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308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorijps@gmail.com

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The Application of Prebiotics in Poultry Production

H. Hajati and M. Rezaei
Department of Animal Science, College of Animal Science and Fisheries,
Sari Agricultural Sciences and Natural Resources University, P.O. Box 578, Sari, Iran

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 (Hatemink, 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 (1994 farm and pet animals), Houdijk (1998, swine), Iji and Tivey (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, 1988). 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.

Corresponding Author: H. Hajati, Department of Animal Science, College of Animal Science and Fisheries, Sari Agricultural Sciences and Natural Resources University, P.O. Box 578, Sari, Iran

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 (eg, 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.
- Should have known structure, which can be documented.
- Should be palatable as food ingredient and large-scale processing must be easy.

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, oligufroctose, inulin); gluco-oligosaccharides, stachyose, malto-oligosaccharides and oligochitosan have also been investigated in broiler chickens (Jiang *et al*., 2006; 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, isomalto-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.

Table 1: Major oligosaccharide candidates for prebiotics

Oligosaccharides	Structure	Linkages	Process	Origin
Xylo-oligosaccharides	(Glu)n	β-1,4	Hydrolysis	Cereals
Lactulose	Gal-Fru	β-1,4	Isomerisation	lactose
Isomalto-oligosaccharides	(Glu)n	α-1,6	Hydrolysis	Algae
Gluco-oligosaccharides	(Glu)n	α-1,2 and α-1,6	Synthesis	Sucrose
Galacto-oligosaccharides	(Gal)n-Glu	β-1,4 and β-1,6	Synthesis	Lactose
Fructo-oligosaccharides	(Fru)n-Glu	(β-2,1)-α-1,2	Synthesis	Sucrose
Oligofructose	(Fru)n-(Fru)n-Glu	(β-2,1)	Hydrolysis	Inulin

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 CO_2, 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 10^4-10^6/g chyme in the stomach to 10^9/g chyme in the ileum (Stewart *et al*., 1993) and 10^8-10^9 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 (Eklund, 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 staphylocus (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 (Niiyama *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, brining 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 owith *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 coliforms 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 (Cyamposis tetragonoloba) bean. By selectively cleaving the mannan backbone chain of guar gum using endo-B-D-mananas, 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 indirectly 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).
3. Modifying metabolic activity of normal intestinal flora (Demigne *et al*., 1986).
4. Stimulation of immune system (Monsan and Paul, 1995).

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

Table 2: Effects of MOS on growth performance of broiler chickens (adapted from Hooge, 2004)

Parameter	Negative control	MOS	Relative change	Note
Body weight (kg/bird)	2.231	2.267	+1.61	29 pen trials
FCR (g/g)	1.808	1.772	-1.99	29 pen trials
Mortality (%)	4.494	3.534	-21.4	21 pen trials
Parameter	Antibiotic control	MOS	Relative change (%)	Note
Body weight (kg/bird)	2.246	2.238	-0.36	21 pen trials
FCR (g/g)	1.822	1.820	-0.11	21 pen trials
Mortality (%)	5.404	4.426	-18.1	16 pen trials

FCR: Feed Conversion Ratio

microvilli (Chio *et al*., 1994) and thereby increases the surface area for digestion and absorption of nutrients.

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 microbs and immune system to reduce colonization by pathogens in certain conditions.

REFERENCES

Bailey, J.S., L.C. Blankenship and N.A. Cox, 1991. Effect of fructodigosaccharide on *Salmonella* colonization of the chicken intestine. Poult. Sci., 70: 2433-2438.

Bengmark, S., 2001. Pre-, pro- and symbiotics. Current opinion in clinical nutrition and metabolic car. 4: 571-579.

Bezkorovainy, A., 2001. Probiotics: Determinants of survival and growth in the gut. Am. J. Clin. Nutr., 73(suppl.): 399S-405S.

Biggs, P., C.M. Parsons and G.C. Fahey, 2007. Effects of several Oligosaccharides on growth performance, nutrient digestibilities and caecal microbial populations in young chicks. Poult. Sci., 86: 2327-2336.

Borg Jensen, B., 1993. The possibility of manipulation of the microbial activity in the digestive tract of monogastric animals. In: Proceedings of the 44th Annual Meeting of European Association for Animal production, Aarus, Denmark, pp: 49.

Bunce, T.J., M.S. Kerley, G.L. Allee and B.N. Day, 1995. Feeding fructooligosaccharides to the weaned pig improves nitrogen metabolism and reduced odor metabolite excretion. J. Anim. Sci., 73(Suppl.85): 70.

Chio, K.H., H. Namkung and I.K. Paik, 1994. Effects of dietary fructolligosaccharides on the suppression of intestinal colonization of *Salmonella typhimurium* in broiler chickens. Korean J. Anim. Sci., 36: 271-284.

Cummings, J.H. and G.T. Macfarlane, 2002. Gastrointestinal effects of prebiotics. Br. J. Nutr., 87(suppl. 2): S145-151.

Demigne, C., C. Yacoub, C. Remezy and P. Fafournoux, 1986. Effects of absorption of large amounts of volatile fatty acids on rat liver metabolism. J. Nutr., 116: 77-86.

Eklund, T., 1983. The antimicrobial effect of dissociated and undissociated sorbic acid at different pH levels. J. Appl. Bacteriol., 54: 383.

Ferket, P.R., 2004. Alternatives to antibiotics in poultry production: Responses, practical experience and recommendations. Nutritional biotechnology in the feed and food industries: Proceedings of Alltech's 20th Annual Symposium, Kentucky, USA, pp: 56-57.

Firman, J.D., 1997. Early Stage Turkey Nutrition and Ideal Protein: Implications for All Aspects of Poultry Production. In: Nutritional Biotechnology in the Feed and Food Industries, Lyons, T.P. and K.A. Jacques (Eds.). Nottingham University Press, Notingham, UK., pp: 57-67.

Flickinger, E.A. and G.C. Fahey Jr., 2002. Pet food and feed applications of inulin, oligofructose and other oligosaccharides. Br. J. Nutr., 87: S297-S300.

Flickinger, E.A., Van, J. Loo and G.C. Fahey Jr., 2003. Nutritional response to the presence of inulin and oligofructose in the diets of domesticated animals: a review. Crit. Rev. Food Sci. Nutr., 43: 19-60.

Ghiyasi, M., M. Rezaei and H. Sayyahzadeh, 2007. Effect of prebiotic (Fermacto) in low protein diet on performance and carcass characteristics of broiler chicks. Int. J. Poult. Sci., 6: 661-665.

Gibson, G.R. and X. Wang, 1994. Bifidogenic properties of different types of fructooligosaccharides. Food Microbial., 11: 491-498.

Gibson, G.R. and M.B. Roberfroid, 1995. Dietary modulation of the human colonic microbiota: Introducing the concept of prebiotics. J. Nutr., 125: 1401-1412.

Harms, R.H. and R.D. Miles, 1988. Research note: Influence of fermacto on the performance of laying hens when fed with different levels of methionine. Poult. Sci., 67: 842-844.

Hatemink, R., 1995. Non digestible oligosaccharides: Healthy food for the colon. Proceedings Symposium Wageningen, 4-5 December, pp: 1-177.

Hayakawa, K., J. Mizutani, K. Wada, T. Masai, I. Yoshihara and T. Mitsuoka, 1990. Effcts of soybean oligosaccharides on human faecal flora. Micro. Ecol. Health Dis., 3: 293-303.

Hillman, K., 2001. Bacteriological Aspects of the Use of Antibiotics and Their Alternatives in the Feed of Non-ruminant Animals. In: Recent Advances in Animal Nutrition, Garnsworthy, P.C. and J. Wiseman (Eds.). Nottingham University Press, Nottingham, pp: 107-134.

Houdijk, J.G.M., 1998. Effects of non-digestible oligosaccharides in young pig diets. Ph.D. Thesis Wageningen University, pp: 1-141.

Houdijk, J.G.M., B.A. Williams, S. Tamming and M.W.A. Verstegen, 1997. Relation between *in vivo* and *in vitro* fermentation of oligosaccharides in weaner pigs. Proceeding of Br. Soci. Anim. Sci. Br. Soci. Anim. Sci., pp: 59.75:170-175.

Hooge, D.M., 2003. Broiler chicken performance may improve with MOS. Feedstuffs., 75: 1.

Hooge, D., 2004. Meta-analysis of broiler chicken pen trials evaluating dietary mannan oligosaccharide, 1993-2003. Int. J. Poult. Sci., 3: 163-174.

Huang, R.L., Y.L. Yin and M.X. Li, 2007. Dietary oligochitosan supplementation enhances immune status of broilers. J. Sci. Food. Agric., 87: 153-159.

Iji, P.A., A.A. Saki and D.R. Tivey, 2001. Intestinal structure and function of broiler chickens on diets supplemented with a mannan oligosaccharide. J. Sci. Food Agric., 81: 1186-1192.

Iji, P.A. and D.R. Tivey, 1998. Natural and synthetic oligosaccharides in broiler chicken diets. World's Poult. Sci., 54: 129-143.

Iji, P.A. and D.R. Tivey, 1999. The use of oligosaccharides in broiler diets. In: Proceedings of the 12th European Symposium on Poultry Nutrition, Veldhoven, The Netherlands: WPSA Dutch Branch, pp: 193-201.

Jiang, H.Q., L.M. Gong., Y.X. Ma., Y.H. He., D.F. Li and H.X. Zhai, 2006. Effect of stachyose supplementation on growth performance, nutrient digestibility and caecal fermentation characteristics in broilers. Br. Poult. Sci., 47: 516-522.

Kannan, M., R. Karunakaran, V. Balakrishnan and T.G. Prabhakar, 2005. Influence of prebiotics supplementation on lipid profile of broilers. Int. J. Poult. Sci., 4: 994-997.

Kermanshahi, H. and H. Rostami, 2006. Influence of supplemental dried whey on broiler performance and cecal flora. Int. J. Poult. Sci., 5: 538-543.

Khksar, V., A. Golian, H. Kermanshahi, Movasseghiand and A. Jamshidi, 2008. Effect of prebiotic fermacto on gut development and performance of broiler chickens fed diet low in digestible amino acids. J. Anim. Vet. Adv., 7: 251-257.

Kim, S.H., S.Y. Park, D.J. Yu, S.L. Lee, K.S. Ryu and D.G. Lee, 2003. Effects of feeding *Aspergillus oryzae* ferments on performance, intestinal microbiota, blood serum components and environmental factors in broiler. Korean J. Poult. Sci., 30: 151-159.

Klis, F.M., P. Mol, K. Hellingwerf and S. Brul, 2002. Dynamics of cell wall structure in *Saccharomyces cervisiae*. FEMS Microbiol. Rev., 26: 239-247.

Li, X., L. Qiang and C.H. Liu Xu, 2008. Effects of supplementation of fructooligosaccharide and/or *Bacillus subtilis* to diets on performance and on intestinal microflora in broilers. Archiv. fur Tierzucht, 51: 64-70.

Liescher, S., 1961. Sind Die Darme Des Mit Muttermilch Emahrten Sauglings Vorherschenden Bifudbakerien Als Nutzliche. Vitaminlieferanten Fur Den Sauglings-organismus Anzusehen. Z. Kinderheik. 85:265. In: Bifidobacteria and Their Role, Rasic, J.L. and J.A. Kurman (Eds.). Experimentia Supplementum. Vol. 39. Birkhauser Switzerland, pp: 77.

Midilli, M., M. Alp, N. Kocabagli, Ö.H. Muglali, N. Turan, H. Yilmaz and S. Çakir, 2008. Effects of dietary probiotic and prebiotic supplementation on growth performance and serum IgG concentration of broilers. South Afr. J. Anim. Sci., Vol. 38.

Mitsuoka, T., 1984. Tamonomy and ecology of bifidobacteria. Bifidobacteria Microflora, 3: 11.

Monsan, P.F. and F. Paul, 1995. Oligosaccharide feed additives. In: Biotechnology in Animal Feeds and Feeding, Wallace, R.J. and A. Chesson (Eds.). VCH Verlagsgesellschaft, Weinheim and New York, pp: 233-245.

Morgan, A., A.J. Mul, G. Beldman and A.G.J. Voragen, 1992. Dietary oligosaccharides. New insights. Agro. Food Ind. Hi-tech., 11: 35-38.

Mul, A.J. and F.G. Perry, 1994. The role of fructo-oligosaccharides in animal nutrtition. In: Recent Advances in Animal Nutrition, Garnsworthy, P.C. and D.J.A. Cole (Eds.). Nottingham, UK, Nothingham University Press, pp: 57-79.

Newman, E.K., 1999. Feeds with antibiotic growth promoters-The oligosaccharide alternative. Biotechnology responds. Alltech's 1999 European, Middle Eastern and African Lecture Toure.

Niiyama, M., E. Deguchi, K. Kagota and S. Namioka, 1979. Appearance of N-labeled intestinal microbial amino acids in the venous blood of the pig colon. Am. J. Vet. Res., 40: 716.

Oku, T., T. Tokunaga and N. Hosoya, 1984. Nondigestibility of a new sweetener, 'Neosugar', in the rat. J. Nutr., 114: 1574-1581.

Panda, A.K., S.V. Rama Rao and M.R. Reddy, 2008. Growth promoters in poultry: novel concepts. 1st Edn., IBDC, pp: 134.

Patterson, J.A. and K.M. Burkholder, 2003. Prebiotic feed additives: Rationale and use in pigs. In: Proceedings of the 9th international symposium on Digestive Physiology in Pigs, Ball, R.A. (Ed.). Banff, Canada: University of Alberta, 1: 319-331.

Peric, L., D. Zikic and M. Lukic, 2009. Aplication of alternative growth promoters in broiler production. Biotech. Anim. Husb., 25: 387-397.

Piray, A.H., H. Kermanshahi, A.M. Tahmasbi and J. Bahrampour, 2007. Effects of cecal cultures and *Aspergillus* meal prebiotic (Fermacto) on growth performance and organ weights of broiler chickens. Int. J. Poult. Sci., Vol. 6.

Roch, C., 1998. Effect of Bio-mos and Flavomycin on Commercial Broiler Performance. In: Biotechnology in the Feed Industry. Proc. Alltech's 14th Annual Symposium. Lyons, T.P. (Ed.). Nicholsville Kentucky, Enclosure code, 52: 163.

Rozen, G.D., 2007. Holo-analysis of the efficacy of Bio-Mos® in broiler nutrition. Br. Poult. Sci., 48: 21-26.

Savage, T.F., E.I. Zakrzewska and J.R. Andreasen, 1997. The effects of feeding mannan oligosaccharide supplemented diets to poults on performance and the morphology of the small intestine. Poult. Sci., 76 (Suppl. 1): 139 (Abstr.).

Scardovi, V., 1986. Bifidobacterium. In: Bergey's Manual of Systematic Bacteriology, Sneath, P.H., N.S. Mair, M.E. Sharpe and J.G. Holt (Eds.). 9th Edn., Williams and Wilkins Publishers, Baltimore, MD., 2: 1418.

Schiffrin, E.J., F. Rochat, H. Link-Amster, J.M. Aeschlimann and A. Donnet-Hughes, 1995. Immunomodulation of human blood cells following the ingestion of lactic acid bacteria. J. Dairy Sci., 78: 491.

Scholz-Ahrens, K.E., G. Schaafsma, E.G.H.M. Van Den Heuvel and J. Schrezenmeir, 2001. Effects of prebiotics on mibneral metabolism. Am. J. Clin. Nutr., 73: 459S-464S.

Song, J.-Y. and W.-F. Li, 2001. The preparation of mannan-oligosaccharide from *Saccharomyces cerevisiae* and its effect on intestinal microflora in chicken. J. Zhejiang University (Agric. and Life Sci.), 27: 447-450.

Spring, P., C. Wenk, K.A. Dawson and K.E. Newman, 2000. The effects of dietary mannanoligosaccharides on cacal parameters and the concentrations of enteric bacteria in the ceca of *Salmonella*-challenged broiler chicks. Poult. Sci., 79: 205-211.

Stewart, C.S., K. Hillman, F. Maxwell, D. Kelly and T.P. King, 1993. Recent Advances in Probiotics for Pigs. In: Recent Advances in Animal Nutrition, Garnsworthy, P.C. and D.J.A. Cole (Eds.). Nottingham University Press, pp: 197-220.

Tissier, H., 1900. Recherche Sur La Flore Intestinale Des Nourissons (Etat Normal et Pathologique). These. University of Paris, Paris, In: Bifidobacteria and Their Role. Experientia Supplement. Rasic, J.L. and J.A. Kurmann (Eds.). Birkhauser Verlag, Basel, Switzerland, 39: 3.

Wada, M., H. Fujita and H. Itikawa, 1987. Genetic suppression of a temperature-sensitive groES mutation by an altered subunit of RNA polymerase of Escherichia coli K-12. J. Bacteriol. Mar., 169: 1102-1106.

Yang, Y., P.A. Iji and M. Choct, 2009. Dietary modulation of gut microflora in broiler chickens: A review of the role of six kinds of alternatives to in-feed antibiotics. World's Poult. Sci. J., 65: 97-114.

Yang, Y., P.A. Iji, A. Kocher, L.L. Mikkelsen and M. Choct, 2008a. Effects of mannanoligosaccharide in broiler chicken diets on growth performance, net energy utilization, nutrient digestibility and intestinal microflora. Br. Poult. Sci., 49: 186-194.

Yang, Y., P.A. Iji, A. Kocher, L.L. Mikkelsen and M. Choct, 2008b. The response of broiler chickens to mannanoligosaccharide, or fructo-oligosaccharide-supplemented diet against pathogenic *Escherichia coli* challenge. Br. Poult. Sci., 49: 550-559.

Yang, Y., P.A. Iji, A. Kocher, L.L. Mikkelsen and M. Choct, 2008c. Effects of mannanoligosaccharides on the growth performance, nutrient digestibility and gut development of young birds given different cereal-based diets. Anim. Physiol. Anim. Nutr. (in press).

Zikic, D., L. Peric, G. Uscebrka, S. Stojanovic, D. Milic and L. Nollet, 2008. Effect of prebiotics in broiler breeder and broiler diets on performance and jejunum morphology of broiler chickens. 1st Mediterranean Summit of WPSA, Book of Proceedings, Porto Carras, Greece, pp: 879-882.