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The Effect of Probiotic and Prebiotic Feed Supplementation on Chicken Health and Gut Microflora: A Review

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Abstract: Commercial poultry production is ranked among the highest source of animal protein in the world. Microbial infections caused by bacteria (*Salmonella*, *Clostridium perfringes*) and parasites particularly *Eimeria* has continued to challenge the poultry industry. Antibiotic Growth Promoters (AGP) have been traditionally used to counter microbial infections in poultry. But due to public health concerns, the use of AGP in poultry is either restricted or out rightly banned in several countries. Hence, this review is aimed at highlighting alternative feed supplements that can enhance performance and protect the chickens from microbial infections. The study found that dietary supplements containing probiotic, prebiotic and enzymes are able to enhance performance while protecting the chickens from microbial infection.

Key words: *Bifidobacteria*, *E. coli*, *Eimeria*, fungi myceliated grains, gut microflora, *Lactobacillus*, microbial infections, prebiotics, probiotics, *Salmonella*

INTRODUCTION

The biggest challenge of commercial poultry production is the availability of good quality feed on sustainable basis at stable prices. In spite of this challenge, commercial poultry production ranks among the highest source of animal protein (Iyayi, 2008). The increase in the size of the poultry industry has been faster than other food-producing animal industries. The trade volume of poultry products has also increased parallel to the rapid growth of global poultry meat and egg production (Windhorst, 2006). Available data indicate that the poultry meat industry has been more dynamic compared with the egg industry over these years (Windhorst, 2006). Feed is the major component of the total cost of production for meat and egg production in the poultry industry.

With improved stock, broiler birds can attain a weight of 2-3 kg within five to six weeks. However, this production capacity is subject to availability of good quality feed and disease control. With the current advent of excluding antibiotic growth promoters in poultry production in Europe and America, the issue of controlling enteric infections caused by pathogenic bacteria without the use of antibiotics becomes challenging. Mortality caused by infection is a major problem in the poultry industry. Such infections are responsible for reduced growth rates and consequent economic losses in poultry. Antibiotics are the main tools utilised to prevent or treat such infections. In animals, antibiotics are also added to the feed as growth promoters and to accelerate the growth of healthy animals. Unfortunately, the long term and extensive use of antibiotics for veterinary purpose may eventually result in selection for the survival of resistant bacteria species or strain (Aarestrup, 1999). Genes encoding for this resistance also can be transferred to other formerly susceptible bacteria, thereby posing a threat to both animal and human health (Montagne *et al*., 2003). Consequently, some countries have banned (Sweden-January 1986) or limited (European union-January 2000, total withdrawal January 2006) the general use of in-feed antibiotics as growth promoters in animals (Montagne *et al*., 2003).

Supplementing the feed of food animals with Antibiotics Growth Promoters (AGP) helps to increase performance and to control diseases. Various mechanisms by which AGP acts have been proposed. Firstly, the nutrients are more efficiently absorbed and less are utilised by the gut wall due to a thinner epithelium. Secondly, more nutrients are available to the host because of a reduced intestinal microflora. Thirdly, there is a reduction in harmful gut bacteria which may reduce performance and cause sub clinical infection. Fourthly, production of growth suppressing toxins or metabolites is reduced. Lastly, microbial de-conjugation of bile acids is reduced. But with the aforementioned limitation of AGP the consequent need for their total withdrawal becomes necessary. Hence, this review is aimed at highlighting alternative feed supplements that have probiotic effects

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and promote growth of broiler chickens, thus achieving both enhanced performance and good health even without the use of antibiotics. In order to find better alternatives to AGP, research has focused on utilization of feed additives such as enzymes, probiotics, prebiotics, symbiotic products and even nutrition to enhance gut health in poultry and prevent or limit production losses due to enteric infections.

Poultry nutrition: Feed is probably the most important entity in the poultry industry that can expose the birds to a wide variety of factors through the gastrointestinal (GI) tract. Intake of exogenous feed is accompanied by rapid development of the GI tract and associated organs. The timing and form of nutrients available to chicks after hatch is critical for development of intestines. Early access to feed has been shown to stimulate growth and development of the intestinal tract and also enhance post hatch uptake of yolk by the small intestine (Uni *et al*., 1998; Geyra *et al*., 2001; Noy and Sklan, 2001; Noy *et al*., 2001; Potturi *et al*., 2005). Birds show slower intestinal development and depressed performance when access to feed is delayed (Corless and Sell, 1999; Vieira and Moran, 1999; Geyra *et al*., 2001; Bigot *et al*., 2003; Maiorka *et al*., 2003; Potturi *et al*., 2005). Such lack of access to feed leads to a depression in intestinal function and bird performance, which may not be overcome at later stage in life (Uni *et al*., 1998; Geyra *et al*., 2001; Bigot *et al*., 2003; Potturi *et al*., 2005). Development of the GI tract may affect the immune status of the bird at early stage in life as it is also the largest immune organ in the body (Kraehenbuhl and Neutra, 1992). Thus, anything that affects the health of the gut will undoubtedly influence the animal as a whole and consequently alter its nutrient uptake and requirements. Diet has significant effect on the immune status as well as overall performance of poultry birds. This can be induced by the presence of soluble or insoluble Non-starch polysaccharides (NSPs) (Iji, 1999; Choct and Annison, 1992a,b; Bedford and Schulze, 1998; Almirall *et al*., 1995; Bustany, 1996; Choct *et al*., 1996, 1999a, 1999b; Jorgensen *et al*., 1996; Leeson *et al*., 2000; Mathlouthi *et al*., 2003; Wu *et al*., 2004). Physical structure (Brunsgaard, 1998; Engberg *et al*., 2004) and form (Hetland *et al*., 2002; Yasar, 2003; Engberg *et al*., 2004; Taylor and Jones, 2004; Bjerrum *et al*., 2005) of the diet. Not only is the gut the major organ for nutrient digestion and absorption, it also functions as the first protective mechanism to exogenous pathogens which can colonize and/or enter the host cells and tissues (Mathew, 2001). As previously stated, the gut is also the largest immunological organ in the body. Thus, it is often implied that a more robust gut will make a healthier animal, which, in turn, digests and utilizes nutrients more efficiently. This link between enzyme activities, gut weight and growth performance has been elucidated by Hetland and Svihus (2001) and Hetland *et al*. (2003). Invariably the various alternatives to AGP as well as means of enhancing performance in poultry while reducing economic losses due to enteric infections is directed majorly at the gut which functions for nutrient digestion and absorption as well as immunological organ. Other feed additives such as probiotics, prebiotics and enzymes can modulate the gut microflora and performance of broiler chickens (Choct, 2009).

Microbial probiotics and other feed supplements:
Probiotics: Probiotics are mono-or mixed culture of living microorganisms, which induce beneficial effect on the host by improving the properties of the indigenous microflora (Ghadban, 2002). Killed bacterial cultures as well as bacterial metabolites have been included in the definition of probiotics (Reuter, 2001). A typical example of probiotics is *Lactobacillus* spp. Poultry feeds containing probiotic microbes are increasing being considered as feed supplement in poultry diets. Willis *et al*. (2011) reported that most medicinal mushrooms contain biologically active substances such as polysaccharides, glycoproteins and other macromolecules, which can serve as good dietary supplements and immuno-modulating agent. The preventive effect of probiotics against *Salmonella* has been reported (Pascual *et al*., 2001). Probiotics have been reported to have favourable effects on performance (Santin *et al*., 2001). The beneficial effect of probiotics is based on their ability to modify the gut microflora. This necessitates that the microorganisms reach the gut in a viable form. The use of treatments such as coating and absorption into globuli has been reported to improve the stability of probiotics (Simon, 2005). The mode of action of probiotics includes; competitive exclusion (Jin *et al*., 2000; Alexopoulos *et al*., 2004; Berchieri *et al*., 2006), microbial antagonism (Conway, 1996; Kelly and King, 2001; Walsh *et al*., 2004; Mountzouris *et al*., 2006) and immune modulation (Cebra, 1999; Perdigon *et al*., 2001; Lan *et al*., 2005).

Several microorganisms have been considered or used as probiotics including fungi particularly mushroom and yeast, bacteria and mixed cultures comprising of various microbes. Willis *et al*. (2008, 2009a, 2009b 2010a, 2010b, 2011) consistently used Fungi Myceliated Grains (FMG) colonized by the edible shiitake mushrooms, *Lentinula edodes* as probiotic for broiler chicken. Ogbe *et al*. (2009) used wild mushroom, *Ganoderma lucidum* for the treatment of *Eimeria tenella* infected chickens. Woo *et al*. (2006) used the probiotic yeast (*Saccharomyces cerevisiae*) and fungi (*Aspergillus oryzae*) for the control of pathogenic bacteria infection in chickens. Similarly, Lee *et al*. (2007a, b) used the probiotic yeast *Saccharomyces boulardii* for the treatment of *Eimeria* infected chickens.

Bacteria are more commonly reported as probiotic than fungi. Two genera of bacteria are mostly reported including lactic acid bacteria of the genus *Lactobacllus* (Sato *et al*., 2009; Taheri *et al*., 2009; Yegani and Korver, 2008; Dalloul *et al*., 2003, 2005; Higgins *et al*., 2008; Haghighi *et al*., 2008; Lee *et al*., 2010) and *Bifodobacteria* (Willis *et al*., 2010a, b; Patterson and Burkholder, 2003). Other bacteria that have been reportedly used, though to a lesser extent in poultry and animal probiotics include *Bacillus*, *Enterococcus*, *Streptococcus*, *Lactococcus*, *Pediococcus* etc (Lee *et al*., 2007a, 2007b, 2010; Patterson and Burkholder, 2003). Microbial probiotics are commonly administered to animals orally either through the feed or drinking water.

Prebiotics: While the life microbes have been increasingly used as probiotics in animal nutrition and health, the macromolecules synthesized by some microorganisms are increasingly been used as prebiotics (Patternson and Burkholder, 2003) especially for immune system modulation. Prebiotics are defined as food ingredients that stimulate selectively the growth and activity of beneficial microorganisms such as *Bifidobacteria* and *Lactobacillus* in the gut and thereby benefit health (Cummings and MacFarlane, 2002). In addition, prebiotics can reduce the numbers of *clostridia* and increase colonisation resistance to pathogens. Prebiotics are assumed to be non-digestible by human or animal digestive enzymes. Thus they can serve as substrate for beneficial bacteria mainly located in the hind gut. Prebiotics may enhance the digestibility and performance parameters by creating the favourable conditions for beneficial bacteria (Steiner, 2006). Several carbohydrates that may be fermented by intestinal microorganisms can be classified as prebiotics (Bauer *et al*., 2006); including NSPs, resistant starch and non-digestible oligosaccharides. Inulin and fructo-oligosaccharides are widely used as prebiotic feed additives (Steiner, 2006). Due to the absence of suitable gastrointestinal enzymes, prebiotic carbohydrates cannot be digested by nonruminants. However, they are exclusively fermented by beneficial bacteria such as *Lactobacillus*, *Bifidobacteria* and *Bacteroides*, thereby having the potential to modulate the composition of microbial communities in the gut (Le blay *et al*., 2000; Mosenthin and Bauer, 2000; Xu *et al*., 2003; Zhan *et al*., 2003; Chen *et al*., 2005). Yang *et al*. (2008) studied the effect of mannanoligosaccharide and fructooligosaccharide on the response of broilers to pathogenic *E. coli* challenge. Patterson and Burkholder (2003) reported that fructo-oligosaccharide products such as inulin, fructooligosaccharide and oligofructose are the dominant prebiotics in use today. However, other macromolecules are increasingly being investigated for their prebiotics activities including mannan oligosaccharides, transgalactooligosaccharide, gluco-oligosaccharide, xylo-oligosaccharide, glycol-oligosaccharide, lactulose, lacttitol, maltooligosacharide, stachyose, raffinose, sucose etc. (Patterson and Burkholder, 2003).

Symbiotic products: These are additives that contain probiotics and prebiotics. Symbiotic products contain viable bacterial cultures that establish early in the gut while the prebiotic present in them serve as a source of nutrient for the probiotics in addition to dietary sources. Some of these products have already penetrated the market (Mohnl *et al*., 2007; Zhang *et al*., 2006).

Enzymes: Important effects of supplementing enzymes include: improved digestibility of nutrients, reduced small intestine fermentation and increased caecal fermentation (Choct *et al.*, 1999a, b). The increased microbial activity in the caeca is likely a result of poorly absorbed products of enzymatic degradation entering the caeca where they stimulate bacterial fermentation (Bedford, 2000). This aspect of enzyme activity may resemble the mode of action of prebiotics. The possibility of producing enzymes targeted at specific results has been reported (Choct, 2006). These include: Enzymes tailored for the generation of specific low molecular weight carbohydrates *in vivo*, which, in turn produce specific health outcomes in the birds. Enzymes targeted at de-activation of anti-nutrients other than Non-starch polysaccharides (NSPs) and phytate. Enzymes targeted at the degradation of non-conventional feed resources to yield metabolisable energy (ME). Use of synthetic enzymes in monogastric diets results in enhanced growth performance and feed conversion, fewer environmental problems due to reduced faecal output. In addition, enzymes increased the accuracy and flexibility in least-cost formulation as well as improved well being of animals. With adequate information and or knowledge on the detailed chemical structures and physiological activities of NSPs in various ingredients, the use of NSPs as energy sources will be made possible, thereby resulting in a more efficient utilisation of non-conventional ingredients such as copra meal, palm kernel cake, sunflower meal, rice husk (rice-mill feed) when incorporated into monogastric diets. The practical application of enzymes on a large scale in the poultry industry was due to the recognition that soluble NSPs present in viscous cereals (wheat, barley, triticle and rye) impair nutrient digestion and absorption. Enzymatic cleavage is the most practical and cost effective way of breaking down NSPs in the GIT of animal (Choct, 2006). The current enzymes are not capable of depolymerising NSPs to their simple constituents during the digesta transit time of poultry (Choct, 2006). The need to expand the utilisation of enzymes which can cater for non-viscous grains, by-products of cereals, the food industry as well as non-conventional raw materials has been emphasised (Choct, 2006).

Effect of probiotics on gut microorganisms and chicken health: The intestinal tracts of newly hatched chickens are basically sterile i.e., containing no microorganism. Through feeding, microbes gradually colonize the Gastro-Intestine Trait (GIT) forming a stable microbial consortium over time. Studies have shown that it takes 2-4 weeks for a stable microbial consortium to form in the GIT of chickens (Lee *et al*., 2010; Amit-Romach *et al*., 2004). During this period of microbial colonization of the chicken GIT, the chicks are exposed to the risk of being colonized by pathogenic organism at a period in their life cycle, when their immunity is low. Through natural selection either beneficial or pathogenic microorganisms are established in the GIT at maturity. When harmful microbes are established they could cause localized or systemic infections, intestinal putrefaction and toxic production (Jeurissen *et al*., 2002; Yegani and Korver, 2008). Examples of pathogenic organism commonly associated with poultry diseases causing economic losses are the protozoa *Eimeria* causing coccidiosis (Willis *et al*., 2008, 2009a, b, 2010a, b, 2011) and the following bacteria *Salmonella*, *E.coli*, *Streptococcus*, *Clostridium perfringes* e.t.c. Microbial infections have resulted in chicks weight loss, death and poor meat quality. On the other hand, beneficial microbes, which are now developed as probiotics suppress/fight pathogenic/ harmful microbes through various mechanism such as competition for food and attachment sites at the GIT (competitive exclusion), production of acidity to make the GIT unsuitable for pathogenic organisms and the stimulation of immunity to fight invading pathogenic microbes. Other documented functions of GIT colonized by beneficial microbes include production of nutrient and vitamins, reduction in meat contamination, enhancement of animal performance, prevention of inflammatory reactions (Patterson and Burkholder, 2003; Yegani and Korver, 2008; Jeurissen *et al*., 2002)

Apajalahti *et al*. (2004) reported that there are about 10^{7}-10^{11} bacteria cfu/g of gut digest and through molecular studies indentified 640 species belonging to 140 genera. The microbial ecology of chicken GIT is quite unique. At maturity, the chicken GIT is quite diverse consisting mostly of bacteria and to a lesser extent protozoa and fungi (Gabriel *et al*., 2006). The diversity/composition of the microbial flora of chicken GIT depends on several factors including diet composition, age of the chicken, breed, geographic location and the specific section of the GIT such as small intestine, ileum, cecum (Apajalahti *et al*., 2001, 2002, 2004). It has been variously reported that each region of the chicken intestine develop its own unique microflora (Yegani and Korver, 2008; Amit-Romach *et al*., 2004; Gong *et al*., 2002a, b). Though, Richard *et al*. (2005) reported that generally the population of the GIT tends to increase from proximal to the distal of the GIT. Apajalahti *et al*. (2002) reported that the population of the bacteria in the ileum was 10^8 and 10^9 cfu/g of the digesta at 1 and 3 days old respectively, whereas at the cecum they were 10^{10} and 10^{11} cfu/g, respectively. Another study carried out by Apajalahti (2004) show that the basal nutrients of poultry diet affect the diversity of bacteria in the GIT with feeds containing corn/sorghum, barley, oats and rye preferentially stimulating the population of *Enterococcus*, *Lactobacillus*, *E. coli* /*Lactococus* and *Streptococcus*, respectively. Recent studies have shown that feeding broilers chicken with fungi myceliated grains have preferentially increased *Bifidobacteria*, while decreasing the population densities of pathogenic *Salmonella* and *Eimeria* (Willis *et al*., 2008, 2009a, 2009b, 2010a, 2010b, 2011; Ogbe *et al*., 2009).

One of the important functions of probiotic microorganism is the stimulation of immunity against invading pathogenic microbes. FMG and other probiotic microorganisms including the normal microflora of the GIT have been shown to stimulate immunity in the host broiler. Several authors have reported the close relationship between the GIT microflora and intestinal immune system in chickens and other animals (Gabriel *et al*., 2006; Lee *et al*., 2010; Huang *et al*., 2004). Guo *et al*. (2004a,b) reported that poultry feed containing mushroom and plant extracts resulted in the enhancement of both humoral and cellular immunity against *Eimeria tenella* infected chickens. Similarly, other author have established immune response enhancement against *Eimeria* in chicken following the administration of mushroom based diets (Willis *et al*., 2010a, 2010b, 2011; Brochers *et al*., 2004; Dalloul *et al*., 2006). Chicken fed with FMG exhibited increased heterphils, macrophages and lymphocytes when compared to the controls (Willis *et al*., 2010a). Heterophils, macrophages and lymphocytes are known to play major roles in the defence against pathogenic microorganisms including *Salmonella* and *Eimeria* (Kogut 2009b; Kogut *et al*., 2005; Kogut and Klasing, 2009a). Shiitake mushroom is known to synthesize the polysaccharide, B-glucan, which has been reported to form biding sites for immune receptors (Mueller *et al*., 2000). Selegean *et al*. (2009) has recently utilized the polysaccharide containing extracellular fractions from oyster mushroom, *Pleurotus ostreatus* to help poultry vaccines stimulate immune system response against microbial infections.

CONCLUSION

Poultry meat is one of the most important sources of animal protein in the world today. As the world population continue to increase so is the demand for poultry meat. Infections caused by pathogenic microorganisms such as Eimeria, Salmonella, Clostridium etc continue to threaten the poultry industry. Such infections are responsible for reduced growth rates and consequent economic losses in poultry. Traditionally, antibiotics growth promoters are used to treat infected chickens. Unfortunately, the long term and extensive use of antibiotics for veterinary purpose may eventually result in selection for the survival of resistant microbial species, thereby posing a threat to both animal and human health. Consequently, some countries have restricted the use of AGP in poultry. In this study we propose the use of feed supplements containing probiotics, prebiotics and enzymes as alternative to conventional AGP.

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