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Probiotics in Broiler Diet: Potential Implications and its Microbial Burden on Meat Yield and Quality - A Review

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ABSTRACT

The objectives of this paper are to review the use of probiotics in broiler diets and its implication on yield and quality and microbial burden of chicken meat. The use of antibiotics as growth promoter for poultry production has been banned in many countries, which in turn comes hand in hand with their prohibition as possible protective agents against infectious diseases and subsequently an increased economic loss for the poultry industry. Nowadays, the use of probiotics as substitute for antibiotics in poultry production has rapidly grown and become an area of great interest, enhancing the performance of broilers and making the products free of any probiotic trace. Probiotics are living microorganisms that can improve chicken health when provision in the diet and can balance the intestinal flora and improving nutrient digestion and absorption; and improve meat yield and quality. Most commonly used probiotics bacteria in poultry production are Lactobacillus, Bifidobacterium and Streptococci species. Recent evidence suggests that the application of probiotics in the feed of broilers can lead to positive outcomes such as increased weight and improved feed conversion ratio, improved intestinal micro flora. Probiotics help in improving digestion, nutrient metabolism and utilization of nutrients by sourcing digestible proteins, vitamins, enzymes and other important co-factors and by decreasing gut pH. On the basis of numerous studies, addition of probiotics to the diets of broiler chickens improved carcass yield and quality, where the dressing percentage was higher for birds fed on diets containing probiotics. Some authors observed higher protein content and lower fat content of breast meat in birds fed probiotic containing diet. Based on different research reports, probiotics decrease the cholesterol content in meat as well as serum. Many research reports found higher antibody titers against influenza disease, infectious bursal disease and Newcastle disease virus in broilers fed diet contained probiotics compared with the controls. According to some authors report the microbiological status of chickens carcasses fed the diet with probiotic had lower numbers of coliforms, salmonella incidence, aerobic plate count and campylobacter than chickens fed the control diet. Moreover, past research findings have shown that the dose and frequency of administration of probiotics in broiler diet are variable depending on birds' age and the action of probiotics. Therefore, the use of probiotics in broiler diet has both meat quality and safety implications.

INTRODUCTION

In poultry industry, antibiotics are used worldwide to promote animal growth performance and prevent and treat diseases (Sneeringer *et al.*, 2015). However, the repeated use of antibiotics in poultry diets results in severe problems like resistance of pathogen to antibiotics, accumulation of antibiotics residue in animal products and environment, imbalance of normal micro flora, and reduction in beneficial intestinal micro flora (Barton, 2000) as well as the concern that some therapeutic treatments for human diseases might be jeopardized due to the appearance of resistant bacteria (Dale, 1992). Some consumer groups are avoiding meat from birds fed with diets containing antibiotics (Tabelas da Avicultura, 1995). Numerous alternatives to growth promoting antimicrobials have been investigated (Huyghebaert *et al*., 2011) and many researchers have tried to investigate natural feed additives alternative to antibiotics in the poultry industry in order to reduce possible harmful effects (Gubta and Da 2013). Those strategies have paying attention on preventing the proliferation of pathogenic bacteria and modulating beneficial gut microflora so that the health, immune status and performance are improved (Adil and Magray 2012).

Probiotics which are live microbial compounds are considered a good alternative to antibiotics, as their use in poultry diets has been associated with positive effects on health and growth in birds. By using probiotics, similar effects as to using antibiotics are achieved but the only difference is that the undesirable effects are avoided (residues, waiting period, resistance, allergies and genotoxicity etc) (Sinovec *et al*., 2000). They have the ability to improves gut micro flora and affect immune systems by secreting beneficial enzymes, organic acids, vitamins and nontoxic antibacterial substances upon ingestion, increase resistance to disease, counteract adverse effect of antibiotic treatment by sustaining the population of beneficial bacteria, and also in nutrient synthesis (Yirga, 2015; Upadhaya *et al.,* 2015). Various types of probiotics are confirmed for inhibiting pathogenic bacteria *in vitro* and *in vivo* through different mechanisms. Probiotics in poultry act as follows: (1) Maintaining normal intestinal micro flora by competitive opposition and exclusion, (2) Changing metabolism by increasing the activity of digestive enzymes and reducing the activity of bacterial enzyme and ammonia production (Kabir *et al.,* 2005 and Kizerwetter *et al*., 2009), (3) Improving digestion and the amount of nutrition; and, (4) activating the immune system.

Most commonly used probiotics bacteria in poultry production are Lactobacillus, Bifidobacterium and Streptococci specie. Other microorganisms such as yeast *Saccharomyces cerevisiae* and some *Escherichia coli* and Bacillus species are also used as probiotics (Khobondo *et al.,* 2015). The effect of probiotics depend on physiological state of the bird type, concentration of probiotic strain persistence of intestine, ability to survive during feed persistence of intestine, ability to survive during feed of the intestine (Mookiah *et al.,* 2014). In addition, some probiotics could produce antimicrobial substances like bacteriocins. Therefore, the potential health benefit will depend on the characteristic profile of the probiotics. Some probiotic strains can reduce intestinal transit time, improve the quality of migrating motor complexes (Husebye *et al.,* 2001) and temporarily increase the rate of mitosis in enterocytes (Banasaz *et al*., 2002 and Halvorsen *et al*., 2000). Therefore the objectives of this paper are to review the use of probiotics in broiler diets and its implication on yield and yield quality and microbial burden of chicken meat.

Over view of probiotics

Probiotics are viable bacterial or fungal which can be incorporated in animal and poultry rations to promote growth performance or reduce pathogenic bacteria, enhance the balance of intestinal flora and exercise valuable effects on the individual in which it has been administered (Collins, and Gibson, 1999). Probiotics defined by the Food and Agriculture Organization and World Health Organization as live microorganisms when administered in sufficient amounts; confer a health benefit (FAO/WHO, 2002). It can be categorized in to probiotic drugs/ probiotic foods (Food ingredients and dietary supplements) and direct feed microbial (Probiotics for animal use) (Sanders, 2009). They are either single or multiple live microbial cultures which promote health benefit to the hosts. They are nonpathogenic and nontoxic in nature, when administered through the digestive route, are favorable to the host's health (Isolauri *et al*., 2004). Probiotics are free of intermingile antibiotic resistant genes, sufficiently stable while manufacturing, processing and storage and do not undergo recombination with pathogenic strains. These live microorganisms can preferably be compatible with feed additives and have good sensory properties. Probiotics contain gram positive bacteria and must have a short generation time. They must attach to intestinal epithelium, have the ability to rapidly colonize the intestine edge of pathogenic microbes and constantly present gut micro flora (Hajati and Rezaei 2010). They have important properties like acid tolerance, bile tolerance and a strong adhesive capability with the digestive tract of the poultry (Choudhari *et al*., 2008). Probiotic bacteria produce antimicrobial substances like volatile fatty acids and bacteriocins and have ability to reduce pH that limit the growth or survival of the pathogenic microbes (Hume, 2011).

The effectiveness of a probiotic supplement depends upon what it contains. A good probiotic should have different character and functions like: adherence to host epithelial tissue, elimination or reduction of pathogen, production of acids, hydrogen peroxide and antagonism to pathogen growth, non pathogenic, non-carcinogenic and improvement of intestinal micro flora (Kaur *et al.,* 2002). There are various different methods for administering probiotic preparations to chickens. It can be given as a powder, capsule, liquid suspension and spray. The amount and interval between doses may vary. According to Chesson (1994) probiotics may be given only once or periodically at daily or weekly intervals. The way of administration and timing are main factors affecting the effectiveness of probiotic supplementation. Administration via the feed, compared to administration in the drinking water, result in a higher increase of average daily gain; besides the supplementation of probiotics during early life is of great importance to the host because harmful bacteria can modulate expression of genes in intestinal epithelial cells, so this can create a favorable habitat (Timmerman *et al.,* 2006).

Effect of probiotics on growth performances and feed utilization in broiler chicken

Many studies confirmed that inclusion of probiotics in to broiler diet improved body weight gain. Mohan (1991) reported improved growth of broilers upon feeding probiotics containing diet. Cho *et al.* (1992) reported growth promoting ability of *Lactobacillus casei* with antibiotics and observed 3.4 to 6.0% increase in body weight gain in broilers. Bhatt (1993) reported significantly higher live weight gains in broiler stock supplemented with *Streptococcus lactis* and *Saccharomyces cerevisiae*. Manickam *et al.* (1994) recorded a significant difference in weight gain between control and an experimental group of broiler when Lactobacillus sporogenes based probiotic was given at 1g per liter of drinking water for a period of 0-6 weeks. Bhatt *et al.* (1995b) studied the effect of dietary supplementation of *Saccharomyces cerevisiae* to broiler chickens and observed increased live weight gain during starter phase. Probiotics help in metabolism of minerals and synthesis of vitamins (Biotin, Vitamins-B_1, B_2, B_{12} and K), which are essential for proper growth and metabolism in chicken (Dhama and Singh (2010). The investigator found that inclusion of probiotic (Lactobacilli and *Bacillus subtilis*) in diet enhances feed efficiency and growth performance in broilers. Broilers probiotic supplemented diet had better weight gain and feed efficiency when compared to the broilers feed without probiotic supplement (Jin, *et al.*, 1996).

The use of probiotics in chicken diet has increased feed intake and/or feed efficiency (Shim *et al*., 2012). According to Larrote *et al* (2015) the Bacillus based probiotics enhanced the feed conversion ratio, as well as bird's body weights and Shareef (2009) reported that probiotic (*Saccharomyces cervisiae*) supplementation of broilers had significantly increased feed consumption. The improvement in performance and productivity of poultry production due to the use of probiotics in feed has increased feed intake and improved feed efficiency (shim *et al* 2012) and increase feed intake along with significant improvement in feed conversion ratio (Landy, and Kavyani, 2013). Shareef and Dabbagh (2009) reported that probiotic supplementation of broilers had significantly increased feed consumption. Cho *et al* (1992) reported that the supplementation of *Lactobacillus casei* improved feed conversion ratio as compared to control and treatments supplemented with antibiotics or other probiotics. Moses (1992) reported that supplementation of probiotics product Biospur in the diet of broiler resulted in improved feed conversion efficiency at 17^{th} week of age. Cavazzoni *et al* (1993) reported 6% improvement in feed conversion efficiency in broilers fed diet supplemented with probiotics (*Bacillus coagulans*) as compared to control.

Effect on carcass yield

Various reports have shown variable effects of probiotics on carcass composition of broilers. Mehr *et al* (2007) observed heavier bodyweight and carcass weights and breast percentage with higher level of probiotic supplementation compared with a lower level and the control treatment. Kalavathy *et al* (2006) reported that addition of effective microorganisms to the diets of broiler chickens improved meat yield and quality. Similarly, Safalaoh (2006) reported that dressing percentage was significantly higher for birds fed effective microorganisms. Also, Safalaoh and Smith (2001) reported that effective microorganism supplementation significantly improved the dressing percentage of broiler chickens.

Abdominal fat deposition and gastrointestinal tracts growth

Some authors have observed that probiotics supplemented diets reduce abdominal fat weight in broilers compared with the controls (Anjum *et al*. 2005; Mehr *et al.* 2007). Anjum *et al*. (2011) reported liver weight was significantly lower in birds supplemented with effective microorganisms than in those not supplemented with effective microorganisms. The authors also observed that gizzard weights did not significantly differ between effective microorganism treated and non treated broiler chickens. And intestine length was significantly lower in effective microorganism treated broiler chickens compared with the control group. Anjum *et al* (1996) found that effective microorganism treated birds had low liver weight, gizzard weight and intestine length. However, Awad *et al* (2009) did not observe differences in the weights (as a proportion of body weight) of caecum, liver, spleen, thymus and bursa of Fabricious between broilers fed a control or a probiotic supplemented diet. Other authors have also reported no effects of probiotic supplementation on lymphoid organs (Ahmadi, 2011; Naseem *et al*., 2012).

Effect on physiochemical property of chicken meat

Endo and Nakano (1999) found higher fat and lower water contents in the meat and liver, and a significantly lower cholesterol content in young broiler roosters given a feed containing a probiotics, whereas Chantsavang and Watcharangkul (1999) found significantly lower ash content and a tendency for the protein content in the breast meat. Pietras (2001) also reported meat of chickens given probiotic (*Lactobacillus acidophilus* and *Streptococcus faecium* bacteria) on the whole rearing period had significantly higher protein content, while crude fat and total cholesterol contents tended to decrease. Improved content of protein and reduced fat in birds fed probiotic containing *Bacillus subtilis* was found by (Kral *et al.,* 2013). Similarly, Hossain *et al.* (2012) observed higher protein content (25.9% vs. 23.4%), however lower breast fat content (0.56% vs. 0.24%) was reported by Abdurrahman *et al* (2016a) with probiotic inclusion in the broiler diet. Considerably reduced fat content in the meat was reported by Inatomi (2015) when broiler chickens were supplemented with a combination of three probiotics (Bacillus mesentericus TO-A, *Clostridium butyricum* TO-A and *Streptococcus faecalis* T-110). The fat content of the breast meat of the birds consumed probiotics and control group were 1.99% and 4.12%, while the content in thigh was 7.10% for the control group and 5.66% for the birds received probiotics.

The color of meat is also important trait for the consumers' perception of meat freshness and quality (Droval *et al*., 2012) which is determined by measuring its lightness, redness and yellowness (Kadim and Mahgoub 2013). Redness is most favored by consumers and lower yellowness values indicate less pale meat (Jiang *et al*. (2014). In a study by Hack *et al* (2015a) the use of probiotic in the water of broilers (*Lactobacillus fermentum*) significantly increased the redness in breast, while there was no effect on the yellowness and lightness in breast and thighs. In another study of (Hack *et al.,* 2015b), however, the probiotic in combination with bee pollen led to significant increase of redness and yellowness in thighs and also increased lightness in both breast and thigh cuts. A slightly reduced lightness in meat following probiotic supplementation was observed in chickens received *Bacillus subtilis* (Aristides, 2012) and *Enterococcus faecium* (Zheng *et al.,* 2015), while combination of *Bacillus subtilis, Clostridium butyricum* and *Lactobacillus acidophilus* (Hossain *et al.,* 2015) had stronger influence on the color of chicken meat. Improvement in color characteristics in breast meat in terms of lightness and yellowness was also reported by Abdurrahman *et al* (2016a). The same authors tested the effect of inulin and Lactobacillus at different feeding levels and found that the combination of both had led to changes in yellowness and lightness values; however, the results depended on the dose of probiotic administered.

Based on the studies, the probiotic administration in poultry showed that pH might be affected, but the results depend on the type of microorganisms and also on the specifics of the experimental design. Ivanovic *et al* (2012) studied the effect of two probiotics supplied in different amount to the diet of broiler chickens and found significant changes in the pH measured 24 hours post mortem in breast and thigh meat, which differed with the type of microorganisms used. Zheng *et al* (2015) reported significantly higher pH in breast meat both 45 min and 24 hours post mortem in broilers receiving *Enterococcus faecium,* which was accompanied by lower drip and cooking losses. Similarly, Abdullah *et al* (2015) observed a significant decrease in the pH during storage of meat from broilers fed probiotic containing diet (*Bacillus subtilis*) for 7 days.

Effect on sensory qualities of meat

Some studies indicated a positive effect of probiotics on sensory characteristics whereas other studies indicated no role of probiotics in this regard. Mahajan *et al* (2000) reported a significant increase in the score of meat quality factors including appearance, texture, succulence, and wholesomeness in broilers fed with probiotic (lactosaccharose). Similar to this report, Zhang *et al* (2005) reported that meat tenderness was increased by inclusion of whole yeast or selected *S. cerevisiae*. Contrary to this report, Loddi *et al* (2000) reported that neither probiotics nor antibiotics affect sensory properties (color and smell intensity, natural taste, tenderness, succulence, wholesomeness, color property and general properties) of the thigh and breast meat.

Jensen and Jensen (1992) studied a positive effect of probiotics containing *Bacillus licheniformis* and *Bacillus subtilis* spores on the flavor of broiler meat after cooling for 5 days. However, Loddi *et al.* (2000) reported that probiotics fed to chickens with water and feed had no effect on sensory characteristics of meat. Mahajan *et al.* (2000) observed the scores for the sensory attributes of the meat balls i.e. appearance, texture, juiciness and overall acceptability were significantly higher, but lower for flavor in the probiotic-Lacto-Sacc fed treatment. Pelicano *et al* (2003) reported that significant improvement in meat flavor fed with probiotics. A study by Ceslovas *et al* (2005) confirmed that probiotic supplementation significantly increased chiken meat tenderness and quality traits. Contrarily, Anna *et al* (2005) observed no significant difference between probiotic treated and untreated treatments on chicken meat sensory parameters.

Influence on meat cholesterol content

Research reports have shown that probiotics decreased cholesterol content of meat as well as serum. Joy and

Samual (1997) reported that probiotics treatment with 100 million organisms gives lower serum cholesterol in treated birds. Also, Lactobacillus fed birds reported significantly lower serum cholesterol at 30 day of age (Jin *et al* (1998), while Kim *et al* (2000) reported significantly lower blood cholesterol in the broiler birds supplemented with probiotics. Pietras (2001) reported that protein content of chicken given probiotics is higher, while their crude fat and serum cholesterol is lower than control treatment. Chitra *et al* (2004) also reported that supplementation of probiotics reported highly significant reduction of serum cholesterol.

Influence on lipid composition and oxidation of chicken meat

Fatty acid composition is an essential component of meat quality, linked with its nutritional value. Past researches on the influence of different probiotics on the fatty acid profile of meat were comparatively scarce, but the overall results show positive effect of the probiotics, mainly related to reduction in saturated and increase of polyunsaturated fatty acids. According to Saleh *et al.* (2012) and Saleh *et al.* (2013) feeding broilers with *Aspergillus awamori* and *Saccharomyces cerevisiae* or their combination led to significant decrease in the saturated fatty acids (C16:0 and C18:0) and increase in C18:1 as well as in the polyunsaturated C18:2, C18:3, C20:4. The same was observed when the diet of the birds contained *Aspergillus awamori* and *Aspergillus niger* in different proportions (0.01%, 0.05%, 0.1%) (Saleh *et al.,* 2011) as well as *Aspergillus awamori* in combination with selenium nanoparticles (Saleh, 2014). Hossain *et al.* (2012a) also observed increase in the C18:3 in breast and C18:2 and C18:3 in thighs with probiotic administration. However, in another experiment (Hossain *et al.,* 2012b) a reduction in the n-6 PUFA in both breast and thigh was observed. Furthermore, Hack *et al* (2014) found a slight increase in mono and polyunsaturated fatty acids while a decrease in the saturated fatty acids in broilers fed probiotics alone or in combination with pollen.

Lipid oxidation is one of the main causes for food quality deterioration. It is usually accompanied by development of off-odors and flavors. Usually the oxidation in foods, including meat is measured by the content of thiobarbituric acid reactive substances (TBARS-test). Recent research report showed either positive or lack of adverse effect of the probiotics on the lipid stability of chicken meat. Saleh *et al* (2011) reported that *Aspergillus awamori* and *Aspergillus niger* significantly decreased the content of TBARS in breast meat of broilers received probiotic and also increased PUFA and fat contents. The low lipid oxidation was accompanied with significantly increased content of muscle tocopherol in the supplemented groups. This was confirmed by other experiments (Saleh *et al.,* 2012; Saleh *et al*., 2014) in birds fed *Aspergillus awamori* alone or combined with canola seed. When using probiotic based on *Lactobacillus fermentum* in the diet, Bobko *et al*, (2015) observed reduced TBARS content in both breast and thigh meat on the first day of storage. Lower TBARS in meat due to probiotics was reported in fresh meat (Hossain *et al.,* 2015) and after 5, 7 and 14 days of storage (Hossain, 2012a; Hossain 2012b). Aristides *et al.* (2012) reported a considerably lower, but not significant, TBARS content in chicken meat with probiotic supplementation. However, Kim *et al.* (2016) did not observe any difference in oxidative stability of chicken meat as affected by dietary probiotic supplementation. It could be seen that the majority of studies on the effect of the dietary probiotics on the lipid profile and oxidation in poultry meat reported increased unsaturation fatty acids. Such results additionally confirm the fact that probiotics exhibit significant antioxidant activity observed in both *in vitro* and *in vivo* experiments (Abdurrahman *et al*., 2016b; Pieniz *et al*., 2014).

Impact on microbial burdens of chicken meat

Giannenas *et al.* (2012) did not observe differences in Lactobacilli, Enterococci and total anaerobe counts, but did observe lower *Escherichia coli* counts in the caecum of broilers fed a probiotic supplemented diet compared with the control. On the contrary, Mountzouris *et al.* (2007) reported that including probiotics in the diet of broilers caused higher concentrations of *Lactobacilli* and gram-positive cocci (e.g., *Enterococci*, *Pediococci*) in the cecal micro flora compared with the controls. Fritts *et al* (2000) reported that carcasses of birds fed diets with probiotic (calsporin) had significantly lower *salmonella* incidence and *coliforms*, *campylobacter* and aerobic plate count than birds fed on control diet. According to Khaksefidi and Rahim (2005), the microbiological status of chickens carcasses fed the diet with probiotic had lower numbers of coliforms and campylobacter than chickens fed the control diet. The authors further stated that all carcasses of chickens on the control diet were positive for salmonella, while only 16 of the 40 carcasses from chickens fed diets containing probiotics were positive.

Impact on intestinal microbiota and morphology

Studies have demonstrated the potential of probiotics to improve the beneficial bacteria and suppress potentially pathogenic bacteria in the intestine (Mountzouris *et al*., 2010). Probiotics beneficially influence the host animal by improving its intestinal balance (Fuller, 1989) and creating gut micro ecological conditions that suppress harmful microorganisms like Clostridium and Coliforms (Shim *et al*., 2010), and by favoring beneficial microorganisms like Lactobacillus and Bifidobacterium. Intestinal morphology including duodenal and ileal villus height and crypt depth as well as villus height to crypt depth ratio are indicative of gut health in broilers. Many research reports revealed that dietary probiotic administration affect villus height and crypt depth in small intestine portions (Abdel-Moneim *et al*., 2020;

Abdel-Moneim *et al*., 2019; Awad *et al.,* 2009 and Olnood *et al*., 2015). Increased villus height and villus height to crypt depth ratio are directly correlated with an increased epithelial turnover (Fan *et al*., 1997), and longer villi are correlated with activation of cell mitosis (Samanya and Yamauchi, 2002). Whereas, shortening of villi and deeper crypts lead to poor nutrient absorption, increased secretion in gastrointestinal tract and reduced performance (Xu *et al*., 2003). Abdel-Moneim *et al* (2019) and Abel-Moneim *et al* (2020) noticed significant increase in villi height of the ileum when bifido bacteria strains were inoculated in the yolk sac at 18 days of incubation while crypt depth was not affected. It seems that probiotics activate mitotic cell division and induce the proliferation of gut epithelial cells, which may explain the increase in villus length (Bai *et al*., 2013; Samanya and Yamauchi, 2002). Similarly, Awad *et al.* (2009) reported that supplementation of Lactobacillus in broiler diet increased villus height and villus height to crypt depth ratio in duodenum and decreased ileal crypt depth. Lactobacillus treatment caused similar changes in intestinal morphology of poultry (Awad *et al*., 2009). Increased villi height is linked with the increase in absorption surface area of villi, which enhance nutrient absorption and subsequent growth performance. However, increased crypt depth may reduce secretion of digestive enzymes, decrease nutrient absorption and eventually lower broilers' growth performance (Singh *et* al., 2011).

In addition to villus length, the shape and pattern of the villi are also important. Pelicano *et al.* (2005) reported that jejunal villi occur in a zigzag form, similar to a wave pattern enabling better absorption of nutrients than those present randomly or parallelly. The zigzag shape of villi reduces ingesta's rate of passage through the gastrointestinal tract compared with the straight form, which enhances the contact between absorption surface area of villi and nutrients. Waved-like arrangement of villi in broiler jejunum has been promoted by Lactobacillus-based probiotic supplement (Pelicano *et al*., 2005).

Role of probiotics in improving digestion

Probiotics help in improving digestion, nutrient metabolism and utilization by offering digestible proteins, vitamins, enzymes and other important co-factors and by decreasing gut pH by production of lactic acids. As source of 'live enzyme factory' (amylase, protease, lipase), it enhances digestion and absorption of carbohydrates, proteins and fats, which also increases the feed conversion efficiency. Probiotics help in metabolism of minerals and synthesis of vitamins (Biotin, Vitamin-B_1, B_2, B_{12} and K), which are responsible for proper growth and metabolism (Dhama and Singh, 2010). The facultative anaerobes (*Bifidobacterium* and *Ljptobacillus*) included in probiotic bacterial consortium reduce the redox potential in the gut and render the environment suitable for obligate anaerobes (Jozefiak *et al.,* 2004; Chichlowski, 2007). An increase in the digestibility of dry matter is closely related to enzymes released by yeast (Lee *et al.,* 2006).

Effect on immune response and survivability

Feeding probiotics to chickens improves antibody titers against viral diseases like Newcastle and infectious bursal diseases and reduces stress. By increasing the immune status it is possible to prevent enteric infections and help reduce the losses due to secondary infections observed in birds commonly during viral diseases or immune suppressive conditions. Naseem *et al.* (2012) and Zakeri and Kashefi (2011) found higher antibody titers against influenza disease, infectious bursal disease and Newcastle disease virus, respectively, in broilers fed Protex containing diets compared with the controls. Moreover, Rhee *et al.* (2004) and Haghighi *et al.* (2005) reported higher blood IgM against SRBC when probiotics were included in a broiler diet. However, Mountzouris *et al* (2010) failed to show improvements in the overall broiler humoral immune status at systemic level in response to probiotic supplementation.

Kabri *et al.* (2004) investigated the effects of probiotics on immune response in the body of chicks and reported a significant increase in antibody production. Dalloul *et al.* (2005) 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.* (2005) reported probiotics increased natural intestinal serum and antibodies for some external antigens in broilers.

Hussein and El-Asry (1991) found that administration of a *Lactobacillus* concentrates at 0.5g/kg starter mixture to broiler chickens decreased the incidence of diarrhea and mortality. Adsul (1993) concluded that the treatment received pure *Lactobacilli* culture showed lesser mortality. Lee *et al.* (1994) noticed that the viability of the broiler treatments given probiotics was not significantly higher than that of the control treatment.

Bhatt *et al.* (1995) observed favorable effect on the livability of broilers after addition of probiotics in the diet of broilers. Kaistha *et al.* (1996) recorded lesser mortality in broilers fed diets supplemented with *Lactobacillus acidophilus*, *Streptococcus uberis and Saccharomycs cerevisiae*. Samantha and Biswas (1997) observed that mortality was reduced in broilers fed diet supplemented with probiotics. Rajmane and Sonawane (1998) reported reduction in chick mortality from 7 to 2% with oral administered probiotics through drinking water at 20g per 1000 chicks.

Effect of dose based application of probiotics

Researchers have shown that probiotics work at different stress level with varied dose rate. The dose and frequency of probiotics administration used for therapeutic and/or growth promotion is variable. The dosage is key factor for effect of probiotics in broilers. The action of probiotics varies with the dose. Studies have been conducted to standardize the dose of probiotics in poultry. But, still this area needs much more study. Krecov and Puijic (1975) fed LBA and Streptococcus at 200 g/400 kg diet to broiler birds and observed that total body weight gain in birds fed with and without LBA were 1570 and 1545 g, respectively. A study conducted by Watkins and Kretzer (1983) that based Lactobacilli feeding to a day old broiler chickens at rate of 510, 710 or 910 CFU revealed a slightly depressed growth at dose rate of 710 CFU and above. Studies also showed that commercial probiotic consumption often increases specific intestinal micro flora. Melluzi *et al* (1986) observed birds fed with 2% of lactic acid bacteria culture had significantly higher body weight than that of control group given reconstituted sterile milk.

Probiotics work on dose wise at particular stage. Majority of research reports suggested that the specific bacteria do not increase unless subjects consume very high dosages of probiotics. Maruta *et al.* (1996) conducted an experiment with probiotics at 3 x 105 Cfu/g dose and reported that a decrease in number and detection rate of pathogenic organism. Singh and Sharma (1996) investigated the effect of feeding *Lactobacillus sporogenes* added at 0, 0.02, 0.03 and 0.04% level in broilers and reported that probiotics combination did not affect feed intake. However, the Lactobacillus feeding improved the feed conversion efficiency significantly at 0.02% level. Kumprecht and Zobac (1996) reported that probiotics having 1.5x10 res to 6 *Streptococcus faecium* C-68 in 1 g feed- 4 g/100 kg decreased the count of *Escherichia coli* in caecal contents by about 50%.

Conclusion and recommendation

Reviewed research works have shown that the application of probiotics in the diet of broilers improves feed intake, feed conversion ratio, stimulates growth rate, improves meat quality traits, health of broilers and reduce enteric pathogens in poultry industry. Probiotics are generally recognized as safe to use without any reported side effects, showing no residue carry over in meat and on human health hazards unlike the use of antibiotics as growth promoters and reduces expenditure on therapeutics.

Standardizing the application dose of various probiotics in poultry needs much more investigation. In future, research on the probiotics in broiler diets should continue in order to establish the most appropriate conditions at which the probiotics would exert their positive effect at the greatest extent in order to produce high quality and healthy meat. Moreover, further research is required on the influence of various probiotics on the fatty acid profile of meat.

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