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REVIEW ARTICLE

NUTRACEUTICAL AND PHARMACEUTICAL IMPLICATION OF PREBIOTICS IN LIVESTOCK AND POULTRY FEED

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In poultry production, dietary prebiotics *viz.* dietary organic acid (OA) supplements, mannan oligosaccharide (MOS), β-glucan and xylanase supplementation are mainly used in order to enhance live body weight gain, dressing percentage, weight of vital organs and muscles and mean villus lengths in digestive tract of poultry birds. Prebiotics can also act as immunostimulants. The term immunostimulant can be used interchangeably with immunomodulator, adjuvant and biological response modifier. Immunostimulators can be in the form of drugs and nutrients. This article highlights role of prebiotics and their physiological implications on body performance.

Key words: Feed, Immunostimulation, Nutraceutical, Prebiotics, Poultry.

INTRODUCTION

Prebiotics are non-digestible feed ingredients that beneficially affect the host by selectively stimulating the growth or activity of one or a limited number of bacterial species, already resident in colon (**Figure 1**) and thus attempt to improve host health (Gibson and Roberfroid, 1995; Ganguly and Mukhopadhayay, 2011; Rashid *et al* 2012). Mainly prebiotics are small fragments of carbohydrates and commercially available as oligosaccharides of galactose, fructose or mannose (Ganguly, 2013a).

Fig. 1. Marketed prebiotics containing products

Among these, mannan oligosaccharide obtained from *saccharomyces spp.* of yeast outer cell wall maintain gut health by adsorption of pathogenic bacteria, containing type-I fimbriae or by agglutinating different bacterial strains and increase villi length uniformity and integrity. Effects of buffered propionic acid in presence and absence of bacitracin or roxarsone were reported earlier in which significant increase in dressing percentage for female broilers and a significant reduction in abdominal fat of males at 49 days tested the effect of dietary lactic acid on performance of broilers from 0 to 6 weeks age. Body weight gain tended to be greater, whereas feed to gain rations were significantly improved where birds were fed 2% lactic acid as prebiotic. Beneficial effects of different organic acids like formic acid, propionic acid, lactic acid ammonium formate and calcium propionate etc. as growth promoter and prebiotic have been studied earlier for having substitute to antibiotic. The effect of yeast cell wall preparation (of *Saccharomyces cerevisiae* origin) as an immunomodulator of the innate immune response was revealed (Paul *et al* 2012). To evaluate its effect on chicken, yeast cell wall preparation (Nutriferm™) was administrated orally to 1 week old broilers @ 0.4 g and 0.8 g per kg feed for 15 days and then switched back to control diet for 20 days. Similar number of birds was kept separately as control with normal feeding regime. Non-specific immunity was assessed in randomly selected treated and control birds at the end of the experimental feeding period ('0' day) by performing neutrophil, monocyte and lymphocyte functional assay *in vitro*. In both dose group super oxide anion production by neutrophil was increased gradually up to '10' day post treatment (DPT) and then decreased to '20' DPT. The increase was significant ($P<0.5$) compared to control birds. In vitro nitrite production by monocyte was found to be high in treated birds than control. In 0.4gm treatment group in vitro non-specific lymphocyte proliferation and IL-2 production was first increased and then decreased abruptly. But in 0.8 g treatment group *in vitro* non-specific lymphocyte proliferation and IL-2 production was increased and then decreased gradually. The findings of the study showed that 15 days oral administration of yeast cell wall preparation on both the doses improve innate immune responses in the broiler chicks.

Xylanase is the name given to a class of enzymes which degrades the linear polysaccharide beta-1, 4 xylan to xylose, thus breaking down hemicelluloses which are a major component of the cell wall of the plants. Xylanases are known to increase protein digestibility of wheat and this is attributed particularly to release of protein from the xylan enriched aleurone layer. Xylanase supplementation improves conjugated bile acid function in intestinal contents and increase villus size of small intestine wall in broiler. Supplementing broiler diets with combination of xylanase and β-glucanase improves the nutritive value of the diet (Veldman and Vahl, 1994). The addition of xylanase improves weight gain, feed intake, feed efficiency, AME and decreased water intake (Wu *et al* 2004) and Vitamin E content of liver in broiler was significantly improved by addition of xylanase (Danicke *et al* 1999; 2001). Nutri-xylanase is a bacterial xylanase processed from *Bacillus subtilis* and produced by a micro-filtration advanced fermentation technique (Ganguly, 2013b).

For studying the effect of xylanase enzyme on body parameters of broiler birds, experimental procedures are available in literature (Bar *et al* 2012). The experimental birds were distributed into five equal groups including control. Studies on body weight gain revealed that at the end of 4^{th} week of experiment, significant difference in body weight gain among the birds of the control and various treatment group was noticed ($P<0.05$) though there was subtle difference among the birds of control and various treatment groups (T_1-T_4). Birds of T_3 group revealed the highest body weight gain followed by the birds of T_1, T_2 and T_4 groups respectively. Birds of the control group revealed the lowest body weight gain. At the end of 5^{th} and 6^{th} week of experiment there was significant difference ($P<0.05$) in the body weight gain among the birds of the control and various treatment group. Birds of T_3 group revealed the highest body weight gain and the lowest body weight gain were observed in the birds of control group.

The effect of purified β-glucan from an edible mushroom (*Pleuratus florida*) as an immuno-modulator on the innate immune responses was studied (Paul *et al* 2013). In broiler chicken (40 No.), purified mushroom glucan was administered orally to 1 week old broiler chicks (20 No.) @ 20 mg/kg feed for 15 days and then switch back to control diet. Similar number of birds was kept separately as control with normal feeding regime. Non-specific immunity and protective ability were assessed in treated and control birds at the end of the experimental feeding period (0 day) by performing neutrophil, macrophage and lymphocyte functional assay in vitro and challenged with virulent field isolate of Newcastle Disease. Superoxide anion production by neutrophil, *in vitro* non-specific lympho-proliferation and IL-2 production were increased gradually up to 10 days post treatment and the increase was highly significant ($P<0.05$) compared to control birds. *In vitro* nitrite production by macrophage was found to be high in treated birds. Also mushroom glucan as a feed supplement significantly provided protection against Newcastle Disease. The result showed the potentiality of β-glucan (mushroom origin) as an immunostimulant in poultry.

Mode of immunomodulation

Prebiotics have the potentiality to enhance many host biological responses and reduce the mortality of fishes caused by invasion by pathogens. However, the anaerobic intestinal tract microbiota of commercially important fishes, such as channel catfish, hybrid striped bass, tilapia and salmonids need to be investigated to determine if there are any particular bacterial species to be enhanced with the use of prebiotics. By increasing the production of volatile fatty acids (VFAs) in the gastrointestinal (GI) tract, hosts benefit by recovering some of the lost energy from indigestible dietary constituents and by inhibiting potential pathogenic bacteria. The VFAs produced are also indicative of the microbial population present in the GI tract (Nisbet, 2002).

An immunomodulator is a substance (*e.g.* a drug) which has an effect on the immune system. Different Immunomodulators stimulate the immune system. Biological activities of immunomodulator are influenced by different physicochemical parameters, such as solubility, primary structure, molecular weight, branching and polymer charge (Bohn and BeMiller, 1995). During the development of immune reactions, immunomodulating the effects of *β*-glucans have been well established (Vetvicka and Sima, 2004). There are two main categories of the immunostimulators:

a) Specific immunostimulators provide antigenic specificity in immune response, such as vaccines or any antigen. For specific immune response, hosts should have prior exposure to an antigen after which recognition and subsequent activation occurs through a coordinated action of B-lymphocytes and T-cells. B cells are lymphocytes that play a large role in the humoral immune response (as opposed to the cell-mediated immune response, which is governed by T cells

b) The non-specific immunostimulators act irrespectively of antigenic specificity to enhance the immune response of other antigens or to stimulate components of the immune system without antigenic specificity, such as glucans, synthetic drug levamisole etc. Many endogenous substances are non-specific immunostimulators. For example, Glucans and mannans possess non-specific immunostimulatory effect. β-glucan is a polymer of glucose consisting of linear backbone of β-1,3 linked D-glucopyranosyl residues with varying degree of branching from the C_6 position (Bohn and BeMiller, 1995). *β*-Glucans are major components of yeasts, mushrooms and fungal mycelia. Mannan is a plant polysaccharide that is a polymer of the sugar mannose. Detection of mannan leads to lysis in the mannan binding lectin pathway.

Immunostimulants as a feed additive significantly provides protection against pathogen and upregulates phagocytosis, bacterial killing and oxidative burst. Probiotic microorganisms in the gut stimulate the immune response of host system in within two ways. They can migrate through the gut wall as viable cells thereby multiplying to a limited extent or the released antigens by the dead organisms are absorbed and stimulate the immune response directly. Probiotics generally find their applications in poultry feed because of their positive effects growth rate, improved feed conversion and improved resistance to diseases. Probiotics have a positive effect on host immune response through increased activity of macrophages shown by enhanced ability to phagocytose organisms or carbon particles, increased production of systemic antibody. *e.g.* IgM and interferon and increased effect of local antibody at mucosal surfaces such as gut wall. The effect of probiotics on the host immune system can be measured by estimating the levels of macrophage enzymes.

Prebiotics have many beneficial effects such as increased disease resistance and improved nutrient availability. As such, prebiotics they have the potential to increase the efficiency and sustainability of livestock and poultry production. The most commonly used prebiotic preparations are fructooligosaccharide (FOS), transgalactooligosaccharide (TOS), inulin, glucooligosaccharide, xylooligosaccharide, iso-maltooligosaccharide, soybeanoligosaccharide, polydextrose, lactosucrose (Vulevic *et al* 2004). Natural sources of prebiotics in vertebrates include chicory, onion, garlic, leek, tomato, honey etc.

Activity on immunostimulation

Glucans having a strong immunomodulating activity have been well studied in fishes and livestock (Anderson, 1992). Only a few immunostimulants can be used for commercial farming purposes (Siwicki *et al* 1998). Many studies have been carried out to measure the effects of glucans on host immunity. Some investigators have adopted the in vitro culture of macrophages with glucan (Cook *et al* 2003), but *in vivo* studies have been carried out by the majority of workers (Sahoo and Mukherjee, 2001; Ortuno *et al* 2002). Under intensive conditions, individuals are more susceptible to microbial infections especially in their growing stages. During stress, immunostimulants can provide resistance to pathogens. There are two types of glucans *α*- and *β*-glucans the numbers of which clarify the type of O-glycosidic bond. Glucans are commercially significant as immunostimulating agents. Different types of *β*-glucans have been used successfully to increase resistance of poultry, fish and crustaceans against bacterial and viral infections (Ganguly and Mukhopadhayay, 2011; Paul *et al* 2013). It has been seen that health, growth and general performance of livestock and poultry may be improved by the use of *β*-glucans (Paul *et al* 2012). Product source, animal species, development stage of the target organism, dose and type of glucan, route, time schedule of administration and association with the other immunostimulants affect immunomodulatory effects of glucans.

The immunostimulatory effects of glucan, chitin, lactoferrin, levamisole, vitamins B and C, growth hormone and prolactin have been reported. These immunostimulants mainly facilitate the function of phagocytic cells and increase their bactericidal activities. Several immunostimulants also stimulate the natural killer cells, complement, lysozyme and antibody responses of host. The most effective method of administration of immunostimulants is by injection. Oral and immersion methods have also been reported, but the efficacy of these methods decreases with long-term administration. Overdoses of several immunostimulants induce immunosuppression. Growth promoting activity has been noted in individuals treated with glucan or lactoferrin. Immunostimulants can overcome immune suppression by sex hormones (Ganguly *et al* 2009; 2010).

For the effective use of immunostimulants, the timing, dosages, method of administration and the physiological condition of the host needs consideration. Immunostimulants can reduce the losses caused by disease in commercial poultry rearing; however, they may not be effective against all diseases (Ganguly *et al* 2009; 2010).

Physiological implications

Literature reveal that calcium formate alone or in combination with other acids when given at the rate of 0.5% and 1.5%, increased FCR and growth performance in broiler chickens up to 35 days of age (Eidelsburger and Kirchgessner, 1994). It was observed mixture of organic acids (ACIDLAC) used as a replacer of AGPs and improved production performance in breeding hens along with other beneficial effects. It is also reported that mixture of organic acids, as a substituted of AGPs improved the performance of birds even in absence of antibiotic (Maiorka *et al* 2004). It was concluded from a dose responsive study (0-0.33%) that MOS @ 0.11%, maximized weight gain in poultry up to 0-8 weeks of age (Savage *et al* 1997). Same type of effect was found with supplementation of 0.1% MOS on hybrid Tom's body weight gain (Stanley *et al* 2004). It is reported from a study from turkeys supplemented with MOS that MOS may be utilized as a alternative to AGPs to improve turkey performance.

It has already been reported that 1% formic acid or 1.45% calcium formate did not affect live weight of broiler chicken (Izat *et al* 1990). It is evident that 80% formic acid and 20% propionic acid mixture added at 1% level to broiler chicken ration did not affect live weight. It has also been reported that formic acid and propionic acid mixture (85% and 15%) added at 1% level to the broiler chicken ration in the initial period did not affect weight gain (Visek, 1978). Reports have also been made about significant increased in body weight gain with the supplementation of 0.5% lactic acid in drinking water (Veeramani *et al* 2003). It was also revealed that increased in body weight with supplementation of lactic acid. The mix of organic acids improves performance of birds (Maiorka *et al* 2004). From a dose responsive study it was concluded (0-0.33%) that MOS @ 0.11%, maximized weight gain in poultry up to 0-8 weeks of age. The same type of effect was found with supplementation of 0.1% MOS on hybrid Tom's body weight gain (Parks *et al* 2001) conducted in turkeys supplemented with MOS that MOS may be utilized as a alternative to AGPs to improve turkey performance (Pelicano *et al* 2005).

Body performance

Dietary supplementation of different feed additives *e.g.* immunostimulants, probiotics and prebiotics usually in small quantities for the purpose of fortifying it with certain nutrients have been found to be beneficial for improving immune status, feed efficiency and growth performance of living beings also. The effect of dietary prebiotics on different body growth parameters of fish is highlighted (Ganguly *et al* 2012). However, the application of prebiotics and supplementary enzymes in fish feed is now gradually gaining importance in commercial livestock farming practices, the article stresses on the effect of prebiotics on live body weight gain, dressing percentage, weight of vital organs and muscles and mean villus lengths in digestive tract of fish along with their application as growth promoters in commercial poultry and aquafeed (Ganguly *et al* 2010).

It was reported that the body weight gain was improved by 12 to 25% and feed consumption was increased by 3 to 21% when chicks at 4 weeks of age fed diets supplemented with enzyme like xylanase. It was noticed that xylanase supplementation improved food conversion ratio by 2.2-2.9% and body weight gain by 0.2-2.5% (Veldman and Vahl, 1994). It was found that addition of xylanase significantly increased the weight gain up to 21 days of age and decreased the feed to gain ratio slightly (Danicke *et al* 2001). Feed efficacy and body weight gain was found to improve with the supplementation of xylanase (Mathlouthi *et al* 2003). It was observed that the xylanase supplementation significantly improved weight gain, feed efficiency and AME (Wu *et al* 2004). It was observed that addition of xylanase and phytase reduced the relative weight gain of the small intestine by 15.5% and 11.4% respectively. It was reported that body weight and feed per gain ratio was improved ($P<0.05$) by xylanase supplementation in the first 2-3 weeks in broilers (Shiyan *et al* 2005). It was noticed that xylanase supplementation increased body weight, feed intake and feed gain ratio. It was observed that xylanase supplementation increased body weight gain from 0-21 days of age of broilers (Liu *et al* 2007). It was found that the supplementation of xylanase enzyme improved ($P<0.05$) growth performance and feed conversion efficiency (Gao *et al* 2008).

It was reported that there was effect on the dressing percentage and weight of different organs and muscles at 21 and 42 days without any major influence on the dressing percentage, organ and muscle weight under different treatment groups with organic acid salts individually and its combination in broiler birds. Higher villus height in duodenum, jejunum in small intestine was reported with most organic acidifier in diet of broiler (Loddi *et al* 2004). Again it was reported higher villus height in the ileum with the diet based on organic acidifier compared with diet fed without MOS + organic acidifier (Savage *et al* 1997). Therefore, the supplementation of organic acidifier may increase villus height of different parts of small intestine. So organic acidifier reduces the growth of many pathogenic and non-pathogenic intestinal bacteria, decreases intestinal colonization and reduces infections process, ultimately decreasing inflammatory process at the intestinal mucosa. It increases villus height and function of secretion, digestion and absorption of nutrients can be appropriately performed by the mucosa. It was also reported positive effects of the use of prebiotics on the intestinal mucosa among which a significant increase in villus height of three segments of small intestine of birds, age one week and supplemented with MOS (Maiorka *et al* 2004). In the present study, use of organic acidifier in diet significantly increased villus height of different segment of small intestine of broiler possibly by reducing intestinal colonization of pathogenic and non-pathogenic bacteria, which is complied in early findings (Savage *et al* 1997; Loddi *et al* 2004). It was reported that in jejunum MOS + OA resulted in higher villi in the jejunum ($P<0.01$) followed by the diets containing MOS based prebiotics while in case of ileum, present investigation was partially corroborated with earlier findings (Pelicano *et al* 2005) who observed higher villi length when birds were fed with prebiotic based on MOS, compared to the control group.

Microorganisms that is sensitive to acid pH and results in higher villi length. Some bacteria may recognize binding sites on the prebiotics instead of intestinal mucosa and the colonization by pathogenic bacteria in intestine is thus reduced. Therefore, besides a lower infection incidence, there is an increase in the absorption of available nutrients, a mechanism that directly affects the recovery of the intestinal mucosa, increasing villi length. These results disagree to those obtained earlier (Santin *et al* 2001) respectively, who found no difference in ileal villi length with the use of probiotics and prebiotics.

Experimental studies were conducted to evaluate the pathomorphological effect of different combination of organic acids *viz.* formic acid, propionic acid and lactic acid as a replacer of growth promoter antibiotics (Roy *et al* 2012). The birds were divided into six equal groups *viz.* negative control (C_1), positive control (C_2) and treatment groups (T_1, T_2, T_3 and T_4). Birds of group C_1 were supplied with diet without any antibiotics or acid, group C_2 with Virginiamycin @ 500 g/quintal feed, group T_1 with 0.3% ammonium formate group T_2 with 0.3% calcium propionate, group T_3 with 0.15% ammonium formate and 0.15% calcium propionate and group T_4 with 0.1% ammonium formate, 0.1% calcium propionate and 0.1% calcium lactate. Body weight gain was higher in C_1 and C_2 compared with treatment groups in first two weeks, but pathological changes were maximum in negative control, *i.e.* after 6 weeks weight gain was significantly better in T_3 and T_4 than in groups C_1, T_1 and T_2 groups. It was reported that combined use of MOS and organic acid salts in poultry feed can be used as an alternative to the antimicrobial and antibiotic growth promoters to achieve good health for sustainable and economic poultry production in India (Das *et al* 2012).

Microbial biomass load in gastrointestinal tract

The findings that MOS and OA successfully reduces bacterial load in the intestine of broiler birds were in accordance with the previous findings (Spring *et al* 2000; Fairchild *et al* 2001). Yeast cell culture residue (YCR) treated broiler chicks resulted lower intestinal coliform population in comparison to control and other antibiotic treated (lasalocid @ 90.7 kg/ton, bacitracin @50 g/ton) groups (Stanley *et al* 2004). Bacteriological studies of intestine of broiler birds revealed reduced *E. coli* compared with the untreated control group. Salmonellae were not found in any group.

No significant difference was observed among control and treatment groups regarding the number of *Clostridium* sp. present in intestine. Above result clearly indicated that consumption of prebiotics mixed with poultry feed reduced load of coliform bacteria in the intestine (Roy *et al* 2012).

CONCLUSION

It can be summarized that supplementation of poultry feed with dietary prebiotics in proper proportions can enhance the immune system of the host by providing increased resistance to infections.

REFERENCES

Anderson DP. Immunostimulants, adjuvants and vaccine carriers in fish: applications to aquaculture. *Ann. Rev. Fish Dis.* 1992;2:281-307. [DOI: 10.1016/0959-8030(92)90067-8]

Bar N, Mukhopadhayay SK, Ganguly S, Pradhan S, Patra NC, Pal S, Goswami J, Singh YD, Halder S. Study on probiotic effect of xylanase supplementation in broiler feed. *Ind. J. Anim. Nutr.* 2012;29(1):100-3.

Bohn JA, BeMiller JN. (1->3)-β-glucans as biological response modifiers: a review of structure-functional activity relationships. *Carbohyd. Polym.* 1995;28(1):3-14. [DOI: 10.1016/0144-8617(95)00076-3]

Cook MT, Hayball PJ, Hutchinson W, Nowak BF, Hayball JD. Administration of a commercial immunostimulant preparation, EcoActiva as a feed supplement enhances macrophage respiratory burst and the growth rate of snapper (*Pagrus auratus*, *Sparidae* (Bloch and Schneider)) in winter. *Fish Shellfish Immunol.* 2003;14(4):333-45.

Danicke S, Jeroch H, Bottcher W, Bedford MR, Simon O. Effects of dietary fat type, pentosan level and xylanases on digestibility of fatty acids, liver lipids, and vitamin E in broilers. *Eur. J. Lipid Sci. Tech.* 1999;101(3):90-100. [DOI: 10.1002/(SICI)1521-4133(199903)101:3<90::AID-LIPI90>3.0.CO;2-Q]

Danicke S, Halle I, Strobel E, Franke E, Jeroch H. Effect of energy source and xylanase addition on energy metabolism, performance, chemical body composition and total body electrical conductivity (TOBEC) of broilers. *J. Anim. Physiol. Anim. Nutr. (Berl)* 2001;85(9-10):301-13. [DOI: 10.1046/j.1439-0396.2001.00318.x]

Das D, Mukhopadhayay SK, Ganguly S, Kar I, Dhanalakshmi S, Singh YD, Singh KS, Ramesh S, Pal S. Mannan oligosaccharide and organic acid salts as dietary supplements for Japanese quail (Coturnix Coturnix Japonica). *Int. J. Livest. Res.* 2012;2(3):211-4.

Eidelsburger U, Kirchgessner M. Effect of organic acids and salts in the feed on fattening performance of broilers. *Archiv Fuer Gefluegelkunde* 1994;58(6):268-77.

Fairchild AS, Grimes JL, Jones FT, Wineland MJ, Edens FW, Sefton AE. Effects of hen age, Bio-Mos and flavomycin, on poult susceptibility to oral Escherichia coli challenge. *Poult. Sci.* 2001;80(5):562-71.

Ganguly S, Dora KC, Sarkar S, Chowdhury S. Supplementation of prebiotics in fish feed - a review. *Rev. Fish Biol. Fisher.* 2012;23(2):195-9. [DOI: 10.1007/s11160-012-9291-5]

Ganguly S, Paul I, Mukhopadhayay SK. Immunostimulants - their significance in finfish culture. *Fish. Chimes.* 2009;29(7):49-50.

Ganguly S, Paul I, Mukhopadhayay SK. Applications and effectiveness of immunostimulants, probiotics, and prebiotic in aquaculture: a review. *Isr J. Aquacul. - Bamidgeh* 2010;62(3):130-8.

Ganguly S, Mukhopadhayay SK. Immunostimulants, probiotics and prebiotics: importance of immunostimulants, probiotic and prebiotic feed supplements in poultry ration & in commercial aquaculture. LAP Lambert Academic Publishing, Germany, 2011.

Ganguly S. Potential non-antibiotic growth promoting dietary supplements for animal nutrition: A Review. *J. Appl. Pharm. Sci.* 2013a;3(7):174-8. [DOI: 10.7324/JAPS.2013.3733]

Ganguly S. Implications for supplementation of dietary enzymes in poultry feed - A Review. *Int. Res. J. Pharm.* 2013b;4(5):10-1. [DOI: 10.7897/2230-8407.04503]

Gao F, Jiang Y, Zhou GH, Han ZK. The effects of xylanase supplementation on performance, characteristics of the gastrointestinal tract, blood parameters and gut microflora in broilers fed on wheat-based diet. *Anim. Feed Sci. Technol.* 2008;142(1-2):173-84.

Gibson GR, Roberfroid MB. Dietary modulation of human colonic microbiota: introducing the concept of prebiotics. *J. Nutr.* 1995;125(6):1401-12.

Izat AL, Adams MH, Cabel MC, Colberg M, Reiber MA, Skinner JT, Waldroup PW. Effects of formic acid or calcium formate in feed on performance and microbiological characteristics of broilers. *Poult. Sci.* 1990;69(11):1876-82.

Liu JR, Lai SF, Yu B. Evaluation of an intestinal Lactobacillus reuteri strain expressing rumen fungal xylanase as a probiotic for broiler chickens fed on wheat-based diet. *Br. Poult. Sci.* 2007;48(4):507-14. [DOI: 10.1080/00071660701485034]

Loddi MM, Moraes VMB, Nakaghi LSO, Tuca FM, Hannas MI, Ariki J. Mannan oligosaccharide, organic acids on performance and intestinal morphometric characteristic of broiler chickens. Abstract of Alltechs 20th Annual Symposium, Nicholasville, Kentucky, USA, 2004; 45.

Maiorka A, Santin AME, Borges SA, Opalinski M, Silva AVF. Evaluation of a mix of fumaric, lactic, citric and ascorbic acids on starter diets of broiler. *Arch. Vet. Sci.* 2004; 9(1):31-7.

Mathlouthi N, Juin H, Larbier M. Effects of xylanase and beta-glucanase supplementation of wheat- or wheat - and barley-based diets on the performance of male turkeys. *Br. Poult. Sci.* 2003;44(2):291-8. [DOI: 10.1080/0007166031000096498]

Nisbet, D. Defined competitive exclusion cultures in the prevention of enteropathogen colonisation in poultry and swine. *Antoine van Leeuwenhoek* 2002;81(1-4):481-6.

Parks CW, Grimes JL, Ferket PR, Fairchild AS. The effect of mannanoligosaccharide, bambermycins, and Virginia-mycin on performance of large white male market turkeys. *Poult. Sci.* 2001;80(6):718-23.

Paul I, Isore DP, Joardar SN, Roy B, Aich R, Ganguly S. Effect of dietary yeast cell wall preparation on innate immune response in broiler chickens. *Ind. J. Anim. Sci.* 2013;83(3): 307-9.

Paul I, Isore DP, Joardar SN, Samanta I, Biswas U, Maiti TK, Ganguly S, Mukhopadhayay SK. Orally administered β-glucan of edible mushroom (Pleuratus florida) origin upregulates innate immune response in broiler. *Ind. J. Anim. Sci.* 2012;82(7):745-8.

Pelicano ERL, Souza PA, Souza HBA, Figueiredo DF, Boiago MM, Carvalho SR, Bordon VF. Intestinal mucosa development in broiler chickens fed natural growth promoters. *Rev. Bras. Cienc. Avic.* 2005;7(4): Campinas Oct./Dec.

Rashid MM, Zahid Hosen SM, Saha D, Khanam UH, Khan MAU, Barua S, Nurul Afser ASM, Emran TB. Pro, pre, synbiotics & human health - a treatise. *Bull. Pharm. Res.* 2012;2(3):129-33.

Roy HS, Mukhopadhayay SK, Niyogi D, Choudhary PK, Ganguly S. Organic acids as a replacer of growth promoter antibiotics in broilers: pathological and bacteriological studies on intestine. *Ind. J. Vet. Pathol.* 2012;36(1):114-6.

Sahoo PK, Mukherjee SC. Effect of dietary beta-1, 3 glucan on immune responses and disease resistance of healthy and aflatoxin B1-induced immunocompromised rohu (Labeo rohita Hamilton). *Fish Shellfish Immunol.* 2001;11 (8):683-95.

Santin E, Maiorka A, Macari M, Grecco M, Sanchez JC, Okada TM, Myasaka AM. Performance and intestinal mucosa development of broiler chickens fed diets containing *Saccharomyces cerevisiae* cell wall. *J. Appl. Poult. Res.* 2001;10(3):236-44.

Savage TF, Zakrzewska EI, Andersen JR. The effects of feeding mannan oligosaccharide supplemented diets to poults on performance and the morphology of small intestine. *Poult. Sci.* 1997;76(1):139.

Shiyan Q, Yubo W, Changhua L, Limin G, Wenqing L, Defa L. Properties of Aspergillar xylanase and the effects of xylanase supplementation in wheat-based diets on growth performance and the blood biochemical values in broilers. *Asian Australas. J. Anim. Sci.* 2005;18(1):66-74.

Siwicki AK, Morand M, Terech-Majewska E, Niemczuk W, Kazun K, Glabski E. Influence of immunostimulants on the effectiveness of vaccines in fish: in vitro and in vivo study. *J. Appl. Ichthyol.* 1998;14(3-4):225-7. [DOI: 10.1111/j.1439-0426.1998.tb00646.x]

Spring P, Wenk C, Dawson KA, Newman KE. Effect of dietary mannanoligosaccharides on cecal parameters and concentration of enteric bacteria in the ceca of salmonella-challenged broiler chicks. *Poult. Sci.* 2000;79 (2):205-11.

Stanley VG, Gray C, Daley M, Krueger WF, Sefton AE. An alternative to antibiotic-based drugs in feed for enhancing performance of broilers grown on Eimeria spp.-infected litter. *Poult. Sci.* 2004;83(1):39-44.

Veldman A, Vahl HA. Xylanase in broiler diets with differences in characteristics and content of wheat. *Br. Poult. Sci.* 1994;35(4):537-50.

Veeramani P, Selvan ST, Viswanathan K. Effect of acidic and alkaline drinking water on body weight gain and feed efficiency in commercial broilers. *Ind. J. Poult. Sci.* 2003; 38(1):42-4.

Visek WJ. The mode of growth promotion by antibiotics. *J. Anim. Sci.* 1978;46(5):1447-69.

Vetvicka V, Sima P. β-glucan in invertebrates. *Invert. Surv. J.* 2004;1(1):60-5.

Vulevic J, Rastall RA, Gibson GR. Developing a quantitative approach for determining the in vitro prebiotics potential of dietary oligosaccharides. *FEMS Microbiol. Lett.* 2004; 236(1): 153-9. [DOI: 10.1016/j.femsle.2004.05.036]

Wu YB, Ravindran V, Thomas DG, Birtles MJ, Hendriks WH. Influence of phytase and xylanase, individually or in combination, on performance, apparent metabolisable energy, digestive tract measurements and gut morphology in broilers fed wheat-based diets containing adequate level of phosphorus. *Br. Poult. Sci.* 2004;45(1): 76-84.

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