The Application of Prebiotics in Poultry Production

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Abstract: For several decades, antibiotics and chemotherapeutics in prophylactic doses have been used in poultry diet to improve their welfare and to obtain economic benefits in terms of improved animal performance and reduced medication costs. With increasing concerns about antibiotic resistance, there is increasing interest in finding alternatives to antibiotics for poultry production. Prebiotics are one of the alternatives that can improve poultry performance through altering gut microflora. Furthermore, high protein prices and environmental concerns have pressured the industry to search for methods for reducing dietary protein levels.

Key words: Prebiotic, poultry, performance, gut microflora

INTRODUCTION
Prebiotics are defined as a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon (Gibson and Roberfroid, 1995). In other words, prebiotics are meant to provide a substrate for beneficial gastrointestinal microbes. Large amounts of bacteria present in the monogastric small intestine and are potentially capable of utilizing these indigestible carbohydrate sources for energy. Recently, some researches (Houdijk et al., 1997; Hillman, 2001) have been conducted to manipulate beneficial bacteria in Gastrointestinal Tract (GIT). Bezkorovainy (2001) suggested that the use of prebiotics is a promising approach for enhancing the role of endogenous beneficial organisms in the gut. They can be used as potential alternatives to growth promoting antibiotics (Hattemink, 1995).

For several decades, antibiotics and chemotherapeutics in prophylactic doses have been used in animal feed to improve animal welfare and to obtain economic benefits in terms of improved animal performance and reduced medication costs. However, there are increasing concerns about the risk of developing cross-resistance and multiple antibiotic resistance in pathogenic bacteria in both humans and livestock linked to the therapeutic and subtherapeutic use of antibiotics in livestock and pets. Enteric diseases are an important concern to the poultry industry because of lost productivity, increased mortality, and the associated contamination of poultry products for human consumption. The European Union has banned all in-feed use of antibiotics from 2006 and the use of antibiotics in feed is being considered for elimination (or intense regulation) in other parts of the world. This perspective has stimulated nutritionists and feed manufacturers to search for new and safe alternatives. The primary alternatives studied include: acidification of the feed by organic acids, feeding probiotic organisms and feeding prebiotic compounds.

In the 1980’s the possible potential effects of prebiotics in animal feeds was already recognized. Since then the interest in the use of prebiotics in animal feed and pet food has resulted in a high research activity. The use of prebiotics in diets for farm animals and pets has been documented by Mul and Perry (1984 farm and pet animals), Houdijk (1998, swine), Ili and Tivery (1998, 1999, poultry), Flickinger and Fahey (2002, pets, poultry, swine and rabbits) and Patterson and Burkholder (2003, swine). The non-digestible inulin-type fructans are found widely in many vegetable feed and food ingredients and are perhaps the most well studied and documented prebiotics in domesticated animals (Flickinger et al., 2003). The use of prebiotics or fermentable sugars instead of antibiotics is going to be popular in birds in order to improve the useful microbial population of the Gastrointestinal (GI) tract (Kermanshahi and Rostami, 2006).

Prebiotics have been shown to alter GI microflora, alter the immune system, prevent colon cancer, reduce pathogen invasion including pathogens such as Salmonella enteritidis and E. coli and reduce cholesterol and odour compounds (Cummings and Macfarlane, 2002). The commercially available fermentation product of Aspergillus orizae, Fermacto referred to as Aspergillus Meal (AM), has no live cells or spores and is proven to enhance the digestive efficiency of the gut (Harms and Miles, 1986). As Kim et al. (2003) reported, Aspergillus oryzae might act as substrates for favourable bacteria such as Lactobacillus in the intestinal microbial system that subsequently reduces Salmonella or E. coli concentrations.

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High protein prices and environmental concerns have pressured the poultry industry to reduce dietary protein levels (Firman, 1997). Thus, low protein diets are of interest and important for feed additive evaluation and animal performance.

The aim of this review is to provide an overview of recent developments on the use and application of prebiotics in poultry feed.

Advantages of prebiotic supplementation: Favourable effects of addition of prebiotics reflect in presence of antagonism towards pathogens, competition with pathogens, promotion of enzyme reaction, reduction of ammonia and phenol products and increase of resistance to colonization.
• Improve gut health (improvement intestinal microbial balance).
• Improve performance.
• Enhance nutrient utilization (e.g., amino acids and proteins).
• Decrease environmental pollution.
• Decrease production cost (Peric et al., 2009; Khksar et al., 2008; Midilli et al., 2008; Ghiyasi et al., 2007).

Piray et al. (2007), reported relative weight of breast and thigh to body weight were significantly (p<0.01) higher in Fermacto® fed broilers as compared to control group. Fermacto® is a microbial feed supplement derived from Aspergillus Mycelium (AM) has been used as an alternative tool for helping newly hatched chicks.

FOS improved broiler’s gain about 5-8% and improved feed conversion ratio by 2-6% (Li et al., 2008; Yang et al., 2009). But, Biggs et al. (2007) obtained research results showing decrease of gain by 2% in group fed FOS in diet. Also, in case of application of MOS, some authors obtained results confirming the improvement of gain and feed conversion in fattening chickens by up to 6% (Roch, 1998; Newman, 1999). Zikic et al. (2008) obtained significantly positive effect of prebiotics on performance and height of intestinal villus in small intestines of broilers. Kannan et al. (2005), reported that supplementation of prebiotic extracted from yeast at 0.5 and 1 g/kg Copra meal at 1 and 1.5/kg and the yeast source at 1 g/kg level helps in reduction of the abdominal fat pad content.

Characteristics of prebiotic:
• Should be neither hydrolyzed nor absorbed in the upper part of the gastrointestinal tract.
• Be a selective substrate for one or limited number of bacteria commensal to caecum/colon, which are stimulated to grow or metabolically activated.
• Able to alter the colonic flora in favour of a healthier composition.
• Induce systemic effects that are beneficial to the host’s health.

Substances used as prebiotic: Non-digestible carbohydrates (oligo and polysaccharides), some peptides, proteins and certain lipids (both ester and ethers) are candidate prebiotic. Lactose is a disaccharide consist of glucose and galactose, which has prebiotic effect in chickens. Since chickens does not have lactase enzyme, lactose enters to the lower segment of the intestine and caeca, where hydrolyzed by microbial activity. The dominant prebiotics are fructo-oligosaccharide products (FOS, oligofructose, inulin); gluco-oligosaccharides, stachyose, malto-oligosaccharides and oligochitosan have also been investigated in broiler chickens (Jiang et al., 2008; Huang et al., 2007).

Fructo-oligosaccharides as prebiotics: Non-digestible carbohydrates include non-digestible oligosaccharides and non-starch polysaccharides, resistant starch (Delzenne and Roberfroid, 1994). All of these non-digestible carbohydrates are expressed as non-digestible polysaccharides because they are not hydrolyzed by endogenous enzyme in the small intestine, but hydrolyzed by colonic bacteria in the large intestine. However, all of these could not be classified as prebiotics but rather colonic food because the process of colonic fermentation in most of these substances is nonspecific (Gibson and Roberfroid, 1995). It should be noted that starch not digested enzymatically but fermented may also be a candidate.

Oligosaccharides are a group of carbohydrates consisting of 2-10 sugar units and each oligosaccharide has a different chemical structure. FOS are named by the chain length (degree of polymerization = DP). Inulin contains 2-60 DP and synthetic fructan (FOS) contains 2-4 DP. Oligofructose contains 2-9 DP and can be obtained by partial enzymatic hydrolysis of inulin. It is well known that oligosaccharides are naturally occurring constituent in plants and vegetable and the most common sources are onions, Jerusalem artichokes, bamboo shoots, chicory roots and bananas. Commercially available prebiotics are mostly fructo-oligosaccharides, isomalt-oligosaccharides, galacto-oligosaccharides, transgalacto-oligosaccharides, inulin and oligofructose etc. (Table 1). Among the candidate of prebiotics, fructo-oligosaccharides are only products that meet the criteria allowing classification as prebiotics (Gibson and Roberfroid, 1995). FOS is one of the most commonly used as prebiotics. Physio-chemical properties of oligosaccharides depend on their chemical structure and composition. Most oligosaccharides are soluble in water or physiological fluids.
Fructo-oligosaccharides and bifidobacteria: The chemical structures of FOS consists of short chain polymers of β 1-2 linked fructose units. FOS are produced commercially either by hydrolysis of inulin or by enzymatic synthesis from sucrose or lactose. They are not hydrolyzed by the enzymes of endogenous origin (Oku et al., 1984). Short chain lengths of chicory inulin up to 20 fructose units are called fructo-oligosaccharides. Specific nondigestible oligosaccharides can selectively proliferate specific bacteria such as bifidobacteria (Hayakawa et al., 1990). A more recent technique is the development of new structurally modified FOS. The Degree of Polymerization (DP) is relatively low (DP < less than 11) and these components may be used as substrates for the development of specific strains of bifidobacteria in the large intestine of pigs. It is assumed that FOS are not digested in the small intestine. So they will reach to the large intestine of pigs where they stimulate the growth of bifidobacteria (Bunce et al., 1995). However, FOS may start to digest (ferment) already in the small intestine. It is also thought that FOS is rapidly fermented in the proximal part of the large intestine of weaned pigs (Houdijk et al., 1997).

Bifidobacteria are anaerobic, gram-positive bacteria and they are found in the gastro-intestinal tract of human infants and adults as well as various warm-blooded animals. The organism was first isolated from the faeces of breast-fed infants by Tissier (1900). FOS is selectively fermented by most strains of bifidobacteria. The predominant species of bifidobacteria in pigs is bifidobacterium psuedolongum (Type A) (Mitsuoka, 1984). Bifidobacteria are saccharolytic organisms and all strains of fermented glucose, galactose and fructose. Glucose is fermented via the fructose-6-phosphate shunt to acetic and L lactic acids. Bifidobacteria do not produce CO₂, butyric or propionic acid. The optimum growth temperature of bifidobacteria is 37-43°C and optimum pH for growth is 6.5-7.1 (Scardovi, 1986). Bifidobacteria populations in the gastrointestinal tract of piglets range from 10^3-10^5/g chyme in the stomach to 10^7/g chyme in the ileum (Stewart et al., 1993) and 10^9-10^10 in the large intestine (Borg, Jensen, 1993). Several studies showed that bifidobacteria and lactobacillus may be beneficial and the dominant bacteria in the colon. Bifidobacteria have an antibacterial effect because they can suppress potential pathogens like E. coli. They do this by producing antimicrobials like bacteriocin or by lowering pH through the rapid production of volatile fatty acids especially acetate and lactate. The undissociated acid which is presented in a higher proportion where pH decreases in the gut can function as antibacterial agent (Ekland, 1983). It is the use of nitrogenous compounds for the growth of bifidobacteria which will lead to less proteinous substances used for energy. Thus, when there is saccharolytic fermentation, the bacteria does not use as much protein for energy. This may result in less amines and branched chain fatty acids. Non-specific immune activity can be increased by feeding fermented milk products with bifidobacterium bifidum (Schiffrin et al., 1995). In an in vitro study, FOS and xylo-oligosaccharides are converted to acids at a high rate by most strains of bifidobacterium, at a lower rate by most lactobacilli, most bacteroides, but not used by eubacteriaceae, most clostridia (except Clostridium butyricum), E. coli and staphylococcus (Wada et al., 1987).

It is well known that amino acids are absorbed from the small intestine. Nitrogenous products produced by microflora from organic nitrogen in colon are absorbed as well (Niyama et al., 1979). Nitrogen absorbed in the colon is as ammonia and urea and excreted as urea via urine. It may be more beneficial for pig that the microbes in the colon grow from protein entering the colon and thereby produce biomass. This will only happen if there is a sufficient quantity of carbohydrate which they can use as an energy source. So the best thing for the animal and human in the colon is to avoid much absorption of nitrogen in the colon and to have the nitrogen excreted with feces as biomass. However, it is in contrast with what is expected nowadays, the absorbed bifidobacterial nitrogen in colon may be beneficial to the pig.

It is believed that dietary prebiotics can increase bioavailability of minerals in the gut (Scholz-Ahrens et al., 2001). Moreover, bifidobacteria produce water soluble vitamin B group (Liescher, 1961).

Mannanoligosaccharides: Mannanoligosaccharides is obtained from yeast cell wall (Saccharomyces cervisiae). They are components of the outer layer of yeast cell
walls and their components include proteins, glucans and phosphate radicals as well as mannose (Klis et al., 2002). The basic composition of the wall consist of mannan (30%), glucan (30%) and protein (12.5%). While the ratio of one component to another remains relatively constant from strain to strain, the degree of mannan phosphorylation and the interaction among the mannan, glucan and protein components vary. Mannanolig-osaccharides contain protein which has relatively high proportion of serine, threonine, aspartic and glutamic acids and a paucity of methionine (Song and Li, 2001).

The exact mechanism through which pathogenic bacteria are inhibited by mannose is unclear, though two theories have been presented. One being that MOS may adsorb bacteria containing type-1 fimbriae inhibiting them from binding to the carbohydrate moieties of the intestinal lining (Hooge, 2003). The other being one of agglutination, that MOS causes pathogenic cells with type-1 fimbriae to aggregate or clump, bringing them out of solution (Spring et al., 2000).

Strains of _E. coli_ and _Salmonella_ were screened to determine the incidence of strains possessing mannose sensitive adhesions (Finucane et al., 1999b). Authors found that 80% of _Salmonella enteritidis_ and 67% of _Salmonella typhimurium_ freely agglutinated with MOS. It is interesting to note that adhesion appears to not with _Clostridium_ or _Helicobacter pylori_, though production improvements have been observed with the use of MOS products. This may implicate other mechanisms of intestinal modification beyond simple type-1 agglutination.

Hooge (2004) reviewed pen trials conducted with a commercially available dietary MOS (Bio-MOS, Alltech Inc.) from 1993-2003 and the meta-analysis showed that Bio-MOS improved the growth performance of birds compared to the negative control (Table 2). Compared to a wide range of antibiotics (including avilamycin, bacitracin, bambermycin or virginiamycin at prophylactic concentrations) a significant decrease in mortality was observed for Bio-MOS treatment (Table 2). The optimal dose of Bio-MOS for broiler production was around 2 g/kg, depending on the production stage of birds (Rozen, 2007). Three major methods of action by which broiler performance was improved by MOS by MOS were: -control of pathogenic or potential pathogenic bacteria which possess type-1 fimbriae (mannose sensitive lectin), -immune modulation and modulation of intestinal morphology and expression of mucin and brush border enzymes (Ferket, 2004). Yang et al. (2008a, 2008b, 2008c) showed that MOS inhibited the development of lactobacilli and coliforms. They reported that the colonization of mucosa-associated coliform was inhibited by MOS as early as 7 days of age.

A large number of reports have suggested that MOS may influence the physical properties of the epithelial lining itself. Histological examination of the duodenal loop (mid-distal) and the jejunum (proximal to the Meckels) revealed an increase in the number of goblet cells with an inclusion level of 0.33% Bio-Mos® (Savage et al., 1997). A lower level of inclusion (0.11%) did not reveal an increase in goblet cell numbers but did show a decrease in crypt size and villus width, suggesting a potential reduction in mucosal turnover rate. An examine by Iji et al. (2001) produced similar results. Researchers reported that with high levels of supplementation, jejunal villi height increased.

**Partially hydrolysed guar gum (PHGG):** One of the most commonly used polysaccharide prebiotic for chickens is guar gum produced from the seed of guar (Cyamopsis tetragonoloba) bean. By selectively cleaving the mannan backbone chain of guar gum using endo-B-D-mannanas, a mixture of galactomannans is obtained, which is known as Partially Hydrolysed Guar Gum (PHGG) (Panda et al., 2008).

**Mechanism of actions of prebiotic:** Prebiotics can either directly bind the pathogens or increasing the osmotic value in the intestinal lumen. However, they have indirect effects through metabolites that are generated by intestinal flora while utilizing prebiotics compounds for their own metabolism. Mechanism of actions of prebiotic can be listed as followed:

1. Lowering the gut pH through lactic acid production (Chio et al., 1994; Gibson and Wang, 1994).
2. Inhibiting/preventing colonization of pathogens (Morgan et al., 1992; Bengmark, 2001).

**Poultry health:** By adding prebiotics to poultry diets, producers can minimize the use of antibiotics and drug resistance to bacteria. Patterson and Burkholder (2003), have reported that prebiotic supplementation can improve health status of the bird’s gastrointestinal tract. FOS reduced the colonization of _Salmonella_ in the chickens’ intestine, especially when the animals received competitive exclusion flora in addition to FOS (Bailey et al., 1991). Supplementation of 0.4% FOS in the diet of broiler chicks significantly increased the number of _Bifidobacteria_ and _Lactobacilli_ and decreased _E. coli_ in the caecum and small intestine. FOS has been observed to alleviate _Salmonella_ induced necrosis of cecal mucosal epithelium, enhances the length of ileal
The effects of MOS on growth performance of broiler chickens (adapted from Hooge, 2004)

<table>
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<th>Parameter</th>
<th>Negative control</th>
<th>MOS</th>
<th>Relative change</th>
<th>Note</th>
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<td>Body weight (kg/bird)</td>
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<th>MOS</th>
<th>Relative change (%)</th>
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<td>Mortality (%)</td>
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FCR: Feed Conversion Ratio

Conclusion: Current trends in poultry production point to reduced use of antibiotic growth promoters and increased use of nonantibiotic feed additives. Prebiotic is one of alternative additives that can be used to improve poultry health and performance. Prebiotic alters the intestinal microbes and immune system to reduce colonization by pathogens in certain conditions.

REFERENCES


