5 log units (Table 1). These studies have enabled to identify the most significant factors that should be taken into account for the successful application of phages and the maximization of reduction of the target microorganisms. These factors include the concentration ratio of the phage to the target bacteria—also known as the multiplicity of infection (MOI), treatment with individual phages or with a cocktail, the optimum exposure time to the bacteria prior to the phage treatment, and the phage administration route. Published studies show that the application of phages in higher concentrations than the targeted microorganism is more successful in reducing the presence of the latter (Bardina et al., 2012). It should also be considered that cocktails of phages are more effective than individual applications (Fiorentin et al., 2005; Andreatti Filho et al., 2007). Additionally, it has been demonstrated that treatment with phages is more effective when it precedes the exposure to the pathogen (Bardina et al., 2012; Wong et al., 2014). On the matter of administration routes, even though most of the studies have been carried out using oral gavages, Carvalho et al. (2010) compared the effectiveness of treatments conducted using a gavage with those in which phages were administered as a feed supplement, approaches that obtained reductions of 1.7 log₁₀ and 2 log₁₀ CFU/mL, respectively. In a different approach, an extremely interesting study showed that the application of phages alongside with probiotics is more effective in reducing *Salmonella* than applying each treatment separately (Toro et al., 2005).

Another alternative to the use of bacteriophages is the application of bacteriophages endolysins (or lysins), which are lytic enzymes encoded by bacteriophages that decompose the bacterial cell wall peptidoglycan during the terminal stage of the phage reproduction cycle. These enzymes present some advantages and disadvantages over living phages that were summarized by O'Flaherty et al. (2009). Some important advantages are: 1) lysins are not self-replicating, meaning they are

more targeted and defined control; 2) resistance to these enzymes has not yet been reported; 3) they can be identified and used from temperate and virulent phages; and 4) lysins have the potential to be used in many environments (humans, animals, food, biofilms, etc.). Among the important disadvantages of lysins are: 1) there is a lack of effectiveness against gram-negative bacteria; and 2) bacteriocins are protein, therefore are susceptible to inactivation. Several reports on the antimicrobial application of endolysins along the food processing line have been carried out, mainly directed to *Staphylococcus aureus* and *Listeria monocytogenes* in dairy products (Oliveira et al., 2012). In addition, a study carried out by Zimmer et al. (2002) reported 2 putative phage lysins from the clostridial phages ΦCP390 and ΦCP26F; after cloning and purification, these lysins were able to lyse their parental *C. perfringens* strain, as well as other strains of the bacterium. It is also important to note that all other *Clostridium* species were resistant to lytic activity, demonstrating species specificity for *C. perfringens*. No other reports of lysins with potential use in broilers were found, perhaps because most of the foodborne contamination is caused by gram-negative bacteria (Zimmer et al., 2002).

It should be stressed that phage therapy still presents limitations, such as variability in the results obtained; this might be explained by different reasons: the development of resistance to phages by target bacteria, low multiplicity of infection, inaccessibility of the target microorganism and the deactivation of phages by the host. The most important limitations to employ bacteriophages in producer farms is the lack of approval and regulation for their use with animals, and acceptance of the therapy by the producer community, given that it is a relatively new technology. However, if such approval is to be achieved, research into the effectiveness of phages in the commercial conditions of factory farming is still required (Grant et al., 2016).

The unique study using bacteriophages in commercial broiler flocks was reported by Kittler et al. (2013) with *Campylobacter* phages. The authors carried out 3 field trials, 2 in the same farm but in different sheds, and the third was carried out in another farm. The herd size for the experimental and control group was, on average, 14,625 chickens/house. The cocktail of 4 phages was supplied via drinking water to a final concentration 10⁵–10⁷ PFU/mL. In the first trial, a reduction of up to 3.2 CFU/g of *Campylobacter* load in the cecal content was achieved, compared to the control. However, no significant reduction was observed in the experimental groups of the other trials, indicating that additional research is required for large-scale application of the phages (Kittler et al., 2013).

Overall, bacteriophages represent a promising alternative for the control of *Salmonella* and *Campylobacter* in farms. However, replicable studies that demonstrate the effectiveness of the technology in intensive production systems are still required.

Table 1. Comparative analysis of the effect of bacteriophages on *Salmonella* counts and incidence in broiler chickens, from literature reports available between 2007–2016.

Reference	Age of animal when challenged with <i>Salmonella</i>	Serovar employed	Concentration of the <i>Salmonella</i> inoculum	Inoculation of phage(s) in relation to the challenge	Concentration of the phage(s)	Phage treatment (I: one phage only; C: cocktail)	Method of inoculation	Results
(Wong et al., 2014)	6 d	<i>S. Typhimurium</i>	10^{10} UFC/mL	2 h post-challenge	10^{12} UFP/mL	I ¹	Oral gavage	Reduction (UFC/mL) of 2.9 log ₁₀ CFU/mL at 6 h post-treatment and undetectable levels after 24 h.
(Gonçalves et al., 2014)	45 d	<i>S. Enteritidis</i>	10^7 UFC/mL	1 hour post-challenge	10^9 UFP/mL	C ²	Oral gavage	Reduction (UFC/mL) in the ceca and crop of 2 log ₁₀ CFU/mL at 3 h post-treatment, and at 6 h reduction of 2 log ₁₀ CFU/mL in ceca and undetectable levels in crop.
(Bardina et al., 2012)	21 d	<i>S. Typhimurium</i>	10^5 UFC/mL	1 d pre-challenge, and d 0,1,2,3,6,8,10,13,15 post-challenge	10^{11} UFP/mL	C ³	Oral gavage	Reductions (UFC/mL) of 4.4 and 3.2 log ₁₀ , at 2 and 6 d post-treatment respectively, after 8 d a reduction of 2 log ₁₀ is maintained.
(Andreati Filho et al., 2007)	21 d	<i>S. Typhimurium</i>	10^5 UFC/mL	Days 0,1,2,3,6,8,10,13,15 post-challenge	11^{11} UFP/mL	C ³	Oral gavage	Reductions (UFC/mL) of 4 and 2 log ₁₀ at 2 and 6 d post-treatment. The reduction of 2 log is maintained until the end of the experiment.
	21 d	<i>S. Typhimurium</i>	10^5 UFC/mL	Days 4 and 5 post-challenge	12^{11} UFP/mL	C ³	Oral gavage	Reduction (UFC/mL) of 1 and 0.5 at 6 and 12 d after treatment.
	6 d	<i>S. Enteritidis</i>	10^3 UFC/mL	1 hour post-challenge	10^8 UFP/mL	I ⁴	Oral gavage	Reduction (incidence) ⁸ of 70% and 0% at 24 and 48 h post-treatment respectively.
	6 d	<i>S. Enteritidis</i>	10^3 UFC/mL	1 hour post-challenge	10^8 UFP/mL	I ⁵	Oral gavage	Reduction (incidence) ⁸ of 65% and 0% at 24 and 48 h post-treatment respectively.
	6 d	<i>S. Enteritidis</i>	10^3 UFC/mL	1 hour post-challenge	10^8 UFP/mL	C ⁶	Oral gavage	Reduction (incidence) ⁸ del 40% and 85% at 24 and 48 h post-treatment respectively.
(Atterbury et al., 2007)	34 d	<i>S. Typhimurium</i>	10^{10} UFC/mL	48 h post-challenge	10^9 UFP/mL	I ¹	Oral gavage	No reduction in <i>Salmonella</i> observed (UFC/mL)
	34 d	<i>S. Enteritidis</i>	10^{10} UFC/mL	48 h post-challenge	10^9 UFP/mL	I ¹	Oral gavage	No reduction significant reduction (UFC/mL)
	34 d	<i>S. Typhimurium</i>	10^{10} UFC/mL	48 h post-challenge	10^{11} UFP/mL	I ¹	Oral gavage	Reduction (UFC/mL) of 2.19 log ₁₀ UFC/mL at 24 h post-treatment
	34 d	<i>S. Enteritidis</i>	10^{10} UFC/mL	48 h post-challenge	10^{11} UFP/mL	I ¹	Oral gavage	Reduction (UFC/mL) of 4.3 log ₁₀ UFC/mL at 24 h post-treatment
(Hurley et al., 2008)	1 d	<i>Salmonella</i> sp.	10^8 UFC/mL	24 h and 28 d post-challenge	10^6 UFP/mL (24 h) 10^8 UFP/mL (28 d)	I ⁷	Water from drinking bowl	No reduction in <i>Salmonella</i> observed (UFC/mL)

Table 1 Continued.

Reference	Age of animal when challenged with <i>Salmonella</i>	Serovar employed	Concentration of the <i>Salmonella</i> inoculum	Inoculation of phage(s) in relation to the challenge	Concentration of the phage(s)	Phage treatment (I: one phage only; C: cocktail)	Method of inoculation	Results
	1 d	<i>Salmonella</i> sp.	10 ⁸ UFC/mL	48 h and 28 d post-challenge	10 ⁸ UFP/mL (24 h) 10 ⁸ UFP/mL (28 d)	I ⁷	Water from drinking bowl	No reduction in <i>Salmonella</i> observed (UFC/mL)
(Borie et al., 2008)	10 d	<i>S. Enteritidis</i>	10 ⁵ UFC/mL	24 h pre-challenge	10 ⁸ UFP/mL	C ³	Water from drinking bowl	Reduction (incidence) ⁸ of 19%
	10 d	<i>S. Enteritidis</i>	10 ⁵ UFC/mL	24 h pre-challenge	10 ⁸ UFP/mL	C ³	Spray on carcass	Reduction (incidence) ⁸ of 30%

¹Phage not identified.

²Cocktail of phages not identified.

³Cocktail of 3 phages not identified.

⁴Phage CB40.

⁵Phage WT45O.

⁶Phages CB40 and WT45O.

⁷Phage SP6.

⁸Reduction of incidence of *Salmonella* compared to positive control. Incidence = number of animals with presence of *Salmonella* per group/No. of animals per treatment group.

CONCLUSIONS AND FUTURE PERSPECTIVES

The gastrointestinal microbiota plays a crucial role in host immune system, its physiological development, health, nutrition and productivity. The manipulation of the microbial community through the inclusion of feed additives such as probiotics, prebiotics, phytobiotics and phages is feasible in order to enhance chicken growth and control either human or animal pathogens. However, it is still required improvements in these approaches to ensure their adequate use in the production chain. Phage-therapy is one of the strategies available to manipulate gut microbiome, which have has promising results and also have some advantages over the others technologies, but research is needed for the use of phages at the productive scale.

SUPPLEMENTARY DATA

Supplementary data are available at *Poultry Science* online.

Supplementary Table S1. Taxonomical affiliation of genera present in the broiler microbiota, cited in this review (according to the NCBI taxonomy database <http://www.ncbi.nlm.nih.gov/taxonomy>).

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CONFLICT OF INTEREST

Viviana Clavijo-López and Martha J. Vives-Florez are members of the spin off SciPhage S.A.S., which works for the development of phage therapy in Colombia.

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