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REVIEW ARTICLE

The use of probiotics as eco-friendly alternatives for antibiotics in poultry nutrition

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Abstract

Antibiotics as growth promoters in poultry have been used for long time for improving feed efficiency and performance. Due to their various side-effects such as antibiotic resistance, destruction of beneficial bacteria in the gut, and dysbiosis, it is required to think about some alternatives. Probiotics are one of the options in this regard for improving poultry production. Probiotics are defined as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host." They are available in various forms for use as feed additives. Probiotics as feed additives aid in proper digestion of feed hence make the nutrients available for faster growth. Immunity can also be improved by addition of probiotics to poultry diets. Moreover, probiotics aid in improving meat and egg quality traits. Various infectious diseases of poultry can be countered by use of probiotics in their feed. A proper selection of probiotic strains is required for gaining optimal effects. This review focuses on the mechanisms of action of probiotics and their importance in poultry feed supplementation for enhancing production and safeguarding health of poultry.

Keywords Probiotics · Mode of action · Beneficial aspects · Health benefits · Poultry

Introduction

Antibiotics were being used as feed additives or growth enhancers since 1940s, when it was found that birds or animals fed diets supplemented with *Streptomyces aureofaciens* having chlortetracycline residues enhanced their growth rate (Eckert et al. 2010). Since 2006, European Union (EU) banned the use of antibiotics as growth promoters or food additives; the prohibition of antibiotics was due to emergence of microbial resistance to antibiotics that were used to treat animal and poultry diseases. In addition, antibiotics cause some other problems such as destruction of some beneficial bacteria in poultry gut. Moreover, it was observed by EU that use of antibiotics reduced growth rate which may be due to rising incidence of subclinical necrotic enteritis and dysbacteriosis (Palamidi et al. 2016). Therefore, probiotics play an important role as the best alternative to antibiotics due to their many beneficial aspects and usage as growth enhancers in humans and animals including poultry and fish (Zorriehzahra et al. 2016). Probiotics are defined as "Live microorganisms which when administered in adequate amounts confer a health benefit on the host" (FAO/WHO 2002). Furthermore, Hill et al. (2014) agreed that the definition of FAO/WHO for probiotics was still relevant, but advised a very simple grammatical correction "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host". Probiotics have also been defined as live microbial feed additives which beneficially affect the host animal via enhancing the balance in the gut and consequently improving feed efficiency, nutrient absorption, growth

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rate, and economic aspects of poultry (Abd El-Hack et al. 2017).

During recent years, nutritionists focused their work on providing novel and alternative supplements growth-promoting and therapeutics to prevent diseases and enhance bird's immunity because of biohazards of using antibiotics like residual impacts on meat and food products (Yadav et al. 2016). In this respect, avian egg antibodies, bacteriophages, cytokines, probiotics, toll-like receptors, and others were explored for their beneficial properties for the safeguarding health of animals, in addition to improving production performance (Tiwari et al. 2014). The beneficial applications and protective impacts of probiotics are visible in several aspects. Probiotics can be used as useful bacteria to eliminate pathogenic bacteria, and the potential use of them helps in enhancing growth and productive performance, quality, and composition of eggs, digestion, and absorption of nutrients, boosting production and safeguarding health of poultry (Ritzi et al. 2014; Alagawany et al. 2016; Popova 2017).

Probiotics involve several species such as beneficial bacteria, fungi, or yeast, and the most commonly used probiotics are strains of *Bacillus subtilis*, *Lactobacillus*, *Bifidobacterium*, and *Streptococcus*, which apart from their growth promoting activities are also capable of reducing many harmful bacteria like *Salmonella typhimurium*, *Staphylococcus aureus*, *Escherichia coli*, *Clostridium perfringens*, etc. (Iannitti and Palmieri 2010). The proper selection of probiotic strains could diminish the side effects of antibiotic usage and has numerous beneficial uses due to its ability to inhibit growth of pathogens. This article highlights the sources of probiotics, mechanisms of action and health benefits, as well as beneficial applications of probiotics in poultry nutrition, production, and health which could be useful for nutritionists, veterinarians, researchers, pharmacists, pharmaceutical industries, livestock, and poultry industry.

Probiotics sources and forms

There are many sources of probiotics such as live bacteria (*Bacillus subtilis*, *Lactobacillus*, *Bifidobacterium*, and *Streptococcus*), yeast (*Saccharomyces cerevisiae*, *Saccharomyces boulardii*, and *Candida*), and fungi (*Aspergillus*). Mostly bacteria belong to *Lactobacillus or Bifidobacterium* species (Chow 2002). Each group contains many kinds of bacteria (*Lactobacillus acidophilus*, *Bifidobacterium bifidus*, etc.) which include different strains. These can be separated from fermented products and human/animal body (gut, breast milk, and feces; Soccol et al. 2010). Further, they can be characterized based on their phenotype and genotype in order to be sure for their non-pathogenic nature and to check their bile tolerance (Kabir et al. 2016). There are other non-conventional sources of probiotic strains such as *Lactobacillus plantarum* and *Leuconostoc mesenteroides* which can be isolated from fresh fruits and vegetables (Sornplang and Piyadeatsoontorn 2016). A probiotic used in animal and poultry field may be composed of a single strain or a mixture of two or more species (David Collins and Gibson 1999). In addition, there are various forms of probiotics such as liquid, powder, gel, paste, and granules which are normally available in the form of capsules, tablets, sachets, etc. (Iannitti and Palmieri 2010). Dry dosage form of probiotics has higher shelf life on storage as well as better tolerance power to gastric environment. Use of hydroxypropyl methylcellulose phthalate 55 as a matrix for tablet form provided good viability of probiotic in poultry (Jiang et al. 2017).

Modes of action

Probiotics can follow various mechanisms based on inhibition of all pathogen via producing organic acids and antibacterial substances, i.e., hydrogen peroxide, bacteriocins, and defensins (Tiwari et al. 2012) (dipicolinic acid and fatty acids); blockading of pathogenic bacterial adhesion to intestinal epithelial binding sites using competitive inhibition (Tiwari et al. 2012); modulating host immune response by impacting regulatory T cells, antigen presenting cells, effector T and B cells, and enterocytes (Oelschlaeger 2010); regenerating of intestinal mucosa (Perdigon et al. 1995), and affecting the digestion by enhancing digestive enzymes secretion and helping in proper digestive process. Probiotics can also regulate the production of anti- and pro-inflammatory cytokine as reported by Roselli et al. (2005); stimulate the production of antibody (sIgA); enhance natural killer and macrophages (NK) cells activity; modulate dendritic cells function and phenotype, accent of AP-1, and NF-kB pathway; and modulate apoptosis and release of nitric oxide (Tiwari et al. 2012). In addition, it can also stimulate function of epithelial barrier; regulate up-regulation of mucous production and motility of intestine; stimulate acidic pH which facilitates absorption of protein and minerals like copper, calcium, iron, manganese, and magnesium (Raghuwanshi et al. 2015). Immunomodulation property of probiotic organisms is exerted through their effect on T helper cells in a strain-specific manner. They can also activate various immune cells (Fong et al. 2016). Probiotic bacteria (bile salt hydrolase active) can also reduce the risk of cardiovascular disease due to their ability to lower cholesterol levels (Jones et al. 2013). In one study, it was demonstrated that probiotic bacteria (*Lactobacillus plantarum* YML009) also have anti-viral potential against H1N1 influenza virus, but mode of action is still to be unraveled (Rather et al. 2015).

Beneficial applications of probiotics in poultry

Figure 1 shows the modes of action and beneficial activities of probiotics in poultry. In recent years, the attention has been grown towards the use of probiotics as an alternative feed additive to antibiotics (Sharifi et al. 2012). Studies confirmed the ability of probiotics to enhance gut microbial balance and consequently animal natural defense system against the pathogenic bacteria (Ohashi and Ushida 2009; Newaj-Fyzul et al. 2014). Natural acclimatization of various lactic acid bacteria to the gut environment and its antimicrobial substances (bacteriocins and organic acids) has supported these organisms with a good advantage comparing with other microorganisms to be used as probiotic additive (Aliakbarpour et al. 2012). Several studies revealed that supplementation of probiotics has positive impacts on growth, feed efficiency, and utilization, besides the mortality rate (Silva et al. 2010; Sen et al. 2012). Since, the probiotics efficiency depends mainly upon selecting more efficient strains, manipulation of gene, the interaction of many strains, and the combination of probiotics and synergistically acting constituents (Song et al. 2014). Using multi-strain probiotics has been proved to be the optimal way of potentiating beneficial impacts of probiotics through improving the growth enhancer bacteria with pathogenic bacteria antagonism in the gastrointestinal tract of birds (Patel et al. 2015).

Probiotics exert several effects on the host such as good effects on mucosal immune system, intestinal luminal environment, and epithelial and mucosal barrier function (Yosef et al. 2012). These additives can treat poor appetite and maldigestion by lowering gut disturbance and can be used as immune enhancers to stimulate the immune system and as new mucosal adjuvants to improve the vaccine specific humoral and cellular immune responses in the host (Borchers et al. 2009). The beneficial applications of probiotics are very promising because of their role as anti-oxidant, anti-inflammatory, anti-allergic, anti-cancerous, strong anti-mutagenic, anti-diabetic, and antiviral potential against many poultry viruses (Alloui et al. 2013). Moreover, probiotic supplements have been used effectively in checking the pathogenic bacteria load of meat and meat products through processing and packaging (Kabir 2009). On the other side, using *Bacillus subtilis* spore (GalliPro®) at 0.2 g/kg as a feed additive reduced the requirements of amino acids and crude protein and, consequently, broiler's feed cost (Zaghari et al. 2015). Apata (2008) mentioned that dietary probiotic supplementation (*Lactobacillus bulgaricus* at 20, 40, and 60 mg/kg feed)

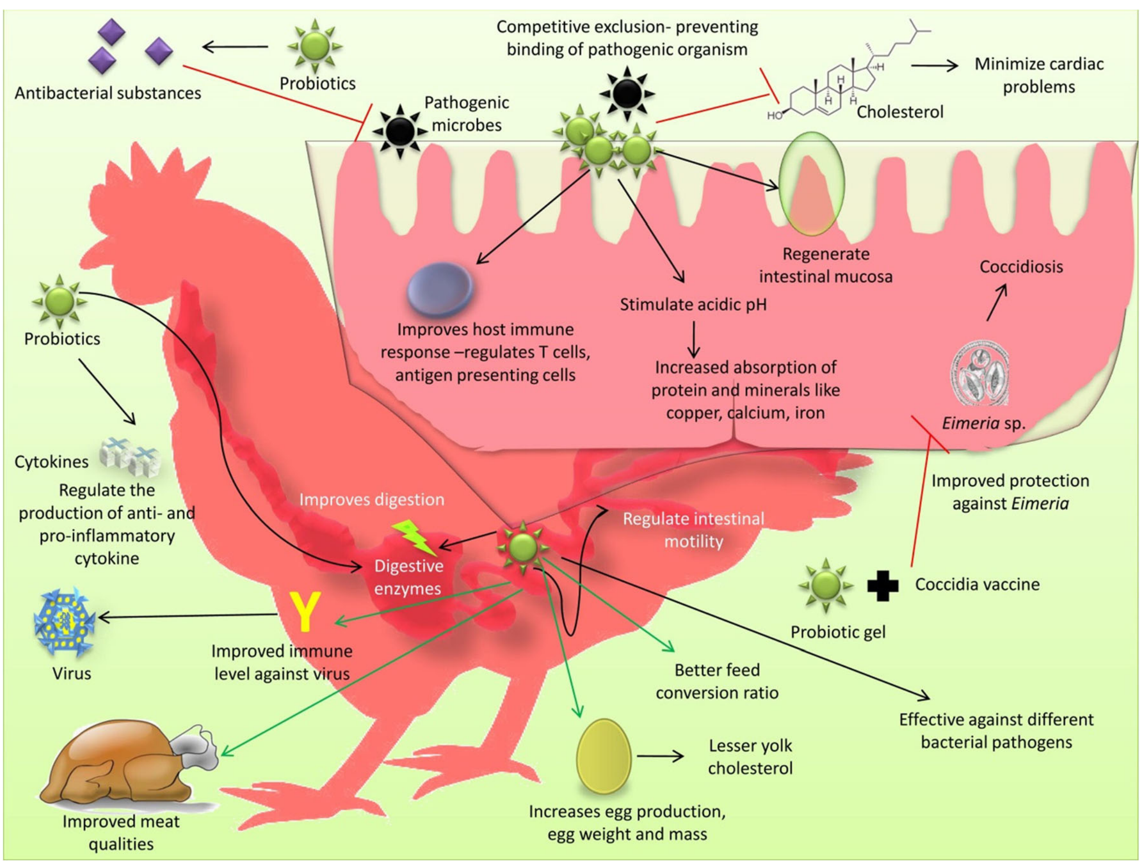

Fig. 1 Modes of action and beneficial activities of probiotics in poultry

increased the apparent digestion coefficients of some nutrients like amino acids and crude protein.

Enhancing growth performance and production

Protexin® as a commercial product (mixture of microorganisms) caused enhancement in laying hen performance and egg quality criteria (Youssef et al. 2013). Egg mass was enhanced in ISA-Brown and Leghorn-laying hens fed diet including probiotics (containing cultures of *Lactobacillus*, *Bifidobacterium*, *Streptococcus*, and *Enterococcus* spp.) at 0.1 and 0.2% in the feed (Yörük et al. 2004). On the contrary, Ramasamy et al. (2009) found that probiotics supplementation had no significant impact on egg yield and egg production. While, supplementation of Lactobacillus culture in hens leads to increase in their egg weight and egg size throughout the laying period (Ramasamy et al. 2009). Probiotic *(Bacillus subtilis)* at 1×10^8 cfu/kg feed had a beneficial impact on layer performance, egg quality criteria, and cholesterol content of yolk lipids (Sobczak and Kozłowski 2015). Supplementation of probiotics such as *Bacillus subtilis* at 8×10^5 cfu/g feed and multistrain probiotics (MP) at 0.4% to basal layer diet enhanced quality of egg, increased egg production, and reduced costs of feed (Ribeiro Jr. et al. 2014). Products of probiotics could provide a good alternative to antibiotics in poultry nutrition and expected to enhance productive performance, egg quality, and feed utilization (Cox and Dalloul 2015).

Bacillus amyloliquefaciens probiotic (BAP) when used as direct fed microbial at the level of 20 g/kg of diet for 35 days showed a positive effect on growth performance in broiler chickens. This is due to good intestinal health leading to better digestion and assimilation of nutrients in the feed (Ahmed et al. 2014). Also, it may be due to better digestibility or due to improved availability of all nutrients (Hrnčár et al. 2016). Broilers fed with *Bacillus coagulans* NJ0516 showed increased amylase and protease activity which led to an enhanced digestibility of starch and protein thus enhancing the growth of broilers (Wang and Gu 2010). Supplementing feed of Ross broilers with fermented *Ginkgo biloba* and *Camelia sinensis* based probiotics both at 0.1 or 0.2% lead to significant improvement in growth performance. Burden of pathogenic *E. coli* was also reduced in their caecal flora (Kim et al. 2016). On the other side, according to Afsari et al. (2014) diet supplemented with beneficial bacteria has no significant effect on egg production or egg size of laying hens. In ovo supplementation (embryonic age of 18 days) of PrimaLac® (*Lactobacillus acidophilus*, *Lactobacillus casei*, *Enterococcus faecium*, and *Bifidobacterium bifidium*) at 1×10^6 cfu probiotic bacteria showed increase in growth performance of first week post-hatch without affecting the rate of hatchability of such eggs (Pender et al. 2016a).

Health and immunity

Products of probiotics could provide a good alternative to antibiotics in poultry nutrition and expected to improve immunity and health status of birds (Cox and Dalloul 2015). In a study of Yang et al. (2012) results showed that the use of *Clostridium butyricum* at 2×10^7 cfu or 3×10^7 cfu/kg feed benefited the balance of the intestinal microbiota and improved the immune functions of broilers. In this regard, Bai et al. (2013) found that using a probiotic product have combined 1×10^7 cfu/g *Lactobacillus fermentum* and 2×10^7 cfu/g *Saccharomyces cerevisiae* at 0.1 or 0.2% in feed could enhance intestinal T cell immune system without any adverse effects on growth performance of broilers from 1 to 21 days of age. Moreover, Lee et al. (2010) assured that chickens fed a diet supplemented with *Bacillus* based direct-fed microbial at 5×10^6 cfu in 0.5 ml sterile distilled water had significantly reduced lesions in the gastrointestinal tract after *Eimeria maxima* infection comparing with those fed the control diet. This may be due to improvement in immunity as evidenced by increased serum nitric oxide level in birds fed with probiotic supplemented feed.

Liao et al. (2015) postulated that supplementing the basal diet of broilers with 2.5×10^8 or 5×10^8 or 1×10^9 cfu *Clostridium butyricum*/kg improved immune function and anti-oxidation. Dietary addition with probiotic products (*Bacillus subtilis*, *Clostridium butyricum, and Lactobacillus acidophilus*) improved humoral immunity and ileal amino acid digestibility of broilers (Zhang and Kim 2014). Al-Fataftah and Abdelqader (2014) demonstrated that dietary supplementation with 1 g/kg of *B. subtilis* to broilers under heat stress conditions was effective in alleviating the harmful influences on growth rate through improving the colonization of beneficial bacteria in the gut and restoring the impaired villus-crypt structure. Use of probiotics in poultry feed also augments vaccine response in them. Probiotic treatment at 200 g/kg of drinking water in broiler chickens was found to enhance the humoral immune response generated through Lasota Newcastle disease vaccine. It was due to their effect leading to increased expression of IL-7 mRNA in Harder's gland, caecal tonsils, duodenum, and ileal Peyer's patch (Hu et al. 2016). Probiotics in the form of gel (Poultry Star®) when given with coccidia vaccine were found to improve protection against *Eimeria* challenge (Ritzi et al. 2016). *Saccharomyces boulardii* and *Bacillus subtilis* B10 modulated the intestinal ultrastructure mediated via increased mRNA expression of occluding, cloudin2, and cloudin3 (Rajput et al. 2013). In laying hens, Ramasamy et al. (2010) found that the content of cholesterol of eggs produced by hens fed diet supplemented with probiotic (*Lactobacillus* culture) was reduced by 10.4% when compared to those of the control hens at 28 weeks of age. Tang et al. (2015) confirmed that supplementation of probiotic (0.1% PrimaLac®) to layer diets significantly ($P < 0.05$) decreased the egg yolk cholesterol and total saturated fatty acids at 28 weeks of age and increased total unsaturated fatty acids at 28, 32, and 36 weeks of age.

Countering infectious pathogens

Dietary addition of probiotic reduced numbers of gut pathogens such as *Salmonella* enteritidis, *Salmonella Gallinarum*, *Salmonella typhimurium*, and *Campylobacter jejuni* (Higgins et al. 2007; Ghareeb et al. 2012; Park and Kim 2015; Oh et al. 2017). Supplementation of the probiotics reduced *Escherichia coli* and total coliform counts and increased the numbers of lactobacilli in the intestine of broiler chickens (Dibaji et al. 2014). In addition, the probiotic mixture (*Lactobacillus pentosus* ITA23 and *Lactobacillus acidophilus* ITA44) improved bacterial count of the cecal contents, by decreasing *Escherichia coli* population and increasing beneficial bacteria (Faseleh et al. 2016). The aforementioned action could be attributed to many mode of actions done by direct-fed microbes, depending on strains/kinds existed in various products used in these studies. Among them, PrimaLac® protected chicken from *Campylobacter jejuni* challenge when given to broilers (in drinking water at 120/1 g/L until day 14, mixed in feed at 454/1000 g/kg until day 28 and 225/1000 g/kg for rest of growth period). This is due to the ability of organic acid and proteinaceous molecules released by probiotic bacteria which kills the pathogenic *Campylobacter* which is hypersensitive to low pH conditions (Ebrahimi et al. 2016). Birds fed *Bacillus subtilis* C-3102 could reduce *Campylobacter* colonization in poultry (Fritts et al. 2000). *Lactobacillus gasseri* SBT2055 inhibited the adhesion, invasion, and colonization of *C. jejuni* (Nishiyama et al. 2014). An in vivo study was carried out in chickens with various *Bacillus* sp., and different isolates were found to protect chickens against *Campylobacter* sp. (Saint-Cyr et al. 2016). Combined administration of *Lactobacillus salivarius* 59 and *Enterococcus faecium* PXN33 caused reduction in colonization of *Salmonella* Enteritidis S1400 in poultry (Carter et al. 2017). A probiotic strain of *E. coli* Nissle 1917 was restructured at the genetic level to secrete Microcin J25, an antimicrobial peptide. A study using 300 turkeys revealed that the use of this constructed *E. coli* could reduce *Salmonella enterica* from the gastro intestinal tract (Forkus et al. 2017). Researchers studied different bacteria as a probiotic culture based on their motility. Three *B. subtilis* isolates were studied for their in vivo ability to reduce colonization of *C. jejuni.* It was hypothesized that the isolate with good motility had increased capability to reduce colonization which might be due to its ability to reach the site of *C. jejuni* faster (Aguiar et al. 2013).

Dietary supplementation of probiotic in water and feed has been found to improve production performance and resistance of poultry birds to *Eimeria* species causing coccidioisis (Ritzi et al. 2014). In ovo administration of 1×10^6 cfu of probiotic bacteria (PrimaLac®) at 18-day embryonic age was found to protect such chicks hatched from mixed *Eimeria* spp. challenge at 3-day post-hatch (Pender et al. 2016b). This may be due to their modulating effect on caecal and ileum immune response genes (Pender et al. 2016a). Probiotic supplements including *Bifidobacterium animalis*, *Enterococcus faecium*, *Bacillus subtilis animalis*, *Lactobacillus reuteri animalis*, or a mix of multi-species probiotic *animalis* at 5×10^8 cfu/kg feed improved growth performance criteria and broilers' intestinal health (Giannenas et al. 2012). Usage of probiotics could also be beneficial in controlling *Listeria monocytogenes* infection in poultry (Dhama et al. 2015). Probiotic PrimaLac® administration in poultry diets has been found to augment antibody production to counter viral diseases like Newcastle disease (ND) and infectious bursal disease (IBD) (Murarolli et al. 2014). Another study was conducted in turkey poults by feeding *Echinacea purpurea* and protexin® probiotic to know the mucosal immunity against NDV. Results indicated probiotic fed poults had better mucosal immunity against ND (Tolouei et al. 2017).

Conclusions and future prospects

Usage of probiotics leads to many health and production benefits in poultry. Definitely, they will serve as the best alternative option for antibiotics in poultry industry. Immunomodulatory benefits are additional advantages for safe meat and egg production along with economic benefits due to prevention of bird loss or treatment expenditure. However, we also see variations in the findings of researchers with some claiming no extra benefit of probiotic usage. Important issue to address in this direction is to find the optimal dose and correct strain of probiotic microorganism for certain required action. Improvement in their form and delivery methods will also help in achieving maximum potential of probiotics. Further area of benefit can also be explored which can be achieved through their potential use with more clinical trials and promising research results. Moreover, better understanding of their mechanisms of action will help in gaining extra advantages from them. Although significant amount of work is available showing positive impact of using probiotics in feed on poultry production, still more research is needed to come out with some standard protocol for their application.

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Compliance with ethical standards

Competing interests The authors declare that they have no competing interests.

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