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Review on the use of probiotics in poultry production (Layers and broilers) as feed additives

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Abstract
In this paper, recent developments in the use and mechanism of probiotics (direct-fed microbial) in poultry are reviewed. Probiotics have been shown to enhance growth performance and feed conversion in broilers as well as egg mass, egg weight, and egg size in layers when added to the diet. The mode of action of probiotics in poultry includes maintaining normal intestinal microflora through competitive exclusion and antagonistic interactions; (ii) altering metabolism by increasing digestive enzyme activity and decreasing bacterial enzyme activity and ammonia production; (iii) improving feed intake and digestion; and (iv) neutralising enterotoxins and enhancing the immune system. Nutritional benefits include higher laying and egg quality, greater daily increments, and improved feed conversion ratio in flocks treated with probiotics (FCR). The quality of meat has also increased. This demonstrates how employing probiotics can improve production outcomes for producers. Poultry immunity is enhanced in addition to these advantages for production by giving the organism the ability to better protect itself against illnesses and stress. The versatility of probiotics and the fact that the bacteria used in their production are an essential component of animal digestive tracts make them safe feed additives, but more research is needed to address the lack of accuracy in the formulation of non-European preparations due to unknown interactions between probiotic bacteria strains as well as their metabolites.

Keywords: Probiotics, poultry, laying eggs, meat, health status

Introduction

History of Probiotics

The first investigator in the area of fermentation and probiotics was Elie Metchnikoff, who worked at the Pasteur Institute in Paris. He reported the existence of increased human longevity by drinking large amounts of soured milk in Bulgarian peasants. This strengthened Metchnikoff's belief that the lower gut and overall health would be affected by microbes from the soured milk. Following this realization, he tested cultures of milk that were fermented by the *Lactobacillus* genus. For instance, *Lactobacillus* bulgaricus later became the strain popular for fermenting yoghurt (Forkus *et al*., 2017; Fuller, 1992; Ran *et al*., 2019) [5, 6]. Probiotics are considered to be live microbial feed supplements that can benefit the poultry, otherwise known as the host. The word “Probiotic” means “for life” and originated from the Greek language (Fuller, 1992) [6].

Other sources have broken the word down more extensively, stating the pro stem is of Latin origin, meaning in favour of, the bios portion which means life, was derived from the Greeks word (Morelli & Capurso, 2012) [9]. The meaning of probiotics has changed over the years (Fuller, 1992) [6]. In 1953, Werner Kollath offered the scientific community the term “probiotic”. His definition of the term stated live micro-organisms are essential for the healthy development of the gut for life.

In 1965, Lilley and Stillwell redefined probiotics, they described probiotics as microorganisms that would aid in the growth of other beneficial microorganisms in the gut (Vila *et al*., 2010) [32].

They are given the majority of the credit for today's meaning, this definition caused it to have the opposite meaning of today's antibiotic (Fuller, 1992) [6]. Antibiotics inhibit the growth of bacteria by introducing a chemical substance (Wang *et al*., 2019) [17].

Morelli and Capurso (2012) [9] described probiotics as the consumption of sufficient live micro-organisms with the Ability to contribute health benefits to the host. This added even more to the refinement of the term probiotic.

The effectiveness of probiotic supplementation can be attributed to the species of microbes and the form of supplementation used, such as wet or powdered (Food and Agricultural Organization and World Health Organization, 2001) [48].

Overview of probiotics in poultry production

Probiotics are feed additives that have gained popularity in poultry production following the ban on antibiotic growth promoters (AGP). They are one of the more universal feed additives and can be easily combined with other additives. Probiotics, above all, have many advantages, including stimulation of the host microflora or immunomodulation. The statement "immunity comes from the intestines" has become more important in the poultry industry because probiotics have proven helpful in the fight against diseases of bacterial origin and against zoonoses. Positive effects on the organism have already been studied at the cellular level, where probiotics were responsible for changes in gene expression, leading to the alleviation of heat stress. In addition to the health benefits, the utility value of the animal's increases in both production and health, and probiotics have improved the taste and quality of poultry products. Future prospects are promising as scientists are working to maximize the positive effects of probiotics by increasing the integrity of probiotics within the bird organism, taking into account, among others, bacterial metabolites. (Huque *et al*., 2018) [15].

Thirty probiotic preparations are currently registered in European Union. It is allowed to use preparations composed of several bacterial strains (Patyra *et al*., 2015) [33]. The most common types of microorganisms used to make probiotics are bacteria such as *Bifidobacterium* spp., Lactococcus spp., *Lactobacillus* spp., *Bacillus* spp., *Streptococcus* spp, as well as yeasts, such as *Candida* spp, the probiotic compositions of the present invention use isolates from probiotic bacteria that are designed to produce enzymes or substances that activate phytases, cellulase proteases or xylanases. Modern probiotics undergo a granulation process, which is a process that uses temperatures that are unfavourable to the bacteria. Therefore, for the production of these feed additives, for example, *Bacillus* spp. producing spores, thanks to heat-resistant spores, the probiotic does not lose its properties, and this makes it possible to create feeds with added probiotics, which are also produced using a granulation process (Ellez *et al*., 2018) [47].

Administration of Probiotics on poultry

The most common method of administering probiotics on poultry farms is to add them to feed, while there are many other methods, such as gavages (vaccines or drops), sprays, granules, tablets, coated capsules, or sachets of powder. In addition to inflicting probiotics in the feed, growers are also opting to administer formulations in the water (Jiang *et al*., 2017) [11]. Each strategy has a different path to a common goal-the pathogen. The routes of action are explained in Figure 1.

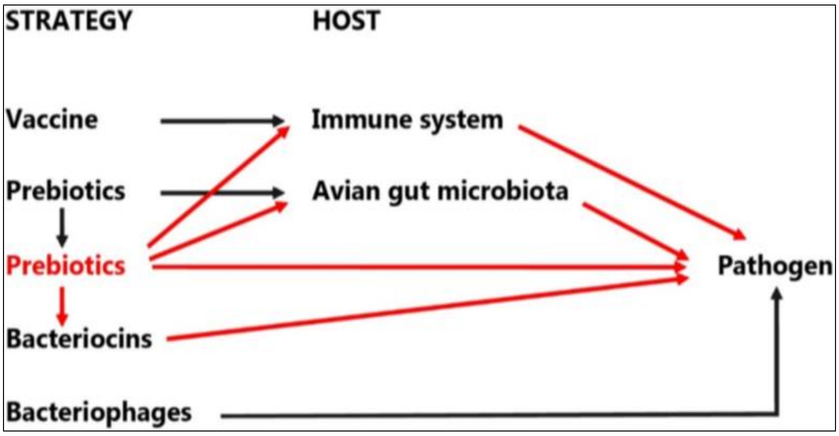

Fig 1: Potential pathways of the strategies in progress to reduce avian gut pathogens. Red arrows represent probiotic pathways (Haddad *et al*., 2016) [3].

Modes of action of probiotics in poultry production

The beneficial effects of probiotics are mediated by their mechanism of action through which they inhibit the growth and proliferation of pathogenic bacteria. The most common manner of inhibition is by lowering the pH of the gut, during *in vitro* studies it was found that primary metabolites, such as organic acids and hydrogen peroxide, are involved in the suppression of bacterial cultures, later volatile fatty acids (VFAs) were found to be equally effective in the suppression of pathogenic gut flora (Chichlowski *et al*., 2007) [1]. Probiotics produce VFAs and organic acids as part of their natural breakdown and metabolism of nutrients in the gut digests. These organic acids lower the pH below that essential for the survival of pathogenic bacteria such as E. coli and *Salmonella* spp. It is now well established that the observed beneficial effects of probiotics are accomplished via lowering the pH through the production of VFAs which inhibit the growth of harmful bacteria (Chichlowski *et al*., 2007; Choudhari *et al*., 2008) [1, 35], another mechanism is through the competition for adhesion sites on the intestinal epithelium, thus preventing colonies of pathogenic bacteria from forming. This competitive exclusion' of harmful bacteria is achieved through colonization of favourable sites of adhesion such as the intestinal villus and colonic crypts, or excretion of the mucins (MUC2 and MUC3) from goblet cells which inhibits the adherence of enter pathogenic bacteria (Chichlowski *et al*., 2007) [1]. Competitive exclusion via probiotics depends upon the ability of the strain to adhere to the gut surface which is a host-specific phenomenon and varies from strain to strain within the same species. Lactic acid bacteria are well known to colonize the caecal wall in the chicken and their competitive exclusion effect has been explained (Yoruk *et al*., 2004) [18]. This stresses the point that a strain adhering well to the gut should be chosen while selecting a probiotic.

Another important mechanism involved in producing beneficial impacts on the host's body is the stimulation of the immune system. An accumulated body of evidence has shown that the protective effect of probiotics is associated with elevated humoral and cellular immune responses, which is achieved through increased production of T lymphocytes, CD + cells and antibody-secreting cells, expression of pro- and anti-inflammatory cytokines, interleukins, IFN-gamma, natural killer cells, antibody production, respiratory burst in macrophages and delayed-type hypersensitivity reactions (Musa *et al*., 2009; Ohashi and Ushida, 2009) [10, 36]. Another mode of action of probiotics is lowering the activities of the intestinal and faecal β-glucosidase and β-glucuronidase bacterial enzymes. These enzymes are involved in the formation of toxins in the body. The *Lactobacillus* culture may reduce β-glucosidase and β-glucuronidase activities by attaching themselves along the chicken intestine, thus preventing colonisation of the bacteria with toxicant-promoting enzymes (Jin *et al*., 2000) [19].

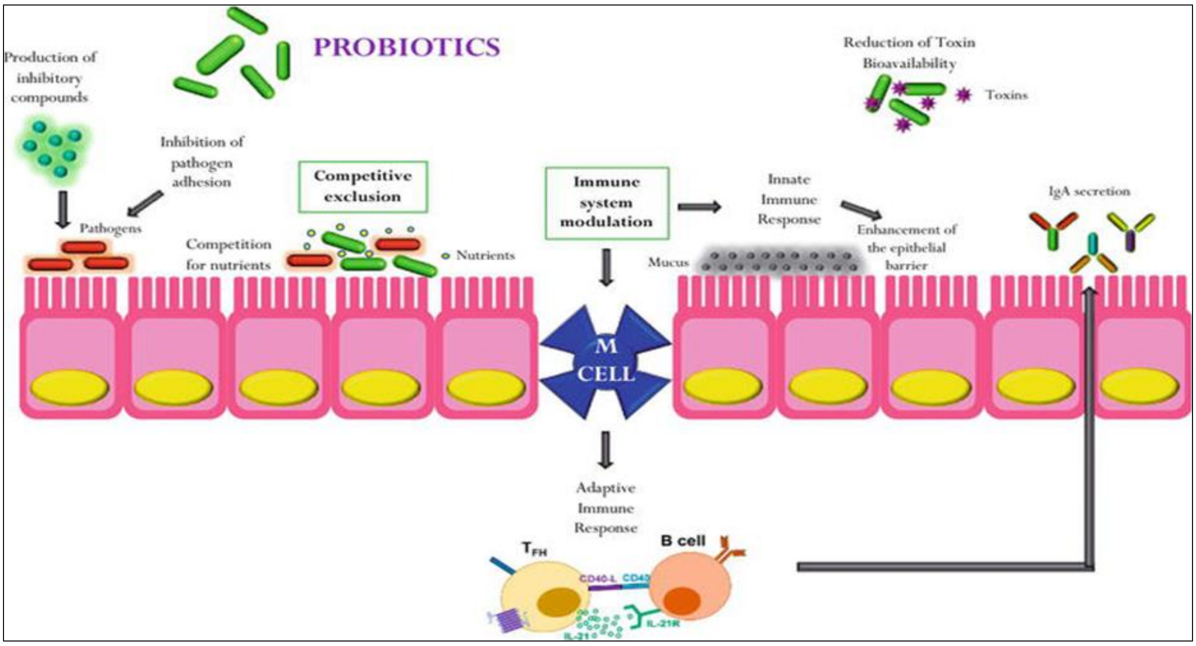

Fig 2: Mechanism of action of probiotics

Probiotics in Broilers

The addition of either pure lactobacilli cultures or mixtures of lactobacilli and other bacteria in broiler diets has produced variable results. Kim *et al*. (2018) [20] showed that supplementation of a commercial probiotic (L . sporegenes) increased the weight gain of chickens given a diet containing 10% mouldy maize at 2 or 6 weeks of age. Consistent improvements in body weight gain of chickens fed a culture of L. sporegenes, have also been reported by Kalbande *et al*. (1992) [8]. Jin *et al*. (1996a) [38], using 200 10-day-old Arbor Acres broiler chicks under a hot and humid environment, found that the weight gain in broilers given feeds incorporated with commercial lactobacilli was significantly higher than that of the control birds ($p<0.05$). Those fed lactobacilli in the feed had a significantly lower feed-to-gain ratio ($p<0.05$). Using adherent *Lactobacillus* cultures isolated from the intestine of chickens, addition to the feed from 0 to 6 weeks of either a single strain of *L. acidophilus* I 26 or a mixture of *Lactobacillus* significantly improved body weight and feed to gain ratios of broilers ($p<0.05$). Jin *et al*. (1997a) [21] also found that the highest growth rate was obtained when broilers were fed a concentration of 0.1% *Lactobacillus* cultures. Yeo and fim (1997) [39] reported that feeding a diet containing probiotics (*L. cusei*) significantly increased average daily weight gain during the first 3 weeks ($p<0.05$) but not during weeks 4-6 of growth. They pointed out that the increase was partly accounted for by increased feed intake.

Effects of probiotics on meat quality

There is widespread agreement that probiotics supplementation could improve the meat quality of broilers. Intramuscular lipid content is involved in determining meat quality particularly nutrition, tenderness, odour, tastes and flavour characteristics. Endo and Nakano (1999) [37] reported a greater tendency of a higher ratio of unsaturated fatty acid to saturated fatty acids in pectoral and thigh meat of broilers fed with probiotics supplemented diet containing *Bacillus*, *Lactobacillus*, *Streptococcus*, Clostridium, Saccharomyces and *Candida*. The results suggested that the fat in meat was converted into favourable fat in the presence of probiotics, which in turn contributed to smoother meat texture. Improved tenderness which was indicated by decreased shear force was reported by Yang *et al*. (2010) [16] when probiotic C. butyricum was added in the diet of broiler, the decrease in shear force observed in the study was positively correlated with increased muscular fat content, the same study also demonstrated that probiotic inclusion in broiler diet modulated fatty acid composition in breast meat of broiler by increasing omega-3 fatty acids concentration especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) while contents of omega-3 fatty acids in the meat of control broilers remain relatively low. Sensory assessment on chicken meatballs conducted by Mahajan *et al*. (2000) [22] revealed that overall organoleptic scores in terms of appearance, texture, juiciness and overall acceptability were higher in probiotic *Lactobacillus* fed broilers than counterparts fed with a traditional basal diet.

Evaluation of the effects of probiotics on the skin colour of broilers elucidated that probiotic *Lactobacillus* saliva could increase xanthophyll accumulation in tissue, thus improving the visual appearance of meat products (Zhu *et al*., 2009) [10]. Meat in broilers fed with probiotics *Lactobacillus* acidophilus, *Lactobacillus* casei, *Bifidobacterium bifidum*, *Aspergillus oryzae*, *Streptococcus* faecium and *Torulopsis* sp displayed higher content of moisture, protein and ash compared to the control (Khaksefidi and Rahimi, 2005) [23]. The results indicated that chicken fed with probiotics has better retention of minerals especially phosphorus, calcium and nitrogen as well as protein efficiency ratio. A higher protein efficiency ratio may subsequently help promote meat yield as observed by Hossain *et al*. (2012) [2] where the addition of probiotics increased breast meat absolute and relative weight. Besides, the carcass quality of broilers was also reported to be improved by probiotics with a lesser occurrence of *Salmonella* contamination.

Probiotics in layers

Egg production supplementation of probiotics in the diet of laying chicken favours egg production performance either by increasing egg number or maintaining production. Some probiotic preparations are also effective in adverse or stressful conditions and are supposed to have no detrimental effects when used in the appropriate amount. Research findings showed the hat use of probiotics in layer diets enhanced egg production (Ribeiro *et al*., 2014; Peralta-Sánchez *et al.*, 2019) [3, 40]. Deviations from these observations also exist, but in many cases, it affects the production positively or at least maintain production as usual. The inclusion of *Lactobacillus* acidophilus (109 cfu/kg feed) significantly enhanced the number of eggs (Abbas *et al*., 2020) [26]. A combination of *Lactobacillus* spp. and *Bacillus* spp. as liquid probiotics mixed culture when fed to the ISA Brown layers showed an increase in egg production (Raka *et al*., 2014) [41]. Abdelqader *et al*. (2013a) [42] showed that feeding of *Bacillus* subtilis (maximum 2.3×108 cfu/g) had significantly better egg production compared to control at doses of 1g/kg feed and 0.5 g/kg feed for a duration of 10 weeks during late production period starting from 64 weeks. Results of a study by Khan *et al*. (2020) depicted that the addition of probiotics (2×109 cfu/g) to the layer diet improved egg production. The probiotic organisms used in the trial were *L. plantarum*, *L. bulgaricus*, *L. acidophilus*, *L. rhamnosus*, *Bifidobacterium bifidum*, and *Streptococcus* thermophiles. Khatun *et al*. (2020) conducted an experiment with three concentrations of dietary probiotic (0, 200 and 400 g/t feed) containing 4×109 cfu/g of B. subtilis involving three different breeds (White Leghorn, Saudi black and Saudi brown). They found statistically no significant difference in egg production performance among dietary treatments. Martinez conducted an experiment using S. cerevisiae fermentation product at a dose of 1.25 g/kg where they found no differences in egg production of the Hy-Line commercial layer (Ray *et al*. 2022) [31].

Probiotics increase egg production, improve egg quality and decrease egg contamination (Haddadin *et al*., 1996; Kurtoglu *et al*., 2004; Van Immerseel *et al*., 2006) [43-45]. Further, it has been reported that probiotics increase egg shell weight, shell thickness and serum calcium in layers (Panda *et al*., 2003) [12]. Balevi *et al*. (2001) [46] found that commercial probiotic (Protexin™) supplemented diets resulted in a decreased broken egg ratio in layers. Yoruk *et al*. (2004) [18] found that egg production increased linearly with increasing levels of probiotics (Lactobacilli spp. + *Enterococcus faecium* + *Bifadobacterium bifidum* + *Aspergillus oryza*) during the late laying period in layers. Panda *et al*. (2003) [12] reported no improvement in FCR, however, increased egg production, shell weight, shell thickness and serum calcium were recorded by feeding a *Lactobacillus*-based probiotic at the rate of 100 mg/kg and 200 mg/kg of feed, although there was no significant difference between the two treatments. In the same experiment, the eggshell and serum calcium improved significantly. Panda *et al*. (2008) [12].

Reducing foodborne bacteria

Intensive genetic selection in broilers and layers in recent years for high-performance traits has resulted in increased susceptibility to infectious diseases. Poultry meat has been associated with the transmission of enteric pathogens, including *Salmonella* and Campylobacter spp (Cox and Pavic, 2009) [14]. Callaway *et al*. (2008) [24] stated that the 'link between human *Salmonella* and host animals is most clear in poultry' and that raw eggs and undercooked poultry are considered to be hazardous. Eggs have been implicated as vehicles in numerous outbreaks of *Salmonella*; in particular, egg the s are the major vehicle of transmission of strains of *Salmonella* enteritis's (Cox and Pavic, 2009) [14]. Probiotics have been extensively used to control pathogenic *Salmonella* in chickens to reduce mortality. *Salmonella* is one of the most important food-borne zoonotic diseases around the world (El-Ghany *et al*., 2020) [25]. *Salmonella* spp. contamination of poultry products primarily originates from the GIT of poultry, specifically the caeca, where there is high microbial activity. To produce *Salmonella*-free meat and eggs.

Probiotics and Their Relationship to Enzymes

Enzymatic production by different strains of bacteria has caused rapid growth and advancement in the field of probiotics. *Bacillus licheniformis* strains have been heavily used in the industry because of its ability to produce amylase, alkaline, protease, and keratinise (Qattan *et al*., 2020) [27] A study conducted by Sohail *et al*. (2010) [28] utilized 250 broiler chicks under either thermoneutral (TN) conditions or heat stress (HS) conditions. The birds were divided into five groups, and after day 21, HS was administered for up to 42 days to some of the treatments. The treatments included the TN basal diet, the HS basal diet, a HS basal diet supplemented with 0.5% MOS, the HS basal diet supplemented with 0.1% PM (Probiotics International consisting of *Lactobacillus plantarum*, *Lactobacillus* acidophilus, *L. bulgaricus*, *Lactobacillus rhamnosus*, *Bifidobacterium bifidum*, *Streptococcus thermophilus*, *Enterococcus faecium*, *Aspergillus oryzae* and *Candida* pintolopesii, with a minimum combined total of 6 × 10 7 colony-forming units cfu/g of product) or the HS basal diet supplemented with a combination of the probiotic. The TN, basal treatment was significantly the highest for the paraoxonase enzyme ($p<.05$) compared with all the HS treatments. However, the probiotic mixture and symbiotic group were numerically the highest in paraoxonase when compared back to the prebiotic and basal HS treatments. Still, it was not completely effective in increasing all enzyme levels measured. The study also measured total probiotics (Protein) resulted in the highest intestinal weight, but also the highest intestinal length in broilers reared to 42 days of age. However, a combination of antibiotics (Flavomycin) and organic acids (Genex) resulted in the highest numerical increase for performance (BW gain, FI and carcass weight). The study also found that carcass yield, liver weight and intestinal pH were not significant across all treatments (Denli *et al*., 2003) [4]. The numerous advantages are overshadowed by a few drawbacks, which include the possibility of lowering semen quality in roosters and the diversity of production processes affecting the persistence of the probiotic.

Conclusion

The beneficial effects of probiotics are the result of improved immune function, better feed utilisation, absorption of nutrients, resistance to infectious bacteria and beneficial changes in the intestinal architecture. As such, probiotics may serve as alternatives to growth-promoting antibiotics. Although the intake of probiotics has been associated with many beneficial effects in poultry production, the exact mechanism through which they produce these beneficial effects is still not completely understood. Moreover, this review paper shows that there is no consensus on the exact dose or type/blend of probiotic (s) to be fed and the duration of feeding. Future research should be focused on the exact mechanism of action of these probiotics through which they produce their beneficial effects, as well as their exact dose, type and duration of feeding.

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