



Probiotics and Poultry Gut Microflora

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Received: 28 Oct. 2019

Accepted: 09 Dec. 2019

ABSTRACT

Poultry production is presently the most effective animal production industry and provides an excellent source of protein production worldwide. The poultry gastrointestinal microbiota includes commensal, mutualistic and pathogenic microbes. The relationship between host and gut microbiota can affect the balance of mutualism and pathogenicity. The imbalanced gut microflora caused by the incidence of disease, hygiene conditions, diet, management practices, and environmental stress affects the survival and productivity of chicken. Maintenance of the gut microbial composition is possible through the regulation of the gastrointestinal microbiota by suppressing the growth of pathogens. For many years, antibiotic growth promoters have been used to manage these problems. Nowadays, because of the emergence of antibiotic-resistant bacteria, other alternatives are being sought. Supplementation of probiotics as feed additives is considered to enhance chicken productivity and to protect the gut from pathogen colonization and help to tolerate environmental stress. The goal of the present article was to review the poultry gastrointestinal microflora and probiotics role in the health and growth of poultry. In addition, this article focused on probiotic microorganisms and their potential characteristics.

Key words: Gastrointestinal microbiota, Poultry, Probiotics

INTRODUCTION

Poultry production is currently the most efficient animal production system and forms the basis of global protein production (USDA, 2019). The advantage of poultry production depends on the ability of chickens to efficiently convert feed into muscle mass. This makes them an effective system for producing high-quality proteins (Phillippa et al., 2018). According to FAO (2012), poultry refers to the domestic birds including domestic chickens (*Gallus gallus domesticus*), turkeys, ducks, geese, dove, and other domesticated birds that are raised to produce eggs and meat. Among these, chicken production is the most popular worldwide. The interaction between the biochemical functions of the poultry and the intestinal microbiota is involved in extracting energy and nutrients from food. Thus, the selection of beneficial microbiota plays an important role in improving production performance, detoxification, modulation of the immune system and protection against pathogens (Clavijo and Florez, 2018). In the poultry, different organs contribute to the digestion and absorption process of nutrients.

Microorganisms present in each organ of the digestive system have independent functions and different taxonomic composition. As a result, gut organs are considered as separate ecosystems for microbes despite the deep interconnection between gut microflora (Wielen et al., 2002).

The microbiota in the poultry gastrointestinal (GI) tract includes commensal, mutualistic and pathogenic microorganisms. The gut microbiota positively influences the GI development, immunological and physiological functions of the gut. In poultry, these microorganisms colonize the GI tract during the early post-hatch period and form a synergistic relationship with the host (Torok et al., 2008). Chicken gut microflora composition changes in relation to the age of chickens, dietary factors, breed, and geographic location. The different factors related to diet, infectious agents, environmental and management conditions negatively affect the balance of poultry gut microbiota, which consequently impairs feed conversion ratio and growth performance (Yegani and Korver, 2008). The balance between pathogenicity and mutualism can be

determined by the relationship between the host and its gut microbiota. Modulation of the GI microbiota by suppressing the growth of pathogens helps to maintain the optimal microbial composition. Hence, the inclusion of antibiotic growth promoters in animal diets improves growth and feed conversion efficiency (Dumoncaux et al. 2006). The emergence of antibiotic-resistant bacteria causes the growing global concerns related to the transmission of these bacteria from animals to humans. This global concern has led to limiting the usage of antibiotics in livestock (Ameta, 2012). Therefore, the alternative attention is concentrated on the use of probiotic microorganisms and other products such as enzymes, organic acids, bacteriocins, bacteriophages and nanoparticles that can similarly enhance poultry productivity and produce safe edible products (Mehdi et al. 2018). In addition, following the European Union ban on the use of prophylactic antibiotics in poultry nutrition, scientists currently enforced to seek alternatives to antibiotic growth promoters to produce safe and efficient poultry meat and egg (Saeed et al. 2017).

Microflora in the chicken gastrointestinal tract

The digestive tract of chickens is comprised of the crop, proventriculus, gizzard small and large intestines and ceca (Nasrin et al., 2012). In addition, gut microflora, gut-associated immune tissue, liver, gall bladder, and pancreas are other important components of the digestive system (Dibner and Richards, 2004). The bacteria are the most abundant microbes of the GI tract. Approximately, there are up to 10^{10} - 10^{11} bacteria per gram of cecal content. Fungi and protozoa are the other gut inhabitant microbes (Albazaz and Buyukunal, 2014). Archaea which is represented predominantly by methanogenic *Methanobrevibacter* are other microorganisms colonized in chicken gut (Saengkerdsud et al. 2007). The specialized microbial communities in the GI tract perform important digestive functions as feed passes (Oakley et al. 2014). In chicken, the main bacterial activities are found in crop, small intestine, and cecum (Albazaz and Buyukunal, 2014). According to the report of (Youssef et al., 2017) inclusion of probiotics on poultry feed resulted in a numerical reduction in intestinal aerobes and fecal coliforms. Furthermore, all probiotics used significantly reduced total aerobic and staphylococci counts in the carcass meat, with a numerical decline in *E. coli* count. A prolonged feed retention time in the crop is associated with significant degradation of starch and fermentation of lactate mediated by the microbial community with the predominance of various *Lactobacillus* species. Also, *Clostridiaceae* family

resides in the crop (Svihus, 2014). The species of *Lactobacillus* and *Clostridiaceae* also are present in the gizzard. However, the existence of pepsin, gastric juices and hydrochloric acid in the gizzard decreases the pH and leads to reduced bacterial populations and fermentation activity (Clavijo and Florez, 2018). In poultry, the lower intestinal tract involves the small intestine, the colon, and two big cecal chambers which are important for the fermentation process (Sekelja et al., 2012). The small intestine is colonized mainly by *Lactobacilli* followed by *Streptococci* and *Enterobacteria*. On the other hand, the caecum is colonized mainly by strict anaerobes and a small number of facultative anaerobes (Cisek and Binek, 2014). The alimentary tract in newly hatched healthy chicken is usually sterile. The development of chicken intestinal microflora depends on their contact with bacteria from the environment within the first days after hatching. Differences in bacteria ingestion from hatching debris, environment, producing facility, feed and water cause variation in the microbial populations (Binek et al., 2000). On the first day of chick's life, the cecal microflora consists mainly of *Enterobacteriaceae*, *Enterococcus* and *Lactobacillus* species. After the second week of age, *Bacteroides* and *Eubacterium* species were established (Borda-Molina et al., 2018). Various species, different individuals of the same species and distinct sections of the GI tract have a different composition of microorganisms. In addition, the gut microflora is unstable over time (Dibner and Richards, 2004).

Impact of poultry gut microorganism on host

The gut is a natural barrier between the host and the intestinal microflora. There are numerous bacterial cell communities and millions of genes in the host. The expression of this amount of genes helps them to perform numerous enzymatic reactions that the host is not able to catalyze. This enables the microflora to influence many aspects of intestinal tract development and to provide metabolic contributions to the host (Yeoman et al., 2012). Generally, the gut microflora has a prominent role in digestion, metabolism, vitamin synthesis, immune stimulation and pathogen exclusion (Amit-Romach et al., 2004). Production of highly specialized hydrolytic enzymes by gut microorganisms allows degradation of complex substrates like non-starch polysaccharides and other indigestible carbohydrates (Sergeant et al., 2014). This hydrolysis allows further fermentation of the feed components by other members of the gut ecosystem that generate short-chain fatty acids, which in turn become accessible to the host as energy and carbon sources.

(Wang et al. 2016). The products and activities of hydrolytic enzymes create an ecosystem that is appropriate for some bacterial genera and hostile to others (Panda et al. 2009). Apart from nonpathogenic microbes, harmful members of the gut microflora may be involved in local or systemic infections, intestinal putrefaction and toxin formation (Yasothai, 2017). Enteric pathogens such as *Escherichia*, *Campylobacter*, *Vibrio*, *Shigella*, *Yersinia*, and *Salmonella* are a major cause of poultry morbidity and mortality throughout the world. Gram-negative enteric pathogens cause diarrhea and fever (Foley et al. 2013).

Probiotic microorganisms

The term probiotic has been defined as “a live microbial feed supplement which beneficially affects the host by improving its intestinal microbial balance” (Fuller, 1989). Probiotics stimulate the growth of beneficial microorganisms, reduce the number of pathogens, and lower the risk of gastrointestinal diseases (Getachew, 2016). These living microorganisms are nonpathogenic and harmless in nature, that are favorable to the host’s health when properly administered through the digestive route (FAO/WHO, 2001). These microorganisms include different species that belong to bacteria, fungi, and yeasts (Chen and Zhu, 2017). Youssef et al. (2017) also reported that probiotics and acidifiers can be used as potential alternatives to antibiotics in broiler diets. Different microbial species or different strains of the same species have different probiotic potential. Specific receptor sites and particular immunological properties are some of the reasons accounting for this difference (Hadisaputro and Harimurti, 2015). Probiotic microorganisms can be isolated from plants, food products, environment, human and animal sources (Hossain et al., 2012). Different studies reported the isolation of potential probiotic strains from the natural poultry gut microflora (Ehrmann et al. 2002, Shin et al. 2008). Competitive elimination of pathogenic microbes, production of antibacterial products (such as bacteriocins and colicins) and immune modulation are the basic mechanisms of probiotics. Live non-pathogenic microbial strains, either single or multi-strain, belonging to the genera *Lactobacillus*, *Streptococcus*, *Bacillus*, *Enterococcus*, *Pediococcus*, *Aspergillus*, and *Saccharo-mycetes* are used in poultry (Dhama et al. 2011).

Role of probiotics in poultry production

The poultry industry is a significant financial activity across the globe. Heavy financial losses occur when birds are subjected to stressful environmental conditions and

disease. The emergence of a wide range of antibiotic-resistant bacteria and pathogens are the main limiting factors for the poultry industry productivity (Kabir, 2009). A stable protective flora is established naturally in the poultry gut. Some dietary and environmental factors such as stress, antibiotic treatment, and excessive hygiene influence the stable protective gut microflora (Donaldson et al. 2017). Probiotic supplements are used to reconstitute the natural flora of chicken. Different strains of bacteria capable of surviving and inhabiting in the gut are used as probiotics. However, probiotics can be harmful to immunocompromised populations. The correct dosage of probiotic administration has not yet been established (Getachew, 2016). Several studies have been described the role of different probiotic *Lactobacillus* strains in chicken productivity and health. A study which involved the use of feed supplemented with *Lactobacillus* culture (1 g *Lactobacillus* culture /1 kg feed) in pure Hubbard and pure Shaver chicks from day 21 to 42 resulted in greater weight gain and heat tolerance in comparison to controls (Zulkifli et al. 2010). *Escherichia coli*, different species of *Salmonella enterica* and *Campylobacter jejuni* are the primary pathogens of poultry farming. The administration of *Lactobacillus* probiotics decreases enteric pathogenic microbes through competitive exclusion in the poultry intestinal tract and improves the intestinal well-being (Hadisaputro and Harimurti, 2015). According to Bansal et al. (2011), broiler chicks fed a diet with probiotic yeast gained significantly higher weight than control groups. In addition, dietary intake of Kefir as a probiotics source resulted in a decrease in chicken liver weight (Vahdatpour and Babazadeh, 2016). Diet supplemented to Protexin® probiotic alone or in combination with Fermacto® prebiotic increased growth hormone level and improved growth performance in quails (Nikpiran, 2014). The administration of probiotic supplements via drinking water significantly improved the weight gain in Kenyan indigenous chicken (Atela et al., 2015). The positive effects on weight gain and feed conversion ratio were observed in quails that received synbiotics (Babazadeh et al. 2011). The addition of probiotics to feed increase feed efficiency, growth performance, egg production, meat and egg quality as well as cholesterol level in poultry products (Getachew, 2016; Popova, 2017).

Role of probiotics in protecting poultry gastrointestinal infection

The probiotic microbes have the capacity to inhibit the development of pathogenic microorganisms in the gut of poultry (Getachew, 2016). Supplementation of probiotic

products allows manipulation of the GI microbiota. For example, *Listeria monocytogenes* is one of the pathogenic microbes that affect the poultry GI tract. Administration of multi-strain probiotic containing different *Lactobacillus* species and *Bacillus amyloliquefaciens* prevents the establishment and spread of this bacterium in the GI tract of broiler chickens (Neveling et al. 2017). In another study, the administration of commercial probiotic preparation formulated from different species of *Lactobacillus* and *S. cerevisiae* reduced the stress of *E.coli* K88 infected Hubbard broiler chicks and reduces *E.coli* proliferation in GI tract (Mohamed and Younis, 2018). According to Forkus et al. (2017), the production of the antimicrobial peptide known as Microcin J25 by engineered *E.coli* inhibits colonization of *Salmonella enterica* in the turkey GI tract. *Clostridium perfringens* is a pathogenic microbe that causes necrotic enteritis in poultry and negatively affects poultry health and productivity. Inclusion of *Lactobacillus johnsonii* BS15 to the feed reduces the incidence of necrotic enteritis and damage of villi by necrotic enteritis in Cobb 500 chicks (Wang et al. 2017). Administration of *Lactobacillus plantarum* K KKP 593/p and *Lactobacillus rhamnosus* KKP 825 via feed or drinking water reduce the number of *E.coli* in ROSS 308 broiler chickens (Michalczuk, 2019). According to Shokryazdan et al. (2017), supplementation of chicken feed with a mixture of *L. salivarius* strains improved populations of lactobacilli and decreased harmful bacteria including *E.coli* and total aerobes. Intestinal microbial modification through early probiotic inoculation has a role in improving the weight gain of the host.

At-hatch administration of beneficial strains has different results compared to the natural acquisition of the same strain from the environment (Baldwin et al. 2018). At-hatch administration as compared to natural acquisition improved feed conversion rate, growth performance, resistance to disease, digestion and absorption of nutrients, and carcass quality (Mohan, 2015). Synthesis of the antimicrobial compounds by the probiotic species, such as *Lactobacillus* spp., *Pediococcus acidilactici*, *Lactococcus lactis*, and *Enterococcus faecium* is one mechanism to prevent pathogens colonization. These antimicrobial products including short-chain fatty acids, bacteriocins, hydrogen peroxide, etc. inhibit or kill bacteria such as *Staphylococcus aureus*, *E. coli*, *Clostridium perfringens*, *Salmonella typhimurium*, *Bacillus* spp., *Listeria* spp., *Klebsiella* spp. and *Proteus* spp. by binding to the specific receptors and causing cell damage (Cisek and Binek, 2014).

Characterization of probiotic microbes

The characterization of probiotic is based on the consensus of scientists on some criteria, with particular attention being paid to the ecological origin of the bacteria, tolerance level to the harsh stomach and small intestine environments and capacity to bind to intestinal surfaces (Koenen et al. 2004). In general, microorganisms with potential probiotic advantages share common characteristics. The common requirements or properties of probiotics are discussed below.

General properties of probiotics

During the isolation process of microorganisms for probiotics, different selection criteria should be used as a reference. According to Kosin and Rakshit (2006) and Fuller (1989) some of the conventional criteria that can be applied for the selection of microbial species as probiotics comprise biosafety, the origin of the strain, resistance to GI tract conditions, intestinal adhesion and colonization, antimicrobial activity, stimulation of immune response, survival and stability throughout processing and storing (Khalil et al. 2018). In order to produce the desired effect, the probiotics strains should have a property to grow and survive in the digestive system of the host as they are exposed to a range of stressful conditions in the gut including lower pH, bile and pancreatic juice (Jose et al. 2015). The effects of simulated gastric juice and bile acids on the growth of probiotics are varied among species and strains. Species or strains with the greatest tolerance to acid and bile are excellent targets for the development of probiotics products. In addition, isolates with high tolerance to heat can be selected to produce probiotics (Hossain et al. 2012). Adhesion of the probiotics microbes to the intestinal mucosa is regarded as a precondition for colonization in the GI tract. This capacity to adhere is one of the most significant requirements for the choice of probiotics (Harzallah and Belhadj, 2013). The selection of probiotics also focuses on the safety of microorganisms. Hence, probiotics should be non-pathogenic and have no adverse effect on the host. The probiotic itself or its fermentation products or cell components should not be pathogenic, allergic, mutagenic, and carcinogenic (Harzallah and Belhadj, 2013). As an advantage, the probiotic strains should act as an adjuvant and stimulate the immune system against pathogenic microorganisms (Jose et al. 2015).

One of the safety considerations for selecting a potential probiotic strain is that it does not contain antibiotic resistance genes that can be transferred to the pathogenic microorganisms (Shakoor et al. 2017).

Probiotics microbes may be subjected to antibiotics in the animal gut when antibiotics are used as medicinal products for animal health. As a result, to be effective, the probiotics strains should possess non-transferable resistance which aids them *in vivo* survival (Shakoor et al. 2017). The resistance of probiotics isolates to some antibiotics is considered as an intrinsic property, presenting no safety concerns in feed or food (Khalil et al. 2018). Antagonistic activity of probiotics microorganisms against pathogens is regarded as a characteristic of probiotic to maintain the gut microflora balanced and to keep the gut rid of pathogens. Probiotics inhibit the growth of pathogenic bacteria through the production of nonspecific antimicrobial compounds such as hydrogen peroxide, short-chain fatty acids, and low molecular weight proteins known as bacteriocins and bacteriocin-like inhibitory substances (Torshizi et al., 2008).

Technological characteristics of probiotics

For the wide-scale distribution of probiotics strains, they must be manufactured under industrial conditions. These probiotic microorganisms have to survive and retain their functionality during storage as frozen or freeze-dried cultures. Similarly, their incorporation into foods or feeds should not provide unpleasant flavors or textures (Saarela et al. 2000). Technological evaluations include pH, salt and bile acid tolerance, hydrogen peroxide production, utilization of different carbon sources, enzymatic activities, hemolytic properties, antibiotics sensitivity, antimicrobial activity and *in vitro* adherence properties (Abiodun et al. 2013). Large scale production of probiotics involves a fermentation process. During fermentation reactions, the probiotics strains may be exposed to different temperature conditions. In addition, the storage and transport process of probiotics products should be under the optimum temperature. Thermophilic organisms have the advantage of tolerating higher temperatures during processing and storage. They have a better chance of remaining viable during the drying process required for prolonged storage and thus become distinctly effective products (Kosin and Rakshit, 2006).

Importance of probiotic research

The animal production system has a considerable impact on the nutrition and health status of consumers. Animal intestinal pathogenic microbes including *Salmonella*, *Campylobacter*, *Yersinia*, and *Listeria* are the major cause of food contamination and zoonosis. Different methods of animal production are introduced to increase productivity, quality, and safety of animal

products, besides protecting animal welfare and the natural environment (Markowiak and Slizewska, 2018). Previously, different medicinal products and antibiotics had been widely utilized to modify the animal gut microflora to enhance productivity and improve animal growth. However, the emergence of drug-resistant microorganisms has been occurred due to the long-term use of antibiotics and other medicinal products which causes a great fear to consumers and it also exerts negative impacts on the environment (Apata, 2012). The usage of probiotics is mentioned as one of the alternatives (Mehdi et al., 2018). Investigation of locally produced probiotics, targeting animals based on their surrounding environment and feed is important to maximize probiotics efficacy and to create market opportunities. Particularly, people in developing countries who do not have access to probiotics and live in different geographical locations will be benefited from locally sourced probiotics (Sybesma et al. 2015).

CONCLUSION

In general, the present review revealed that an effective dose of probiotics can have a dominant role in the improvement of intestinal microflora and production performance. In addition, it can inhibit the development of pathogenic microorganisms in the gut.

DECLARATION

Competing interests

The authors have no competing interests.

Authors' contribution

Kibirnesh Tegenaw designed the review, collected the information, and wrote the manuscript. Dr. Kagira and Prof. Nega collected the information and revised the manuscript.

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