A Review of Dietary Probiotics in Poultry

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Abstract

The use of probiotics has rapidly grown in recent years, enhancing the performance of broilers and leading to the production of products free of any probiotic trace. Recent evidence suggests that the use of microbial probiotics can play a significant role in the future of the poultry industry. Although this method is not complete yet, it can be employed by human societies as a useful tool to maximize poultry products, enhance the health, and ensure the safety of food sources. Some reports show that the application of probiotics as supplements of the feed of broilers can lead to positive outcomes such as increased weight and improved feed conversion ratio (FCR).

Keyword: Probiotic, Broiler, Hen, Turkey, Growth, Health


Introduction

Probiotics are nonpathogenic living organisms present in some foods which have positive effects on the health of the hosts if they enter the body in sufficient amounts. Imani et al1 concluded that probiotic therapy as an inexpensive and non-invasive strategy can reduce pathophysiologic symptoms and improve various liver diseases with no complications. Khani et al2,3 reported various potential positive effects, including the improve health of the digestive system, increase body immunity, reduced symptoms of lactose intolerance, decreased allergy, reduced risk of particular cancers, treatment of colitis, reduced serum cholesterol concentration, reduced blood pressure in those with hypertension, alleviated respiratory and Helicobacter pylori infections. In nature, the infants of animals receive protective flora from mothers or the environment. Nevertheless, contemporary childbirth and postpartum care methods limit contact with mothers and provide synthetic foods and environments. As a result, some natural parts of the microflora of infants' digestive system, which cause resistance to diseases do not exist anymore. Diet, antibiotics, and stress also affect the flora in adults. The application of probiotic supplements can compensate for this deficiency. Therefore, the use of these compounds does not create something, which does not naturally exist. Rather, it fully regenerates the protective ability of flora.4 Numerous studies have been conducted on probiotics with different results. The present study and similar studies attempt to provide a scientific basis for the concept of probiotics and delineate the way modern probiotics are manufactured. This hopefully paves the way for required studies and provides a basis for a more rational approach to the selection of probiotic strains in the future.

Prebiotics, Probiotics, and Symbiotic

Prebiotics are food nutrients which are indigestible or minimally digestible in the digestive system and their positive effect on human health is through the stimulation of growth or increase in the activity of a limited number of probiotic bacteria in the large intestine. This role is played by minimally digested and fermentable carbohydrates in the small intestine, leading to the growth of Bifidobacteria and some gram-positive bacteria. In fact, carbohydrates pass the small intestine, move to lower parts, and become accessible to the bacteria in the large intestine. Lactulose, galactooligosaccharides, fructooligosaccharides, inulin, and their hydrolyzed metabolites, maltooligosaccharide, are among prebiotics which are usually used in human nutrition. Probiotics improve the performance and health status of birds through competitive exclusion and creating a balance in the microbial population in the digestive system.5 Based on Fuller's6 definitions, probiotics are microbial nutritional supplements which exert positive effects on the host by improving the microbial balance in the intestine. This definition emphasizes the living nature of probiotics. Symbiotics are a combination of beneficial probiotic species and prebiotic carbohydrates, and the simultaneous consumption of these compounds has...
useful synergistic effects. In fact, the superiority of and major reason for the use of symbiotics are that, without a prebiotic, a probiotic would have little survival in the environment because prebiotics are the food source of probiotics.7 Due to the beneficial and synergistic effects of using a mixture of probiotics and prebiotics in good, it is recommended to refer to them as symbiotics.

Pourazari et al8 concluded that a stressful environment causes various diseases in broilers. Probiotics, prebiotics, and essential oils of Thymbus vulgaris L. can be used in poultry farming as an alternative to antibiotics. Feeds contained 1-2 g/kg Fermacto, 1-2 g/kg of Bioplus, and 0.5-1 g/kg of T. vulgaris L. essential oil while the feed of the control group did not contain any additives. Compared to the control group, Fermacto, Bioplus, and T. vulgaris L. essential oil led to weight gain and feed intake. The feed was increased with 2 g/kg of Fermacto. Moreover, 1 g/kg of T. vulgaris L. essential oil improve feed conversion ratio (FCR) but reduced the relative weight of thighs and wings. A significant difference was observed between groups in terms of blood parameters. Therefore, Fermacto, Bioplus, and T. vulgaris L. essential oil microorganisms improve mean daily weight gain of broilers with little effect on the carcass, organs, and plasma components. Technomouse prebiotic supplement significantly affected weight gain, feed intake, FCR, blood constitutes, and immunoglobulins in turkeys. Still, feed protein level significantly affected weight gain and FCR in the final stage, uric acid concentration on day 98, and IgM titer on day 49. Therefore, this study provides evidence supporting the fact that prebiotic supplements in turkey feed with different protein levels do not affect growth performance, blood biochemistry, or hemagglutination inhibition. The protein level of feed had more effects on the examined parameters compared to prebiotics.9 Karimi et al10 investigated the effects of some probiotics, including Bioplus, PrimaLac, Tipax, and Protexin on ostriches and found that, in treatments using Bioplus and PrimaLac, total cholesterol level (157 and 210 mg/dL) was increased compared to the control group (119 mg/dL), but total cholesterol was slightly decreased for the group receiving Tipax (79 mg/dL) in its feed (P > 0.05). Consequently, commercial probiotics had different effects on growth performance, carcass characteristics, and hematological parameters.

Probiotics Microbes and Their Characteristics
These microorganisms are nonpathogenic and are not related to bacteria causing diarrhea. They can’t transfer antibiotic-resistance genes and maintain genetic stability. Probiotic microbes can resist to gastric acid, bile, and digestive enzymes, and can attach to the intestinal wall and fight off pathogens. They have anti-mutagenic effects and play a role in reducing serum cholesterol. Probiotic microbes also stimulate the immune system without causing inflammation and have anti-cancer effects. Furthermore, they can increase bowel movement, maintain the health of mucus, and improve the bioavailability of food components.11

Probiotics’ Mechanism of Action
Increasing colonization resistance or direct pathogen inhibitory effects are important factors which enable probiotics to reduce the incidence rate and duration of diseases. Various types of probiotics are demonstrated for inhibiting pathogenic bacteria in vitro and in vivo through various mechanisms. Probiotics in poultry act as follows: (1) maintaining normal intestinal microflora by competitive opposition and exclusion,12,13 (2) changing metabolism by increasing the activity of digestive enzymes and reducing the activity of bacterial enzyme and ammonia production14; (3) improving digestion and the amount of nutrition15 and (4) activating the immune system.16-18 Probiotics can be used to control zoonoses and common diseases in poultry. In traditional conditions, competitive exclusion in poultry shows the consumption of intestinal microbes naturally found in poultry and chicks which are ready to be placed in the nest of a brood of chicks.19,20 First discussed this when they attempted to control Salmonella infantis in Finnish broilers. They discovered that small amounts of Salmonella challenge (1-10 cell per culture) were sufficient for the onset of salmonellosis in chicks. Moreover, they determined that chicks are most predisposed to Salmonella contamination in the first week of hatching. The consumption of Lactobacillus species did not create protection. Therefore, they had to evaluate the uncontrolled number of intestinal bacteria in adult chicks which were resistant to Salmonella infantis. This method was later called Nurmi method or competitive exclusion. The competitive exclusion method of inoculating one-day-old chicks with grown microflora clearly shows the effect of intestinal microbes on intestinal function and disease resistance.21,22 Although competitive exclusion is compatible with the definition of probiotics, this method provides grown intestinal microbes instead of adding one or multiple bacterial species to microbes created for each chick. The inoculation of one-day-old chicks grown with competitive exclusion or more classic probiotics acts as a good model for determining the activity and effectiveness of these microorganisms. This is also commercially important due to the predisposition of one-day-old chicks to infection. Using this model, some probiotics are demonstrated for reducing colonization and transmission of Salmonella and Campylobacter.23,24

Based on consumption rate, probiotics reach a large amount of acid lactic bacterium to the intestinal route. These microorganisms are known for adjusting the intestinal environment and reaching enzymes and other useful matters to the intestines.25 Feeding chicks with Lactobacillus acidophilus supplement or the mixture of Lactobacillus cultures significantly (P<0.05) increases amylase levels after 40 days of feeding. Entering probiotics (a mixture of multi-species of Lactobacillus and Streptococcus faecium) increases the activity of carbohydrate in the small intestine of piglets. Lactobacillus colonizes in the intestine and may secrete enzymes, thereby increasing the activity of intestinal amylase.26,27 It is established that probiotics alter intestinal pH and microflora in order to facilitate the activity of intestinal enzymes and digestion.28 Moreover, probiotics may reduce the production of ammonia in intestines and thus improve the health status of birds.29

Probiotic is a general term, and products can include yeast
cells, bacterial cultures, or both, and microorganisms are stimulated to adjust the intestinal environment to facilitate health status and improve nutritional function. Mechanisms through which probiotics improve the function of FCR are altering the intestinal flora, increasing the growth of anaerobic microorganisms and gram-positive bacteria which produce lactic acid and hydrogen peroxide, preventing the growth of intestinal pathogens, and increasing the digestion and consumption of nutrients. Therefore, major outcomes of using probiotics include an increase in growth, reduction in losses, and improvement of FCR.

The Role of Probiotics in Changing the Composition and Metabolism of Microbial Flora
Intestinal microbial flora is often constant in each person, although it differs across individuals. Still, the prescription of probiotics in infants and adults leads to a change in the microbial profile and metabolic activities of the stool. Although these variations are small, they are usually sufficient for correcting the course of the disease in case of prescription in pathogenic conditions. In most cases, the prescription of probiotics increases the number of Bifidobacteria and Lactobacillus, reduces the pH of stool, and decreases the activity of bacterial enzymes.

Criteria for Selecting Probiotics in the Poultry Industry
Probiotic bacteria must follow the following conditions: They must be natural flora of the intestine; they must be able to attach to intestinal epithelium in order to overcome potential barriers such as low gastric pH, presence of bile acid in the intestine, and competition with other microorganisms in the intestinal route. The competition of most species selected by laboratory and in vivo assays has been evaluated to control their continuation in chicks. Moreover, probiotics must exert their potential positive effects (e.g. improving nutrition and increasing the immune response) in the host. As a result, they must be made appropriate for industrial processes technologically and under normal storage conditions.

The Effect of Probiotics Against Salmonella Contamination
The use of probiotic spray for newly hatched chicks through administration in the first drinking water is a very efficient method for controlling the colonization of Salmonella in poultry intestine. Blankenship et al. reported that the prevalence of Salmonella in ceca and processed carcass was significantly decreased from 41% in control flocks to 10% in treated flock. This shows that chicks treated with probiotics can serve as a useful means for reducing salmonella contamination. The same authors used mucosal competitive exclusion (MCE) to treat newly hatched chicks through water spray and reported that initial feed, water, and litter contamination were at a low frequency (>10%) while eggshell fragments and chick paper pads were frequently contaminated (<50%). After three weeks of growth, litter contamination, skin with feathers, and ceca significantly differed (P < 0.05), while no reduction was observed in the treated flock compared to the control flock.

Competitive Exclusion
Tortuero used live bacteria and observed that the use of Lactobacillus leads to functions similar to those obtained following the use of antibiotics. Competitive exclusion indicates the prevention of a bacterium's entry to the attachment sites by filling the accessible space. In this way, the pathogenic bacterium is viewed as a competitor and removed by competition over attachment site.

Digestion and Enzyme Activity
In vitro studies have shown that digestive enzymes of Lactobacillus species are enriched in the intestine. Szylit et al. reported that 2 out of 5 Lactobacillus strains separated from a male chick showed amylase activity. Jin et al. reported that amylase activity in the small intestine is increased due to the Lactobacillus population fed to broilers, but has no effect on lipolytic and proteolytic activities. Collington et al. showed that including a probiotic (mixture of various species of Lactobacillus plantarum, L. acidophilus, Lactobacillus casei, and Streptococcus faecium) in pig's diet led to considerable carbohydratase activities in the mucosal membrane.

Bacterial Enzyme Activity
Goldin and Gorbach reported that nitroreductase, azoreductase, beta-glucuronidase activities in the intestine can lead to a decrease by feeding with L. acidophilus supplements. Similar results have been observed in humans. A similar reduction in beta-glucuronidase has been seen in chicks fed with 40% yogurt in drinking water and in pigs.

Ammonia Production
It is reported that probiotics containing L. acidophilus, S. faecium, and Bacillus subtilis decrease ammonia concentration in the excreta and little of broilers.

Increasing Feed Intake and Digestion
Pet's intestinal bacterial flora plays a significant role in feed absorption and digestion. They participate in the metabolism of nutrients such as carbohydrates, proteins, lipids, and minerals, as well as in the synthesis of vitamins. Nahanshon et al. detected that adding Lactobacillus in corn/soy or corn/barley/soy diet stimulates appetite and increases fat, nitrogen, calcium, phosphorous, copper, manganese, and their retention in layers.

Enterotoxin Inactivation
The substance produced by a probiotic may be inactivated with 40% yogurt in drinking water and in pigs. The same studies with Lactobacillus bulgaricus indicated that these small microscopic organisms produce a metabolite which inactivates the enterotoxin release by various forms of E. coli.

Stimulation of Body Immune System
Immune resulting from the exposure of intestine to various forms of antigen, such as pathogenic bacteria and protein in feed, is important in young animals' defense against intestinal
infections. Dun et al reported that birds treated with *Lactobacillus reuteri* further showed ideal and deeper cryptal villi which improve T cell function responses and increase the secretion of the IgM anti-*Salmonella* antibody.

Studies on Probiotics and Symbiotic in Poultry

Effects of Probiotics on Performance and Growth

Hosseini et al examined the effect of adding probiotics containing *Streptococcus* and *Bifidobacterium* on the performance of broilers. In terms of feed intake, carcass percentage, and abdominal fat weight, no significant difference was observed between treatments. Mean live weight and FCR in the 0-21 day period was significantly (*P < 0.05) improved in groups receiving probiotics compared to the control group, but this effect was not significant throughout the entire experiment.

Mahajan et al reported a significant (*P < 0.05) increase in the weight of edible viscera, warm carcass weight, cold carcass weight, and carcass percentage of broilers fed with probiotics (*Lactobacillus + Saccharomyces*). Jin et al observed a significant increase in the weight of chicks fed with various levels of probiotics compared to the control group. The positive effect of probiotics on chick weight was also reported by other researchers, e.g. Midilli and Tuncer and Kabir et al.

However, others did not observe such an increase in chick weight.

The effect of using organic acids and probiotics was examined on the performance and carcass characteristics of broilers. The examined properties were feed intake, body weight gain, FCR, and carcass characteristics. In terms of weight gain in the growth period, breast percentage, and abdominal fat percentage, no significant difference was observed between treatments (*P > 0.05*). For feed intake, weight gain, FCR, and abdominal fat percentage, the treatment containing probiotic and that containing a mixture of probiotic and organic acids yielded the best results. Results of this experiment showed that the use of organic acids and probiotics lead to better performance in the examined properties in male Ross 308 broilers compared to the feed containing antibiotics.

Effects of Probiotics on Immune Responses

Rowghani et al examined the use of probiotics and other additives in the feed of broilers and their effects on broilers’ immune system. In this study, the resistance of the immune system was examined with the use of probiotic, ToxiBan (commercial compound #1), Formicin (commercial compound #2), and a mixture of probiotic and ToxiBan in the feed of broilers. FCR was significantly better (*P > 0.01*) in ToxiBan treatment compared to other treatments. In general, in this study, a significant difference was found between various treatments in terms of body weight, feed intake, FCR, and blood antibody titer (*P < 0.01*).

Kabir et al investigated the effects of probiotics on immune response in the body of chicks and reported a significant increase (*P < 0.01*) in antibody production. They also considered the difference in the weight of spleen and bursa to be related to the difference in antibody production level in the group fed with probiotics and the control group. Dalloul et al explored the effects of feeding with *Lactobacillus* probiotic on the intestinal immune response of broilers during the course of *Eimeria acervulina* infection and showed that the probiotic continues providing some immune indices by adjusting immunity despite the relatively high amount of *E. acervulina*. Haghighi et al showed that probiotics increase natural intestinal serum and antibodies for some external antigens in broilers. Khaksefidi and Ghoorchi reported that antibody titer in 50 mg/kg probiotic-supplemented group is significantly higher 5 and 10 days after immunization compared to the control group when sheep-red-blood cells (SRBC) is injected in 7 and 14 days of age.

The Effect of Probiotics on Intestinal Microbiology and Morphology

Gharib et al compared the effect of probiotic and prebiotic on experimental contamination with *Campylobacter jejuni* in broilers. Experimental treatments included positive control (contaminated), negative control (non-contaminated), probiotic (PrimaLac), and prebiotic (Fermacto). The highest and lowest FCR at 49 days of age were observed in positive control and negative control groups, respectively (*P < 0.05*). Moreover, the treatment of negative control had the highest weight gain and feed intake at 49 days of age from among treatments (*P < 0.05*). Kabir et al indicated that probiotics can remove harmful pathogens through competition for attachment to the wall of the small intestine. Furthermore, broilers fed with probiotics underwent intestinal histological changes, including a variation in the length of villi and increased cell surface. Broilers fed with *Lactobacillus* strains have a smaller number of coliforms in their stool.

Effects of Probiotics on Meat Quality

Kabir and Kabir et al examined the effect of probiotics on the microbiology and gustatory factors of broiler meat and showed that the consumption of probiotics enhances the quality of meat before and after freezing. Mahajan et al reported a significant increase in the score of meat quality factors, including appearance, texture, succulence, and wholesomeness in broilers fed with probiotic (lactosaccharose). However, flavor and taste showed lower scores.

Factors Affecting the Probiotic Effect

The interactions between a microbial additive and the host and its digestive system microflora lead to various effects with a very complex nature. Some factors affecting this final effect are examined below.
Quality Assurance
The survival property of a probiotic product is of vital importance. However, the number of living microorganisms is not always the same as the number reported on labels. Another complicating factor is the difference in strains which can occur in one species. It is quite likely that the consumption of 2 probiotics produced from similar and equal bacterial species should lead to different results. Therefore, when comparing 2 probiotic products containing similar species, it is essential to know whether the employed cultures for the production of one probiotic product originate from one strain.\(^1\)

Consumption Amount and Method
Experiments on rats and humans reveal that the effect of probiotics is ceased when consumption is stopped. Similarly, in pigs and poultry, probiotics cannot be found in the digestive system seven days after consumption.\(^4\)

Age and Type of Animal
Animals’ digestive system microflora, physiology, and immune status change and their status is not the same during infancy, after weaning, and in puberty. As the flora in the infancy period is still changing, as a general principle, affecting the flora is easier over this period compared to other stages of life, since flora becomes relatively stable later. Therefore, it can be recommended that the consumption of probiotics should start as quickly as possible after birth. Alterations in diet composition may also occur in this period. Today, we are aware that variations occur in human infants' digestive system flora after weaning and the consumption of formula or solid food. It has been shown that milk contains an agent which promotes the growth of Bifidobacteria and can affect the response emerging after the consumption of probiotics containing these organisms. The debilitative effect of additives containing lactobacillus acidophilus on enzymatic activity depends on the type of diet. No such effect has been observed in rats receiving a diet containing seeds, whereas a positive response has been achieved in rats receiving a diet containing meat.\(^4\)

Flora Composition in the Animal Host’s Digestive System
It is possible that probiotics somehow act by affecting the microflora composition in the host’s intestine. In his way, the prerequisite for obtaining a positive response after the consumption of a probiotic compound would be the microbial presence with an undesirable effect such as reduced growth. Therefore, it is possible that no effect would be exerted by probiotics if a growth-debilitating organism is not present. Similarly, if the probiotic organism is naturally acquired, no response would be obtained following its consumption. The need for consuming probiotic additive is due to today’s unnatural life imposed on humans and animals. A clear example is a poultry whose eggs are removed from the brooding hen and kept in a clean incubator.\(^6\)

Type of Product
The importance of establishment may be decreased upon the continued consumption of probiotics, because it provides the opportunity for the presence of a large number of probiotic microorganisms in the digestive system without needing establishment or growth. Some probiotic organisms such as Aspergillus oryzae - which are unlikely to grow in rumen or affect rumen's metabolic activity - must act in this way. Nevertheless, even if probiotics are designed such that they would not have to be consumed continually, the maximization of survival in digestive system and performance of the above-mentioned experiments may prove useful in the selection of the most effective strains.\(^4\)

Production Methods
The behavior of an organism in the digestive system can be affected by its method of growth and harvest from the environment; for instance, attachment to mucous is affected by the energy source of carbohydrate used for growth or the presence of milk at the site of attachment. The growth of E. faecium in milk enhances the immunization against diarrhea caused by E. coli in pigs. The suspension of probiotic organisms in milk may also improve their ability to attach and, therefore, a better establishment in the digestive system. The phase of the growth cycle in which the probiotic organisms are obtained may also affect their attachment to the mucosal cell.\(^6\)

Conclusions
Today, the increases in population and necessity of access to healthy sources of protein have caused the ever-increasing popularity of the poultry industry. The dense growth of poultry increases the risk of various microbial infections such as Salmonella, Campylobacter, and Clostridium perfringens. Antibiotics are widely used to improve growth factor and prevent and treat various infections. The presence of antibiotic residuals in meat and egg, followed by antibiotic resistance, threaten the health of consumers. Considering this increasing trend of antibiotic consumption and the ever-increasing prevalence of antibiotic resistance, alternative compounds such as probiotics and prebiotics are being employed today. Their consumption as nutritional supplements in poultry diet is expanding due to their health-promoting effects, such as increasing growth, improving eggs, fortifying the immune system, and enhancing the health status.

Authors’ Contributions
All authors equally contributed to the current study.

Conflict of Interest Disclosures
The authors declare they have no conflicts of interest.

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