INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print) 2222-5234 (Online)
http://www.innspub.net
Vol. 5, No. 1, p. 373-380, 2014

RESEARCH PAPER OPEN ACCESS

Effects of mannan oligosaccharides on ileal digestibility of nutrients and microbial populations in the ceca of broiler chickens

M. Afrouziyeh[1*], SH. Hanifian[2], M. Taghinejad[1]

[1]*Department of animal science, College of Agriculture, Tabriz Branch, Islamic Azad University, Tabriz, Iran,*

[2]*Department of Food science and Engineering, College of Agriculture, Tabriz Branch, Islamic Azad University, Tabriz,Iran*

Key words: Broiler, Ileal digestibility, mannan oligosaccharides, microbial populations, prebiotic.

http://dx.doi.org/10.12692/ijb/5.1.373-380 Article published on July 12, 2014

Abstract

Different levels of dietary mannan-oligosaccharide (MOS) and Avilamycin administered to commercial broilers were evaluated for their efficacy on performance, ileal digestibility of nutrients and microbial activity in the digestive tract. Dietary treatments included an antibiotic-free diet (CTL–), a positive control (CTL+, 10 mg/kg of Avilamycin), and three antibiotic-free diets containing Active Mos (MOS, 0.1, 0.2 or 0.3% of the diet). Each treatment was randomly assigned to 4 floor pen replicates (20 birds each). In grower and finisher period, weight gain and feed conversion ratio improved by addition of 2 and 3 g/kg prebiotic or avilamicin into basal diet compared with control diet ($P<0.05$). At d 28, the population of lactobacilli and Bifidobacteria were highest in birds fed 2 and 3g/kg MOS respectively. There was no significant difference in CP digestibility between birds consuming different treatments ($P>0.05$). The digestibility of OM was enhanced ($P<0.05$) with dietary supplementation of 2 g/kg MOS as compared to the control diet.

* **Corresponding Author:** M. Afrouziyeh ✉ afrouziyeh@iaut.ac.ir,

Introduction

Since the early 1950s, antibiotics have been widely used in poultry feeds, at first to control disease, but subsequently, subtherapeutic levels of antibiotics have been used to increase growth rates and improve feed efficiency. The introduction of antibiotic growth promoters (AGP) in livestock production has lead to substantial economic benefits (JETACAR, 1999), but with advances in animal genetics, nutrition, and vaccination programs, the magnitude of benefits from AGP has lessened. Currently, the subtherapeutic usage of antibiotics in livestock production is under severe scientific and public scrutiny, because AGP have been linked to the development of antibiotic-resistant pathogenic bacteria, which pose a threat to human health (Smith *et al.*, 2003). As result of such concerns, in 1997, the European Union initiated a ban on subtherapeutic usage of the antibiotic avoparcin in animal production, and all AGP were banned on January 1, 2006 (Burch, 2006). Although a complete ban on AGP has not been implemented in many countries, international pressure and public health concerns are likely to lead to such a scenario. Consequently, the poultry industry must develop alternatives to AGP to address public health concerns without compromising the efficiency of poultry production. Compounds that may have prebiotic effects are one possible way of improving intestinal health and performance in the absence of antibiotic growth promoters. A prebiotic compound was defined by Gibson and Roberfroid (1995) as "a nondigestible feed 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 and thus improves gut health." Mannan oligosaccharides (MOS) are among the classes of prebiotics that beneficially affect gut health, but they do so by different modes of action (Ferket, 2004). Research comparing BioMos (Alltech Inc., Nicholasville, KY), a commercial mannan oligosaccharide, to AGP shows that it can effectively suppress enteric pathogens, enhance the immune response, and improve the integrity of the intestinal mucosa in broilers (Spring *et al.*, 2000; Iji *et al.,* 2001). However, the effects of mannanoligosaccharides on the beneficial microorganisms in the chicken gut are not very consistent (Spring *et al.*, 2000; Fairchild *et al.,* 2001; Fernandez *et al.* 2002; Denev *et al.*, 2005). The addition of specific mannanoligosaccharides (MOS), derived from the outer cell wall of *Saccharomyces cerevisiae*, to a broiler chicken diet has been reported to improve their growth performance (Hooge,2004;Rosen, 2007). It appears that the response in growth performance to MOS is more pronounced in early life (Tucker *et al*, 2003; Jamroz *et al,* 2003; Yang *et al*, 2005). Decreased improvement in the growth performance of broiler chickens associated with age may be related to a less-balanced gut microflora in younger than older birds. During the growth of the birds, the gut microflora changes; for example, it takes about 2wk for lactobacilli to become the predominant bacteria (Barnes *et al*, 1972). It is well established that bacteria with type 1 fimbriae will bind to mannose-based receptors in the intestine. Furthermore, it has been demonstrated that MOS can act as a receptor analogue to prevent harmful bacteria possessing type-1 fimbriae from attaching to the gut wall, thereby helping birds to reach and maintain a healthy gut (Spring *et al*, 2000). More recently, it has been reported that the actual form of MOS will define their function and efficacy (Newman, 2006). However only a few studies have looked at the effects of MOS on the development of a normal gut microflora of birds (Kocher *et al*, 2005). In addition to measuring growth performance, which was the emphasis of most of the earlier studies with poultry, the effects of mannanoligosaccharides on ileal digestibility of crude protein and cecal microbial populations of *Bifidobacterium* and *Lactobacillus* spp. and *Escherichia coli* in chicks at different ages were determined. The present experiment was designed to test the hypothesis that the addition of an optimum dosage level of MOS to broiler chicken diets could lead to less pathogens in the intestinal microflora and an improved gut function, allowing for more consistent production responses.

Materials and methods

Bird Management and Experimental Design

Six hundred one day old Ross 308 broiler chicks were randomly assigned into 5 treatments. Each treatment includes 6 replicates and there were 20 chicks in each replicate. Feed and water were provided ad libitum. The broilers were raised on floor pens and maintained on 23-h continuous light with 1-h dark. The ambient temperature started at 33 ˚C (from day 0 to 3) and was gradually reduced according to usual brooding practices until 22 ˚C in day 49. This research project was carried out in compliance with the Guide to the Care and Use of Experimental Animals of the Canadian Council on Animal Care (Canadian Council on Animal Care, 1993). All chicks received common starter diet containing 23% crude protein from day 1 – 10, then, they received the experimental diets, which was formulated to be isonitrogenous, isoenergetic and to meet or exceed Ross 308 nutrient requirements (with 21% CP in grower diet from day 11 - 28 and 19% CP in finisher diet from day 29 – 49). The 5 experimental diets included the following: 1) negative control diet (CTL–), antibiotic-free; 2) positive control diet (CTL+), containing 10 mg/kg of Avilamycin; 3) CTL– with the addition of Active Mos (MOS, 0.1, 0.2 and 0.3% of the diet. Feed consumption and BW (by pen) were recorded at weekly intervals. Feed conversion ratio (FCR) calculated as the unit of eaten feed per unit of body weight gain.

Microbial Populations of Cecal Digesta

At 28 and 42 d of age, the cecal contents from each bird were aseptically transferred into sterile plastic bags and stored at –20°C for microbiological analysis. One gram of each cecal content sample was decimally diluted (from 10-1 through 10-8) using 0.85% sterile saline solution. The enumeration of different groups of bacteria was performed by plate count method using VRBA (Merck, Germany) supplemented with methylumbelliferyl-β-D-glucuronide (Scharlau, Spain) at 35°C for 24 to 48 h for E. coli (Hitchins *et al.*, 1998) and Rogosa agar (pH 5.5) at 30°C for 5 days in CO2 enriched atmosphere using Anaerocult C in anaerobic jar (All Merck, Germany), for lactobacilli (Gardiner *et al*, 1998), while bifidobacteria were enumerated on RB agar, at 38°C for 3 days in the anaerobic jar (Mikkelsen *et al.*, 2003). The mean value of the duplicate plate counts was then recorded for each sample.

Determining Ileal Digestibility of Nutrients

One week before determining of the ileal digestibility of nutrients (d 28) the experimental diets were supplemented with 0.4% of Cr2O3 as an indigestible marker. On d 35, three birds per pen (3 chicks per pen with 4 pens per treatment) were killed by intracardial injection of Ketamin. All of the ileal digesta between the yolk sac and the terminal ileum (4 cm above the ileal - cecal junction) were obtained immediately and carefully (Gong *et al*, 2002). The digesta from each of the 3 birds per pen was pooled as one sample into a plastic bag and immediately stored in -20 °C. Before analyses, the digesta samples were defreeze and ground through a 1.00 mm mesh screen, and then mixed thoroughly. Dry matter, organic matter (OM) and crude protein (CP) contents were determined (AOAC, 2007). Samples also were analyzed for chromic oxide (Fenton and Fenton, 1979). The apparent ileal digestibility values for dietary crude protein and organic matter were calculated as follows $DD = 1 - [(ID \times AF) / (IF \times AD)]$ Where DD is the apparent digestibility of a nutrient in diet; ID is the concentration of an indigestible marker in diet; AF is the nutrient concentration in ileal digesta; IF is the indigestible marker concentration in ileal digesta; and AD is the nutrient concentration in diet (Huang *et al,* 2005).

Statistical Analysis

Data achieved from plate counts were transformed into logarithmic scale prior to statistical analysis. Data were analyzed as a one-way ANOVA using the GLM procedure of SAS (SAS Institute, 2002), with pen serving as the experimental unit for performance parameters and bird as the experimental unit for ileal digestibility and microbiology parameters.

Results and discussion

Growth performance

The effects of dietary addition of avilamycin and different levels of MOS on broiler performance are shown in Table 1. In grower period, weight gain

improved by addition of 2 and 3 g/kg prebiotic or avilamicin into basal diet compared with control diet (P<0.05) and feed conversion ratio was significantly lower in diet supplemented by 3 g/kg prebiotic or avilamicin compared with control diet (P<0.05). In finisher period, supplementation of 2 and 3 g/kg prebiotic or avilamicin significantly improved feed conversion ratio and weight gain compared with control diet (P<0.05). In studies with broilers fed MOS, AGP, a combination of MOS and AGP, or an AGP free diet, Waldroup *et al* (2003) reported no improvement in growth performance and feed efficiency. However, based on a metaanalysis of 44 research trials with broilers, Hooge (2004) shows that birds fed MOS showed improved growth performance and feed efficiency compared with those fed AGP-free diets; performance was similar between MOS and AGP.

Table 1. Effects of antibiotics and different levels of mannan oligosaccharide on weight gain (g), feed consumption (g), and feed conversion of broiler chickens.

Treatments[1]						
	CTL-	CTL+	MOS (g/kg)			SEM[2]
Age			1	2	3	
Weight gain (g)						
d 1-10	160	164	161	164	165	1
d 11-28	854^{c}	879^{a}	863^{bc}	873^{ab}	883^{a}	2.7
d 29-42	1180^{c}	1310^{a}	1210^{bc}	1280^{ab}	1290^{a}	12
Feed consumption (g)						
d 1-10	243	244	239	246	241	1.3
d 11-28	1390	1380	1370	1400	1380	5.6
d 29-42	2190	2260	2140	2220	2250	16.4
Feed conversion rate						
d 1-10	1.51	1.49	1.48	1.50	1.46	0.008
d 11-28	1.63^{a}	1.57^{b}	1.58^{ab}	1.60^{ab}	1.56^{b}	0.0075
d 29-42	1.85^{a}	1.72^{b}	1.75^{ab}	1.74^{b}	1.74^{b}	0.0139

[1] CTL-: control diet without antibiotic and MOS, CTL+: control diet with antibiotic (Avilamycin). MOS: Mannanoligosaccharides.

[2] Standard error of mean.

Table 2. Effects of antibiotic and different levels of mannan oligosaccharides on ileal digestibility of crude protein (CP) and organic matter (OM) in broiler chickens.

	Treatments					
	MOS (g/kg)			CTL-	CTL+	SEM
	1	2	3			
Apparent digestibility of CP (%)	76.92	81.60	78.86	75.82	76.24	0.66
Apparent digestibility of OM (%)	79.06^{ab}	88.43^{a}	83.39^{ab}	70.69^{b}	78.79^{ab}	1.57

[1] CTL-: control diet without antibiotic and MOS, CTL+: control diet with antibiotic (Avilamycin). MOS: Mannanoligosaccharides.

[2] Standard error of mean.

Microbial composition

The populations of lactobacilli in the cecal digesta are shown in Figure 1. At both d 28 and 42, birds fed the CTL+ diet had the lowest population of lactobacilli. At d 28, the population of lactobacilli in birds fed 2g/kg MOS and at d 42 in those fed 3g/kg MOS exceeded that of birds fed control diets.

At d 28 the population of bifidobacteria in the cecal digesta was highest (P<0.05) in birds fed 3g/kg MOS. Fernandez *et al*. (2002) and Denev *et al*. (2005) reported increases in lactobacilli and bifidobacteria

populations in the ceca of broilers fed MOS compared with an AGP free diet. Sims *et al.* (2004) observed increased cecal population of bifidobacteria in turkeys fed MOS compared with an AGP-free diet, but there were no differences in cecal load of lactobacilli. Spring *et al.* (2000) also reported no effect of MOS on lactobacilli populations in the ceca of broilers. In studies with turkeys, Fairchild *et al.* (2001) reported that intestinal populations of lactobacilli and bifidobacteria did not differ among an AGP-free diet or those containing MOS or flavomycin. Factors contributing to variability in the effects of MOS on population of beneficial bacteria in the gut may include differences in experimental conditions, diet formulation, seasonal effects, and health status of the flock.

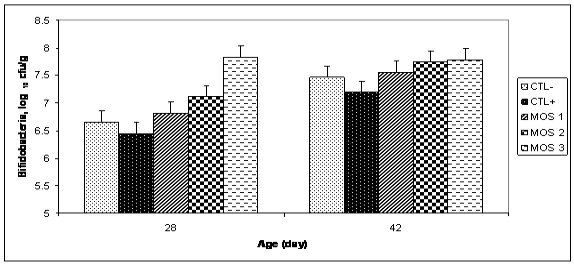

Fig. 1. Effects of antibiotic-free diets (CTL-); antibiotic-supplemented diets (CTL+, 11 mg/kg of Avilamycin); antibiotic-free diets supplemented with Active Mos (MOS) at 0.3, 0.2 and 0.1% in the diet on the populations of bifidobacteria in the cecal digesta of broiler chickens. a–bValues with different letters within a group are different (P<0.05).

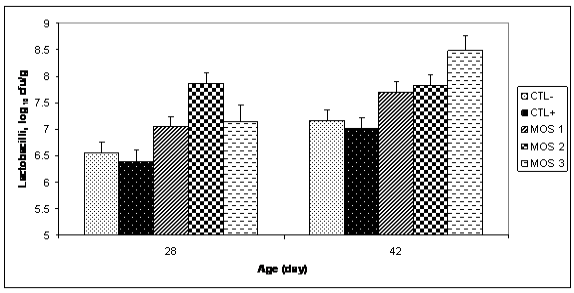

Fig. 2 Effects of antibiotic-free diets (CTL-); antibiotic-supplemented diets (CTL+, 11 mg/kg of *Avilamycin);* antibiotic-free diets supplemented with Active Mos (MOS) at 0.3, 0.2 and 0.1% in the diet on the populations of lactobacilli in the cecal digesta of broiler chickens. a–bValues with different letters within a group are different (Bonferroni t-test, P <□0.05).

Ileal digestibility

Table 2 shows effects of dietary addition of avilamycin and different levels of MOS on ileal digestibility of nutrients. There was no significant difference in CP digestibility between birds consuming different treatments (P>0.05). The digestibility of OM was enhanced (P < 0.05) with dietary supplementation of 2 g/kg MOS as compared to the control diet. However, the difference between diet contain 2 g/kg MOS and diet contain 3.0 g/kg MOS was not significant.

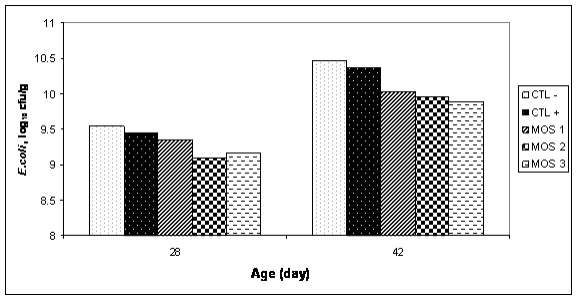

Fig. 3. Effects of antibiotic-free diets (CTL-); antibiotic-supplemented diets (CTL+, 11 mg/kg of *Avilamycin*); antibiotic-free diets supplemented with Active Mos (MOS) at 0.3, 0.2 and 0.1% in the diet on the populations of E.coli in the cecal digesta of broiler chickens.

Several studies have investigated the effects of oligosaccharides on digestibility of DM, energy, calcium, CP and phosphorus (Huang *et al*, 2005; Li *et al*, 2007). However, this kind of studies is scarce. The finding of improved digestibility of OM is in agreement with the results of Huang *et al.* (Huang *et al*, 2005), who found that dietary supplementation of oligosaccharides improved nutrient digestibility and feed efficiency in broiler chickens. They suggested that an increase in the digestion and absorption of nutrients is a major mechanism responsible for the enhanced growth performance of broilers in response to dietary oligosaccharide supplementation. Dietary supplementation with prebiotics has been shown to improve the health status of the gastrointestinal tract; therefore, these substances are being actively investigated as indirect growth promoters (Patterson and Burkholder, 2003). The nutrient digestibility enhancement in broilers supplied with oligosaccharide diets may be due to an improvement

in gut health (Tuohy *et al*, 2003). According to previous study (Lemieux *et al*, 2003), the enhanced ileal digestibility of nutrients in the broilers fed the diets containing oligosaccharide might be explained by the following findings. First, oligosaccharide supplementation reduced the number of pathogenic bacteria (e.g., *E. coli*, *Salmonella typhimurium*) and increased the beneficial bacteria (acid producer bacteria e.g., *Lactobacilli*) numbers in the intestine. Second, oligosaccharides may stimulate the secretion of digestive enzymes from the stomach, pancreas, and intestinal mucosa. Mannanoligosaccharides provide nutrients effectively stimulate the growth of beneficial microflora in the small and large intestine and the result would be better balance of bacterium population. These new bacteria population produce different digestive enzymes which add to existing broiler endogenous enzymes (Potter and Shelton, 1984). The findings of a possible synergism between improved gut microflora and digestibility of organic matter and birds performance is also an important aspect of this trial.

References

AOAC: Official Methods of Analysis of AOAC International 2007. 18th ed. Rev. 2. AOAC Int., Gaithersburg, MD.

Barnes EM, Mead, Barnum. 1972. The intestinal flora of the chicken in the period 2 to 6 weeks of age, with particular reference to the anaerobic bacteria. Br. J. Nutr. **13,** 311–326.

Burch D. 2006. Anticipated effects of the withdrawal of antibiotic growth promoters (AGPs) from pigs in the European Union on 1st January 2006.http:www.octagonsevices.co.UK/articles/withdrawalAGP.htm. Accesses Aug. 2006.

Canadian Council on Animal Care. 1993. Guide to the Care and Use of Experimental Animals. Vol. 1. Can. Counc. Anim. Care, Ottawa, Ontario, Canada.

Denev SA, Dinev I, Nikiforov I, Koinarski V. 2005. Effects of mannan oligosaccharides on composition of cecal microflora and performance of broiler chickens. Pages 351-353 in 15th Eur. Symp. Poult. Nut., Balatonfüred, Hungary. World,s Poult. Sci. Assoc., Budapest, Hungary.

Fairchild AS, Grimes JL, Jones FT, Wineland MJ, Ednes FW, Sefton AE. 2001. Effects of hen age, Bio-MOS and flavomycin on poult susceptibility to oral Escherichia coli challenge. Poult. Sci. **80,** 562-571.
http://dx.doi.org/10.1093/ps/805.562

Fenton TW, Fenton M. 1979. Determination of chromic oxide in feed and feces. Can. J. Anim. Sc. **58,** 631-633.
http://dx.doi.org/104141/cjas79-081

Ferrandez F, Hinton M, Van Gils B. 2002. Dietary mannan oligosaccharides and their effect on chicken caecal microflora in relation to Salmonella Enteritidis colonization. Avian Pathol. **31,** 49-58.
http://dx.doi.org/101080/03079450120106000

Gardiner G, Ross RP, Collins JK, Fitzgerald G. Stanton C. 1998. Development of a Probiotic Cheddar Cheese Containing Human-Derived Lactobacillus paracasei Strains. Applied and Environmental Microbiology **64,** 2192-2199.

Gibson GR, Roberfroid MB. 1995. Dietry modulation of the human colonic microflora: introducing the concept of prebiotics. J.Nutr. **125,** 1401-1412.

Gong J, Forster RJ, Yu H, Chambers JR, Wheatcroft R, Sabour PM, Chen S. 2002. Moleculer analysis of bacterial population in the ileum of broiler chickens and comparison with bacteria in the cecum. FEMS Microbial. Ecol. **41,** 171-179.
http://dx.doi.org/10.1111/j.15746941.2002.tb00978.x

Hitchins AD, Feng P, Watkins WD, Rippey S. R, Chandler LA. 1998. *Escherichia coli* and the

Coliform Bacteria. In: Jackson, G.J., Merker, R.I., Bandler, R. (Eds.), Bacteriological Analytical Manual, 8th ed. FDA publication, USA, 156-170 p.

Hooge DM. 2004. Meta-analysis of broiler chicken pen trials evaluating dietary mannan oilgosaccharides, 1993-2003. Int. J. Poult. Sci. **3,** 163-174.
http://dx.doi.org/10.3923/ijps.2004163.174

Huang RL, Yin YL, Wu GY, Zhang YG, Li YJ, Li LL, Li MX, Tang ZR, Zhang J, Wang B, He JH, Nie XZ. 2005. Effext of dietary oligochitosan supplementation on ileal digestibility of nutrients and performance in broilers. Poult. Sci. **84,** 1383-1388.
http://dx.doi.org/10.1093/ps/849.1383

Iji P, Saki AA, Trivery DR. 2001. Intestinal structure and function of broiler chickens on diets supplemented with a mannanoligosaccharide. J. Sci. Food Agric. **81,** 1186-1192.
http://dx.doi.org/101002/jsfa.925

Jamroz D, Wiliczkiewicz A, Orda J, Wertelecki T, Skorupinska J. 2003. Effect of a feed antibiotic or mannan oligosaccharides in broiler chickens. Poster in Alltech's 20th Annu. Symp. Nutr. Biotechnol. Feed Food Ind., Lexington, KY. Nottingham Univ. Press, UK.

JETACAR. 1999. The use of antibiotics in food-producing animals: Antibiotic-resistant bacteria in animals and humans. Rep. Joint Expert Advis. Comm. Antibiot. Resist. Commonw. Aust., Canberra.

Kocher A, Denev SA, Dinev I, Nikiforov I, Scheidemann C. 2005. Effects of mannanoligosaccharides on composition of the cecal microflora and performance of broiler chickens. Pages 216–220 in Proc. 4 BOKU-Symp. Tierernа¨hr., Tierernа¨hr. ohne antibiotische Leistingsfo¨rderer. Univ. Bodenkunde Wien., Vienna, Austria.

Lemieux FM, Southern LL, Bidner TD. 2003. Effect of mannan oligosaccharides on growth performance of weanling pigs. J. Anim. Sci. 81: 2482–2487.

Li XJ, Piao XS, Kim SW, Liu P, Wang L, Shen YB, Jung SC, Lee HS. 2007. Effect of citho-oligosaccharide supplementation on performance, nutrient digestibility, and serum composition in broiler chickens. Poult. Sci. **86,** 1107-1114.
http://dx.doi.org/10.1093/ps/866.1107

Mikkelsen LL, Bendixen C, Jakobsen M, Jensen BB. 2003. Enumeration of Bifidobacteria in Gastrointestinal Samples from Piglets. Applied and Environmental Microbiology, **69(1),** 654–658.
http://dx.doi.org/10.1128/AEM.69.1.654-6582003

Newman K. 2006. Quantifying the efficacy of MOS. Feed Mix. **14,** 2–4.

Patterson JA, Bulkholder KM. 2003. Application of probiotics and prebiotics in poultry production. Poult. Sci. **82,** 627-631.
http://dx.doi.org/10.1093/ps/824.627

Potter LM, Shelton JR. 1984. Methionine, cystine sodium sulfate, and Fermacto-500 supplementation of practical-type diets for young turkeys. J. Poult. Sci. **63,** 987-992.
http://dx.doi.org/10.3382/ps0630987

Rosen GD. 2007. Holo-analysis of the efficacy of Bio-Mos in broiler nutrition. Br. Poult. Sci. **48,** 21–26. http://dx.doi.org/101080/00071660601050755.

SAS User's Guide. 2002. Version 9.0 ed. SAS Inst. Inc., Cary, NC.

Sims MD, Dawson KA, Newman KE, Spring P, Hooge DM. 2004. Effects of mannanoligosaccharide, bacitracin methylene disalicyclate , or both on live performance and intestinal microbiology of turkeys. Poult. Sci. **83,** 1148-1154.
http://dx.doi.org/10.1093/ps/83.71148

Smith DL, Johnson JA, Harris AD, Furuno J. P, Perencevich EN, Morris JG. 2003. Assessing risks for a pre-emergent pathogen: Virginamycin use and the emergence of streptogramin resistance in Enterococcus faecium. Lancet Infect. Dis. **3,** 241-249. http://dx.doi.org/101016/S1473-3099(03)00581-4

Spring P, Wenk C, Dawson KA, Newman KE. 2000. The effects of dietary mannan oligosaccharides on cecal parameters and the concentrations of enteric bacteria in the ceca of salmonella challenged broiler chickens. Poult. Sci. **79,** 205-211. http://dx.doi.org/10.1093/ps/79.2205

Tucker LA, Esteve-Garcia E, Connolly A. 2003. Dose response of commercial mannan oligosaccharides in broiler chickens. Poster presented at WPSA 14th Eur. Symp. Poult. Nutr., Lillehammer, Norway. World's Poult. Sci. Assoc., Norwegian Branch, Lillehammer.

Tuohy KM, Probert HM, Smejkal CW, Gibson GR. 2003. Using probiotics and prebiotics to improve gut health. Therapeutic Focus. **8,** 692–700.

Waldroup PW, Fritts CA, Yan F. 2003. Utilization of Bio-Mos mannan oligosaccharide and Bioplex copper in broiler diets. Int. J. Poult. Sci. **2,** 44–52. http://dx.doi.org/10.3923/ijps.2003.4452

Yang Y, Choct M, Iji PA. 2005. Effect of dietary mannanoligosaccharide level on performance and gross morphology of digestive tract segments of broiler. Pages 72–75 in Proc. 17th Annu. Aust. Poult. Sci. Symp., Sydney, NSW, Australia. Poult. Res. Found., Univ. Sydney, Australia.