POULTRY GUT MICROBIOTA – COMPOSITION AND ITS ROLE IN HEALTH, IMMUNITY AND PRODUCTION PERFORMANCE

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Abstract

In animals and birds, gut health is crucial for overall health, better feed efficiency, and production performance. In livestock species, the gut microbiota is essential and plays a crucial role in maintaining gut health. During the last decade, the advances in next-generation sequencing technologies resulted in rapid identification and analysis of gut microbiota composition in animals and birds. These technologies helped identify even non-culturable microorganisms also. The healthy gut microbiota is known to improve the production performance of chicken by reducing the colonization of pathogenic bacteria and maintaining the integrity of the gastrointestinal tract. Moreover, several researchers identified diverse microbial populations in the gut of chickens raised in contact with adult hens and commercial chickens. The differences in the identification strategies of microbiota and many host and environmental factors might contribute to the diversity in chicken gut microbial composition. Early acquisition of microbiota in chicken is known to stimulate the gut immune system's development, imparting resistance to enteric diseases like *Clostridium perfringens* induced necrotic enteritis in chickens. Although *Lactobacilli* spp. and *Bifidobacterium* spp. are predominant gut microbiota species, several hundreds of beneficial microbiota have been identified. These newly identified bacterial species could be tested as probiotics either singly or in combination for improving the overall gut health of chickens, thereby increasing the production performance in commercial chicken.

Key words: Gut health, Immunity, Microbiota, Poultry, Probiotics

Introduction

Since chickens' domestication started, eggs and meat from chickens became the most common protein source for human consumption worldwide. As a result of domestication, chickens altered their natural behaviour and adapted to a living environment different from their ancestors. The present-day lifespan of commercial broiler chickens is 5 weeks, and that of layers is 1 year, whereas red jungle fowl can live for 15-20 years. Also, there is a considerable difference in the physiology and microbiota of the chicken raised in commercial hatcheries than that of chicks brooded by an adult hen, which might be responsible for stronger disease resistance (Rychlik, 2020).

In terms of feed efficiency, poultry is the most efficient feed converters with a feed conversion ratio (FCR) of 1.8-2.0 among other livestock species. The poultry industry's major challenge is maintaining high feed efficiency, especially in the intensive production system. The birds are more prone to infectious diseases in areas where climatic conditions are changing. The poultry gut health is crucial for maintaining high FCR and in the prevention of gut-related infectious diseases. The compromised gut will predispose the birds to enteric diseases like necrotic enteritis caused by gram-positive bacteria *Clostridium perfringens* type A and type C, parasitic diseases like coccidiosis caused by *Eimeria* spp. Also, the

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structure, composition, and gut microbial function are vital for the poultry health since this microbiota is essential and will influence the development of intestinal epithelium and maintain intestinal homeostasis (Kers *et al.*, 2018).

Also, the intestinal microbiota modulates gut health, thereby stimulating gut immunity and resistance against microbial infections by several mechanisms like the competitive exclusion of pathogenic bacteria by preventing colonization, thereby preventing the expense of energy required by the host against fighting infectious diseases. Thus, a “healthy” gut microbiota saves energy for the host, which can be converted into an improvement in the birds’ productive performance (Diaz Carrasco *et al.*, 2019). Currently, the advances in microbiology and biotechnology techniques, the advent of omics like metagenomics and next-generation sequencing (NGS) technologies made it possible for detailed characterization of the gut microbiota of chickens without the need for culture quickly and robustly with low cost compared to traditional methods of isolation and identification of unknown microorganisms. The present review compiled the information from several studies of the poultry microbiome (Barnes, 1979; Zhu *et al.*, 2002; Lu *et al.*, 2003; Gabriel *et al.*, 2006; Lee and Newell, 2006; Qu *et al.*, 2008; Danzeisen *et al.*, 2011; Yeoman *et al.*, 2012; Oakley *et al.*, 2013; Zhao *et al.*, 2013) and focused on the gastrointestinal tract microbiota, their role in gut health and immunity against enteric diseases, the role of prebiotics, probiotics and other alternatives to antibiotics in the maintenance and improvement of gut health for enhanced production performance in poultry.

Chicken gut microbiota

There are numerous microbial communities in the chicken intestinal tract that maintain gut health by modulating various physiological functions, including metabolism and nutrition. A strong linkage has been observed between

chicken gut microbiota and its digestive process, absorption of nutrients, and productivity, which is influenced by the diversity and composition of gut microbiota (Stanley *et al.*, 2013; Mancabelli *et al.*, 2016). Several hundreds of bacterial species have been identified in the intestinal microbiota of chicken, with the majority belonging to the division Firmicutes, Proteobacteria, Bacteroidetes, and Actinobacteria (Oakley *et al.*, 2014; Clavijo and Flórez, 2018). Several workers observed that the microbial composition differs across the various compartments of the chickens’ gastrointestinal tract (GIT) (Sekelja *et al.*, 2012; Yeoman *et al.*, 2012; Stanley *et al.*, 2014; Yan *et al.*, 2017). In chickens, the diversity of gut microbiota is found to increase during the early weeks of life, and also, the colonization patterns observed to differ between layers and broiler chickens.

In chickens, various compartments of the GIT are populated with complex microbial communities, including protozoa and fungi, and dominated by bacteria. Among the bacterial communities in the chicken intestine, *Lactobacilli* are found to be predominant species in the proximal parts of the intestine of adult chickens (Videnska *et al.*, 2013; Wang *et al.*, 2017), and the composition and complexity of the microbiota found to increase considerably in the caecum and colon. Shang *et al.* (2018) reported that the chicken intestinal tract harbors more than 500 bacterial species. Although *Lactobacillus* is the bacterial genus in the crop and gizzard in mature birds, the high bile acid concentrations and low pH may also favor the growth of *Bifidobacterium* sps. The low pH of gastric juices in the gizzard, which is also described as the teeth of the GIT, limits the total number of bacterial cells below 10^8 /g (Gong *et al.*, 2007; Yeoman *et al.*, 2012; Han *et al.*, 2016). The ileum possesses the greatest microbial diversity and density where the bacterial genera *Lactobacillus*, *Clostridium*,

Enterococcus, and *Turicibacter* are in high numbers among other genera (Fig. 1) (Han *et al.*, 2016; Siegerstetter *et al.*, 2017; Borda-Molina *et al.*, 2018). Strict anaerobic bacteria of the genera *Clostridium*, *Enterococcus*, *Bacillus* and *Ruminococcus* were abundant in birds' ceca (Zhao *et al.*, 2013; Borda-Molina *et al.*, 2018). Also, several workers (Jeurissen *et al.*, 2002; Rehman *et al.*, 2007; Kohl, 2012) published the data regarding the composition of different bacterial communities present in various compartments of chicken GIT (Stanley *et al.*, 2014; Waite and Taylor, 2014). It has been observed that the gut microbiota can have negative effects like over stimulation of the immune system, breakdown of bile, production of harmful amino acid catabolites apart from providing beneficial activities to the host (Gaskins *et al.*, 2002). A 'healthy gut' microbiota is considered a net benefit to the chicken.

Development and maturation of gut microbiota

The microbial colonization and development in GIT of birds is observed to occur from the moment of hatching and can even begin before, through the passage of microbes through the eggshell pores (Roto *et al.*, 2016; Lee *et al.*,

2019). The strict hygienic practices followed in large scale commercial hatcheries reduce the pathogenic microbial load in the hatcheries. The newly hatched birds can acquire their microbiota from an artificial environment on the farm instead of the natural maternal source (Donaldson *et al.*, 2017). However, it is noticed that part of the microbial communities that colonize in early embryos can be inherited from maternal hens during different developmental stages (Ding *et al.*, 2017). Immediately after hatching, GIT of chickens becomes successively colonized by *Enterobacteriaceae* at first days of age, then Firmicutes (approximately from 7 days of age) (Ballou *et al.*, 2016) and also with the microorganisms at the farm comes from the rearing environment and bacteria present in food and water (Kubasova *et al.*, 2019).

Gut microbiota in chicks in contact with adult hens

Several authors observed a vast difference in the microbiota composition in chickens raised in commercial hatcheries to that present in chicks in contact with an adult hen. Since some of the ubiquitous bacteria such as *E. coli* and *Lactobacilli* are always present in the environment and the chance that these bacteria

Fig. 1. Distribution of the most abundant gut microbial taxa along the intestinal tract of a chicken (Picture was taken from Maki *et al.*, 2019)

come into contact with newly hatched chicks is high from the first days of life. The farm atmosphere and the animal house can also contain spores of facultative anaerobes like those of Clostridiales, families *Lachnospiraceae* and *Ruminococcaceae*. This could be the reason for the colonization of *Lachnospiraceae* and *Ruminococcaceae* after *E. coli*. More genera have been documented in several workers' reviews (Videnska *et al.*, 2014; Awad *et al.*, 2016; Ranjitkar *et al.*, 2016). Kubasova *et al.* (2019) documented about 45 genera of bacteria in the cecal microbiota of contact chicks in comparison to control chicks. These genera include members of the phylums *Actinobacteria*, *Bacteroidetes*, *Proteobacteria*, *Deferribacteres*, *Spirochetes*, *Tenericutes*, *Synergistetes*, *Euryarcheota*, apart from the Firmicutes, which are common gut microbiota. The authors opined that these 45 genera present in commercial hatcheries and chicken farms should be provided to newly hatched chickens as probiotics. Further, they opined that sixty-eight genera commonly present in high density in contact and control chicks are less important for consideration as probiotics, as most of the farm environmental sources are rich enough with this microbiota. The authors observed *E. coli*, *Proteus*, and *Salmonella* as more abundant in control chickens' microbiota. These genera are found to be suppressed by 45 genera provided to chicks by hens.

Role of microbiota on immunity and gut health

A healthy gut implies morphological integrity and physiological functions of the intestinal tract, including tissue metabolism, efficient immune responses, improved barrier functions, sustained inflammatory and energy balance, and adequate healthy microbiota. The commensal microbiota can stimulate the development of the immune system, including the formation of the mucus layer, epithelial monolayer, the development and maturation of

the intestinal immune cells such as cytotoxic T cells (Tc) and helper T cells (Th), phagocytic cells and antibody-producing cells, and the lamina propria (Dibner and Richards, 2005; Shakour *et al.*, 2009; Oakley *et al.*, 2014). This tissue builds immune barriers between the host and the microbes, and overcomes the effects caused by undesirable gut microorganisms. In the cecum and colon (distal gut), the microbiota also capable of producing energy, some vitamins, amino acids, and short-chain fatty acids (SCFA) from the undigested feed, which eventually become available for the host (Gaskins *et al.*, 2002; Dibner and Richards, 2005).

Several studies have focused on understanding the relationships between compositions of the native gastrointestinal tract microbes and colonization of the GIT by pathogens. It has been documented that the mechanism of competitive exclusion by commensal gut microbiota acts as an anti-infectious barrier by inhibiting the pathogen adherence, their growth, and by the production of bacteriocins and other toxic metabolites by fermenting complex polysaccharides. The initial interactions between gut microbial community and host innate immune system can lead to subsequent adaptive immune responses, either cell or humoral immune responses (Pan and Yu, 2014). Although several host and environmental factors can influence the establishment and evolution of the gastrointestinal microbiota, it appears that the gut microbiota-immune system interactions are dominant at some stages. The host immune systems react to the gut infections by several complex interconnected pathways that involve both the innate and adaptive immune systems. For example, beta-defensins, small antimicrobial peptides present on the epithelial surface of the intestine forms an important part of innate immunity (Shimizu *et al.*, 2008). These beta-defensins are produced in response

to challenge by lipopolysaccharide, involving Toll-like receptors (TLR-4) and the nuclear factor kappa light chain enhancer of activated B cells (NF- κ B) transcription factors (Akbari *et al.*, 2008). The expression of these defensins in the gut is affected by microbial exposure, and microbial exposure reduction resulted in lower expression of duodenal and ceca beta-defensins at the early growing period of birds (Butler *et al.*, 2016). These initial interactions of the gut microbiota and host immune system can alter the composition of microbiota, resulting in subsequent effective adaptive immune responses (Pan *et al.*, 2014).

The need for early microbial colonization of GIT

To develop a healthy gut immune system, it is imperative for early microbial colonization of the GIT. In several studies, it has been observed that germ-free chickens are more susceptible to intestinal infections. Hence, microbial exposure is necessary for the appropriate development of the gastrointestinal tract immune system (Dibner *et al.*, 1998; Shanahan, 2002). Also, it is considered that the development and maturation of the immune system is highly dependent on antigen encounter. In a study, Cheled-shoval *et al.* (2014) compared intestinal mucosa of germ-free chickens with conventional birds at 7day of age which revealed the low density and number of acidic and neutral goblet cells, sialylated and sulfated acidic mucin staining, and the major intestinal mucin expression MUC2. It suggested a less mature or underdeveloped intestinal mucosa in germ-free animals. Nevertheless, gut microbiota plays a crucial role in the development and maturation of the immune system and would potentially impact optimal health and productivity.

Gut microbiota and antibiotics

The antibiotics are commonly used at low concentrations in the worldwide poultry

industry as growth enhancers. Administering antibiotics in the early stages of chickens known to alter the gut microbiota and associated with immune dysfunction, including the development of autoimmune diseases (Arrieta *et al.*, 2014), alteration in the composition and diversity of the gastrointestinal tract microbial community (Khadem *et al.*, 2014; Neumann and Suen, 2015; Pourabedin *et al.*, 2015; Soler *et al.*, 2016) increased susceptibility of the host to gut infectious pathogens (Arrieta *et al.*, 2014; Kumar *et al.*, 2018).

In contrast, few studies were performed to identify the biochemical compounds present in broiler chickens' intestine by metabolomics approach, where the chickens were fed a standard diet supplemented with different antibiotic growth promoters like bacitracin or virginiamycin. These studies demonstrated that the supplementation of antibiotic preparations had profound effects on the levels of a wide variety of chemical metabolites, particularly amino acids, fatty acids, nucleosides, and nicotinamide-related compounds supporting the idea of an anti-inflammatory effect of these antibiotic growth promoters (Gadde *et al.*, 2018) and also in the production of inflammatory mediators like cytokines (Calder, 2017). Although the birds fed with bacitracin methylene disalicylate showed microbial diversity and species richness similar to control animals, the composition of microbiota in the cecum and ileum were differentially affected. Diaz Carrasco *et al.* (2018) and Kumar *et al.* (2018) observed that bacterial phyla were not affected by bacitracin in feed. However, changes in the bacterial population were observed at the lower taxonomic level. These changes in bacterial communities are correlated with the expression of various cytokine genes such as, IL-10, IL-4, IFN- γ , especially at initial stages of growth. Corthay *et al.* (2006) observed that commensal bacteria can modulate cytokine production in the gut with a significant role in both innate and adaptive immune responses.

Table 1. Showing the distribution of common gut microbiota across the various compartments of chicken GIT

GIT location (per g of content)	Bacterial phyla	Bacterial genera
Crop (10^8 - 10^9 / g)	Firmicutes	<i>Lactobacillus</i>
Gizzard (10^7 - 10^8 / g)	Actinobacteria	<i>Bifidobacterium</i>
	Proteobacteria	<i>Enterobacter</i>
	Firmicutes	<i>Lactobacillus</i> , <i>Enterococcus</i>
Small Intestine (Ileum; 10^8 - 10^9 / g)	Firmicutes/Low G+C, Gram-positive bacteria	<i>Enterococcus</i> , <i>Clostridium</i> , <i>Lactobacillus</i> , <i>Candidatus</i> , <i>Arthomitus</i> , <i>Weisella</i> , <i>Bacillus</i> , <i>Ruminococcus</i> , <i>Eubacterium</i> , <i>Staphylococcus</i> , <i>Streptococcus</i> , <i>Turicibacter</i> , <i>Methylobacterium</i>
	Cytophaga/Flexibacter/Bacteroides/ High G+C, Gram	<i>Bacteroidetes</i> , <i>Flavibacterium</i> , <i>Fusobacterium</i> , <i>Bifidobacterium</i>
	Proteobacteria	<i>Ochrobacterium</i> , <i>Alcaligenes</i> , <i>Escherichia</i> , <i>Campylobacter</i> , <i>Hafnia</i> , <i>Shigella</i> ,
	Actinobacteria/ Cyanobacteria	<i>Corynebacterium</i>
Caeca (10^{10} - 10^{11} / g)	Methanogenic Archaea (0.81%)	<i>Methanobrevibacter</i> , <i>Methanobacterium</i> , <i>Methanothermobacter</i> , <i>Methanosphaera</i> , <i>Methanopyrus</i> , <i>Methanothermus</i> , <i>Methanococcus</i>
	Firmicutes/ Low G+C, Gram-positive bacteria (44–56%)	<i>Anaerotruncus</i> , <i>Ruminococcus</i> , <i>Faecalibacterium</i> , <i>Lachnospiraceae</i> , <i>Bacillus</i> , <i>Streptococcus</i> , <i>Clostridium</i> , <i>Megamonas</i> , <i>Lactobacillus</i> , <i>Enterococcus</i> , <i>Weisella</i> , <i>Eubacterium</i> , <i>Staphylococcus</i> , <i>Streptococcus</i> ,
	High G+C, Gram-positive bacteria (23–46%) Actinobacteria <i>Corynebacterium</i> Proteobacteria (1–16%)	<i>Ochrobacterium</i> , <i>Alcaligenes</i> , <i>Escherichia</i> , <i>Campylobacter</i>
Large Intestine	Firmicutes	<i>Lactobacillus</i>
	Proteobacteria	<i>Escherichia</i>

(Source: Shang *et al.*, 2018)**Identification and characterization of gut microbiota**

For improving the poultry gut microbiome, it is imperative to understand the roles of the chicken

gut microbiota and the methods used in microbiome research. Previously, culture-based techniques have been used to identify and characterize the microbial diversity of the avian

gut. However, this method does not apply to non-culturable microbial species. Currently, the advent of omics and metagenomics, and advances in NGS high-throughput technologies capable of rapidly obtaining a complete census of a bacterial community led to important new insights into the biological and ecological roles of the GI microbiota (Shang *et al.*, 2018). It has been observed that there is a wide variation in the microbiota composition of chickens across and within studies (Stanley *et al.*, 2013; Brooks *et al.*, 2015; Reardon, 2016). Several factors associated with host and environment such as feed access, housing, litter, the environment can alter the microbiota composition within and across the different avian species (Stanley *et al.*, 2013; Waite and Taylor, 2014; Thibodeau *et al.*, 2017). Much of the microbiomes have been characterized using 16S rDNA sequencing, and it is also observed that differences in the composition of gut microbes might be due to differences in the sequencing platforms used, the choice of PCR primers, cycle numbers, nucleic acid extraction protocols, and sample storage can create variation in outcomes between studies as documented by several workers (Pissavin *et al.*, 2012; Lozupone *et al.*, 2013; Brooks *et al.*, 2015; Hermes *et al.*, 2015; Laukens *et al.*, 2016; Allali *et al.*, 2017).

Alteration of gut microbiota composition

During the last decade, several articles have been documented on the composition of chickens' intestinal microbiota and its associations with production performance (Nava *et al.*, 2007; Brisbin *et al.*, 2008; Kohl, 2012; Stanley *et al.*, 2012a; Yeoman *et al.*, 2012). The differences in the abundance of bacterial species for broilers with high and low growth, feed efficiency have been studied (Stanley *et al.*, 2012a; Singh *et al.*, 2014). Necrotic enteritis associated with *Clostridium perfringens* infection causes severe production losses in broilers and is a major microbiota research area (Hook *et al.*, 1996;

Van Immerseel *et al.*, 2004). *C. perfringens* infection is associated with alteration in the composition of the microbiota, but it remained unclear whether these are cause or effect of *C. perfringens* proliferation (Stanley *et al.*, 2012b; Antonissen *et al.*, 2016; Moore, 2016). Moreover, the indiscriminate usage of antibiotics resulting in the development of antimicrobial resistance and the ban on antibiotic growth promoters in the European Union has prompted research into developing alternative strategies aiming at stimulation or colonization of the chicken intestine with beneficial microbiota (Stanley *et al.*, 2014). It believes that the early colonization of the chicken intestine with non-pathogenic healthy microbiota is of great importance for chicken health and productivity since it can alter the intestine's morphology and physiology, and susceptibility to infectious diseases (Kers *et al.*, 2018). At present, the administration of prebiotics, probiotics, consortia of microorganisms either singly or in mixed cultures directly on or inside the egg are being evaluated as strategies to favor the intestinal colonization of birds with a "healthy" microbiota early from the moment of hatching (Baldwin *et al.*, 2018; Rubio, 2019).

Prebiotics

In a study, Xu *et al.* (2003), reported that the poultry feeds supplemented with dietary prebiotics such as 0.4% fructo-oligosaccharides (FOS) resulted in a significant increase in body weight gain, feed efficiency and stimulated the growth of beneficial bacteria like *Bifidobacterium* and *Lactobacillus*. Also, supplementation of mannan-oligosaccharides (MOS) in broiler diets has significantly improved energy, protein, fiber, and carbohydrate digestibility and utilization in several studies (Kumprecht and Zobac, 1997; Samarasinghe *et al.*, 2003; Yang *et al.*, 2008a, b).

Probiotics

A probiotic is defined as a live microbial feed supplement that beneficially affects the host

Table 2. List of commonly used microorganisms as probiotics for the prevention of subclinical necrotic enteritis (SNE) in chickens

Bacteria	Species
Bacillus	<i>Bacillus cereus</i> 8A, <i>Bacillus licheniformis</i> , <i>Bacillus pumilus</i> , <i>Bacillus subtilis</i> , <i>Bacillus coagulans</i>
Lactic acid bacteria	<i>Lactobacillus acidophilus</i> , <i>Lactobacillus amylovorus</i> , <i>Lactobacillus animalis</i> , <i>Lactobacillus fermentum</i> , <i>Lactobacillus johnsonii</i> FI9785, <i>Lactobacillus johnsonii</i> BS15, <i>Lactobacillus mucosae</i> , <i>Lactobacillus plantarum</i> , <i>Lactobacillus reuteri</i> , <i>Lactobacillus salivarius</i>
Enterococci	<i>Enterococcus faecium</i> , <i>Enterococcus faecalis</i> , <i>Enterococcus durans</i>
Bifidobacteria	<i>Bifidobacteria animalis</i> sub species <i>lactis</i> , <i>Bifidobacteria thermoacidophilum</i>
Yeast	<i>Saccharomyces cerevisiae</i> , <i>Candidatus savagella</i>

(Source: Khaliq *et al.*, 2020)

animal by improving its intestinal microbial balance (Fuller, 1999). Probiotics have been used widely in the poultry industry as a potential alternative to antibiotics. Administering *Lactobacilli* has been shown to decrease poultry colonization by intestinal pathogens *Salmonella* and *Campylobacter* reduction in the adhesion of *C. perfringens* to immobilized canine mucus (Rinkinen *et al.*, 2003). Although some studies are inconclusive, oral uptake of *Lactobacilli* and bifidobacteria decreased the number of intestinal *C. perfringens* bacteria and spores in humans and mice in other studies (O'Mahony *et al.*, 2001). Hofacre *et al.* (1998) showed that a commercial probiotic preparation reduced the gross lesions of necrotic enteritis in chickens, but the protection was far less than that conferred by competitive exclusion flora.

It is observed by several workers that, *Lactobacilli* present in the environment in an amount that should fully cover the requirements of chickens (Cressman *et al.*, 2010; O'Brien *et al.*, 2016). Enterococci and Bacilli are less common microbiota members. Moreover, these species observed that when inoculated in pure cultures in newly hatched chicks, they do not colonize (Kubasova *et al.*, 2019). Unlike the conflicting results when using probiotics consisting of a single or limited number of bacterial

strains (Weinack *et al.*, 1985; Baldwin *et al.*, 2018; Beirao *et al.*, 2018; Mazanko *et al.*, 2018) there is consensus on the efficacy of commercially available probiotics products such as Broilact or Aviguard in conferring increased resistance to *Salmonella* in chickens (Ferreira *et al.*, 2003; Milbradt *et al.*, 2014). These products and *Lactobacillales* and *Clostridiales*, contain different *Bacteroides* species, *Megamonas*, *Megasphaera*, *Dialister*, *Phascolarctobacterium*, *Sutterella*, *Parasutterella*, *Blautobacterium* etc. (Kubasova *et al.*, 2019), i.e., the same bacteria which are transferred by contact from hens to chicks.

For the improvement of healthy gut microbiota, special attention should be drawn on the concept of probiotics and competitive exclusion, wherein new probiotic species should be identified and the efficacy of currently available probiotics should be always tested. Also, the use of novel bacteria belonging to the genera such as *Megamonas*, *Megasphaera*, or *Bacteroides* needs further investigation.

Plant extracts

Plant extracts, known as phytobiotics are widely used in traditional medicine systems (Savoia, 2012) particularly for their role as antimicrobial, antiparasitic, anti-inflammatory, and antioxidant

Fig. 2. Figure showing the beneficial effects of chicken gut microbiota

activities (Steiner, 2009; Hashemi and Davoodi, 2010; Vondruskova *et al.*, 2010). These plant extracts, in general, are considered safe and effective against certain bacteria and have been used as growth promoters and health protectants (Hashemi and Davoodi, 2010; Abreu *et al.*, 2012), particularly in Asian, African, and South American countries, and in recent years are gradually being used in developed countries. The authors also reported the effects of phytogetic feed additives on chickens' production performance (Hashemi and Davoodi, 2010). It was observed that plant extracts possess antibacterial activities with minimum inhibitory concentrations ranging from 100 to 1000 µg/mL according to *in vitro* bacterial susceptibility tests (Simoes *et al.*, 2009). Several studies conducted during the last few years reported that the use of raw plant extracts as poultry feed supplements (Redondo *et al.*, 2014) might positively affect bird's health and productivity. There is significant scientific evidence that suggests the use of tannins, polyphenolic secondary metabolites to enhance nutrition and animal health in both cattle and poultry (Frutos *et al.*, 2004; Yang *et al.*, 2015). Numerous *in vitro* and *in vivo* studies have verified the activity of tannins against several types of intestinal pathogens, including helminths (Athanasiadou *et al.*, 2008), viruses (Ueda *et al.*,

2013), and bacteria such as *Campylobacter* and *Clostridium perfringens* (Saif *et al.*, 2008; Costabile *et al.*, 2011; Anderson *et al.*, 2012).

Essential oils

Essential oils (EOs), the organic compounds from plants, are assumed to possess antimicrobial properties and are also involved in plant defense (Fraenkel, 1959; Tajkarimi *et al.*, 2010). Recently, many reports demonstrated the antimicrobial activity of EOs to control bacterial pathogens (Baratta *et al.*, 1998; Kalemba and Kunicka, 2003; Burt, 2004) and possess broad range antibacterial properties (Deans and Ritchie, 1987; Oussalah *et al.*, 2007). Although the evidence about the inhibitory spectrum of EOs is contradictory, some studies concluded that Gram-positive bacteria are more resistant than Gram-negative bacteria (Lin *et al.*, 2008). However, most works reported that Gram-positive bacteria are more susceptible to EOs than Gram-negative bacteria (Burt, 2004; Inouye *et al.*, 2001) for their difference in the cell wall structure (Mann *et al.*, 2000; Inouye *et al.*, 2001). Several studies documented the use of EOs to reduce the incidence of necrotic enteritis in chickens and to control the proliferation of *C. perfringens* (Timbermont *et al.*, 2010; Jerzsele *et al.*, 2012; Yang *et al.*, 2015). Also, several

reports have been published about the antibacterial effects of *Piper nigrum*, *Origanum vulgare*, *Syzygium aromaticum* and *Thymus vulgaris*, and their components, thymol, carvacrol, and eugenol, against *Clostridium* species (Paster *et al.*, 1990; Dorman and Deans, 2000; Mitsch *et al.*, 2004).

Conclusion

The gut microbiota plays an essential role in maintaining the integrity of the gastrointestinal tract of a chicken. Several hundreds of healthy gut microbiota have been identified with the metagenomics approach. Although there is considerable variation in the diversity and composition of gut microbiota in the chickens of the outdoor, backyard, or commercial chickens, it is not possible to conclude that the chicken's microbiota is present raised in contact with adult hens is of probiotic potential. Several factors like age, housing, litter, feed access, and the environment will influence the gut microbiota's

development and maturation. It is a need of the hour to characterize the culturable healthy gut microbiota with the potential to colonize the GIT of birds and to prevent enteric gut diseases especially *Clostridium perfringens* mediated necrotic enteritis in chicken with new advances in identification and characterization of bacteria. Novel bacterial species can be tested individually or in defined mixtures in chickens, either as potential next-generation probiotics or as starter cultures followed by testing different feed formula in pre-colonized chickens under standardized conditions. Understanding the principles of chicken gut colonization by bacterial microbiota may allow for the construction of a new generation of probiotics and improve gut health, reduce the need for antibiotic therapy and improve chicken welfare in the broadest possible terms.

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