

Probiotics in Poultry Nutrition

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Contributor: Rajesh Jha, Pravin Mishra

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1. Introduction

The European Union-wide ban on the use of antibiotic growth promoters (AGP) in farm animals in 2006 was a stellar step toward tackling the claimed antibiotic resistance issue ^[1]. Though many jurisdictions followed suit, due to the sparse regulation and lack of quantitative monitoring data on AGP, antibiotics have still been used as a growth promoter in many countries ^[2]. However, with the emerging public health concern about antibiotic resistance, it has become imperative to find an alternative approach to grow healthy animals ^[3]. Moreover, eliminating the use of antibiotics has spurred considerable consequences such as compromised animal performance and increased incidence of animal diseases ^{[4][5]}. Enteric diseases have become one of the prime concerns in the poultry industry after the exclusion of AGP. The industry has been suffering from unsatisfactory production efficiency, bacterial overgrowth in the small intestines, nutrient malabsorption, and associated food contamination ^{[6][7]}. Several feed additives in poultry have been tried as an alternative to AGP with varying degrees of success ^[8]. These commonly used feed additives can be classified into eight principle classes ^[9].

Probiotics are live bacteria, fungi, or yeasts that supplement the gastrointestinal flora and help to maintain a healthy digestive system. The joint Food and Agriculture Organization of the United Nations (FAO) and World Health Organization (WHO) working group have defined probiotics as “live microorganisms that, when administered in adequate amounts, confers a health benefit on the host” ^[10]. Probiotics can be provided as a live microbial feed supplement, also known as direct-fed microbials (DFMs), in the poultry diet or water or can be administered to the developing embryo using in ovo feeding technology ^[11]. Probiotics and DFMs are interchangeably used for beneficial microbes by poultry scientists ^{[12][13][14][15][16]}, though their functions and intent of use differ. Siragusa delineated the relationship between them as “Probiotics for livestock are termed direct-fed microbials or DFM” ^[17]. DFMs are beneficial microbe-containing feed additives that can complement the use of antibiotics and restore gut functions by stabilizing the gut microflora, enhancing animals’ performance ^[18] and altering the rumen fermentation pattern in ruminants ^[19]. DFMs gained popularity because of their prophylactic efficacy against bacterial infections of the gut and immunomodulating activity ^[12]. DFMs function to normalize gut microbiota and prevent gut infection; in contrast, probiotics exert a broader array of benefits as functional foods ^[20] providing health gain and reducing the risk of diseases. Thus, although probiotics and DFMs have a different meaning, this review has used these terms interchangeably for practical application purposes, as those were reported by different workers.

The use of probiotics in poultry has increased steadily over the years due to the higher demand for antibiotic-free poultry and its well-researched benefits. The probiotic market reached 80 million USD in 2018, and this increasing trend of adding probiotics in poultry feed is expanding the global probiotics market, which is projected to reach 125 million USD by 2025 at a compound annual growth rate of 7.7% ^[21]. The benefits include enhanced growth and laying performance, improved gut histomorphology, immunity, and an increase in beneficial microbiota. This article is focused on the role of probiotics on gut microbiota. Role of probiotics on other variables, please refer to the original article ([10.3390/ani10101863](https://doi.org/10.3390/ani10101863)).

2. Gut Microbiota

Diverse gut microbiota plays a significant role in host metabolism, growth performance, nutrient digestion, and overall health of birds ^[8]. The composition of chicken gut microbiota depends on age, especially at the early stages of life, genotype, farming conditions/environment, and diet/feed additives ^[22]. Sometimes, the gut microbiota composition can be

altered severely by non-infectious or infectious stressors. Consequently, this dysbiosis can impact intestinal morphology and activities (e.g., increased permeability of the intestine, higher risk of bacterial infection, sepsis, inflammation, and reduced digestion) [23].

Probiotics can affect the health, performance, and disease risk of the hosts, as they can amend the dysbiosis and improve the balance of gut microbiota in healthy hosts by reducing the proliferation of pathogenic species and increasing the beneficial bacteria [4][8]. The most commonly used probiotic species belong to the genera *Lactobacillus*, *Streptococcus*, *Bacillus*, *Bifidobacterium*, *Enterococcus*, *Aspergillus*, *Candida*, and *Saccharomyces* [24] and exert preferential health benefits on the host through the competitive exclusion of deleterious bacteria and the immune modulation in the gut [4]. Several studies have found effects of probiotics supplementation on the gut microbiota, enzyme activities, and microbial fermentation in the digestive tract in broiler chickens [25][26][27][24][28][29][30].

Mountzouris et al. [31] assessed the effects of a multi-bacterial species probiotic, which contained 2 *Lactobacillus* strains, 1 *Bifidobacterium* strain, 1 *Enterococcus* strain, and 1 *Pediococcus* strain. Four hundred day-old male Cobb broilers were allocated to four experimental treatments for six weeks of study. The body weight, ADFI, and FCR were determined weekly, and cecal microflora composition, the concentration of SCFA, and activities of 5 bacterial glycolytic enzymes (α -galactosidase, β -galactosidase, α -glucosidase, β -glucosidase, and β -glucuronidase) were determined at the end of the study. The results showed that probiotic treatment had significantly higher specific activities of α -galactosidase and β -galactosidase than the control birds. Overall, the probiotic treatment displayed a growth-promoting effect that was comparable to avilamycin (an AGP) treatment. It suggests that probiotics modulate the composition and activities of the cecal microflora of broiler chickens.

Since newly hatched broiler chickens demonstrate a delayed commensal colonization and low bacterial diversity, they are ideal for controlling development and studying the composition of the intestinal microbiota. Nakphaichit et al. [25] evaluated the role of *L. reuteri* in newly hatched broiler chicks for the first-week post-hatch. The growth performance and ileum microbiota of the chickens were monitored for six weeks. The results showed the number of total bacteria in ileum samples at d 42 was five times higher in the probiotic group than in the control group. Four additional strains were analyzed in another study with 294 day-old Cobb broiler chickens [32]: *L. johnsonii*, *L. crispatus*, *L. salivarius*, and one unidentified *Lactobacillus* spp. The microbial profile and production performance were evaluated. The results showed that the addition of probiotic *Lactobacillus* spp. to feed increased the number of total anaerobic bacteria in the ileum and ceca, and the number of lactic acid bacteria and *Lactobacilli* in the ceca. Furthermore, all four probiotics tended to reduce the number of Enterobacteria in the ileum compared with the control treatments. An important feature of *Lactobacilli* is the ability to auto- and co-aggregate. Typically, bacteria demonstrating a high auto-aggregation capacity show a good adhesion to the mucus.

Martínez et al. [26] studied the probiotic potential of *Propionibacterium acidipropionici*. *P. acidipropionici* LET105 and LET107 were administered at a concentration of 106 cfu/mL in the drinking water. This supplementation showed the normal development of lactic acid bacteria and *Bifidobacteria* but a slow colonization by Bacteroides. Eventually, this increased the lactic acid production and lowered butyric acid production with a rise in mucus secretion, which increased the protection against pathogens.

The probiotic supplementation of broilers with *B. licheniformis* and *B. subtilis* did not show a significant effect on the ileal and cecal microflora [33]. This non-significant effect on total aerobic and Salmonella count in the gut was also found when a mash diet supplemented with *Lactobacillus acidophilus*, *L. casei*, *Enterococcus faecium*, and *Bifidobacterium thermophilus* was fed to Ross 308 broiler chickens [34].

L. salivarius expressing 3D8 scFv has been found as beneficial in the study [35], where it showed the supplementation of that strain increased the abundance of Firmicutes, Proteobacteria, Actinobacterias, and Bacteroides in the fecal samples. Considering the abundance at the genus level, *Lactobacillus* was found as the most abundant genus, constituting 22.8% of the microbiota in the fecal samples in the *L. salivarius* 3D8 scFv treated chickens. A combination of *L. salivarius* and *Pediococcus parvulus* also improved the weight gain, intestinal morphology, and immune response [36]. Neveling and co-authors have shown that a combination of *Lactobacillus crispatus*, *L. salivarius*, *L. gallinarum*, *L. johnsonii*, *Enterococcus faecalis*, and *Bacillus amyloliquefaciens* inhibited the colonization of Salmonella in the GIT of broilers. Broilers treated with the multi-species probiotic had higher levels of lysozyme in their serum and higher T lymphocyte responses compared to control birds.

Probiotics favor the growth of bacteria of specific genera. When broilers challenged with *Salmonella enteritidis* were fed with a *Bacillus coagulans*-containing diet, this diet helped increase the *Lactobacilli* and *Bifidobacterium* but lowered the coliform and salmonella concentration in the cecum [37]. Besides that, this reduced the salmonella loads in the liver of the chickens.

The probiotic bacteria can initiate gene exchange with the gut microbiome and transfer genetic attributes to the surrounding bacteria. Their intimate cell-to-cell contact with other bacterial inhabitants of intestinal ecosystem increases the odds of genetic exchange of plasmids [38]. This conjugation process transfers the genes responsible for the acquired resistance of the probiotic microbe against antibiotics to the natural commensal microbes of the gut [39]. Studies related to human probiotics have identified different antibiotic resistance determinants in the genome of probiotic species of the *Lactobacillus*, *Bifidobacterium*, and *Bacillus* genera which have potential to transfer genetic resistance genes to other bacteria [40][41]. However, enough concern has not been observed about antibiotic resistance gene transfer in poultry through probiotic supplementation.

3. Conclusions

Probiotics are considered a captivating feed additive because of their immense empirical benefits: improvement in the gut microbiological homeostasis, immune response, growth, and laying performances. The use of probiotics in poultry production may address the public health concerns of antimicrobial resistance development to some extent, as this could replace the use of some subtherapeutic antibiotics. Studies showed a range of variation in the incurred benefits because of the differences in the methodologies of the experiments (e.g., the strains of probiotics, the dose of probiotics, the age, the breed of birds, the species, the inoculation level of challenging pathogens, and external factors). Many studies have attempted to compare the benefits among different inclusion levels of probiotics. However, no conclusive recommendation can be made regarding the optimal dose of probiotics, as the reported investigations were conducted under conditions with various confounding factors—e.g., variations in diet, husbandry, and stressors. Though the benefits are evident in different studies, details about probiotics' mechanisms of action are yet to be unraveled. Future studies should be directed to find the mechanism of action of probiotics, determine the optimal dose for single- or multi-strain probiotics, measure the effect in birds with flaws in gut integrity and enteric diseases, eliminate the risks of antibiotic resistance gene transfer, and set selection criteria for new probiotic species. Some human studies have shown that probiotic supplementation may incur some health risks. Similar studies in poultry are necessary to find the negative consequences of probiotic use as well.

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