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Full Length Research Paper

The effects of mannan-oligosaccharides on cecal microbial populations, blood parameters, immune response and performance of broiler chicks under controlled condition

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A study was conducted to evaluate the effects of mannan- oligosaccharides (MOS) on cecal microbial populations, immune responses to phytohaemagglutinin-P (PHA-P) and sheep red blood cell (SRBC) and performance (weight gain and feed conversion ratio) of broiler chicks under strict controlled condition. Sixty four day-old male broiler chicks were randomly assigned to 12 battery cages pens of 4 chicks each and were fed from 1 to 42 days of age. Two basal diets were formulated for starter (1 to 21 days) and grower (22 to 42 days) period and a graded level of MOS (0.5, 1 and 1.5 g/kg) were added to basal diet to make diets 2 to 4. Body weight gain and feed conversion ratio were measured at 21, 35 and 42 days of age. Immune response to PHA-P was measured at 35 and to SRBC at 28 and 42 days of age. Cecal contents were assayed for *lactobacilli* and *Escherichia coli* at 42 days of age. Plasma triglyceride, cholesterol and HDL concentration was measured at 42 days of age. Body weight gain and feed conversion ratio did not differ among dietary treatments. Carcass, breast, tight, gizzard, duodenum, jejunum and ileum relative weight and duodenum, jejunum and ileum relative length were not affected by treatments. MOS increased immune response to PHA-P and SRBC numerically. Plasma triglyceride and HDL concentration did not differ among the treatments but plasma cholesterol concentration decreased linearly by adding MOS to the basal diet (P<0.05). Results showed no significant differences in *lactobacilli* and *E. coli* content of ceca among the treatments. Results of this experiment showed that MOS could affect the immune response and performance and as well as plasma cholesterol regardless of strict controlled condition.

Key words: Mannan-oligosaccharides, broiler chicks, performance, immune response, plasma factors.

INTRODUCTION

The subtherapeutic usage of antibiotic growth promoters (AGP) is under intense scientific and public scrutiny because their use has been linked to the development of antibiotic -resistant pathogenic bacteria, which pose a threat to human health (Smith et al., 2003). At the same time, food safety remains a major public health concern worldwide. Prebiotics are defined as “a nondigestible 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) . Mannose-based carbohydrates occur naturally in many products, such as yeast cell walls or different gums, which are available at reasonable prices. The observation on the effects of mannanoligosaccharides (MOS) on broiler performance is controversial. Hooge (2004) reported that growth performance and feed efficiency were improved in birds fed MOS compared with those fed basal diet. Based on the published research, it appears that MOS have variable effects on broiler performance. These may be attributed to differences in

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the type of product, experimental conditions, diet formulation, and health status of the birds. It is reported that (Sims et al., 2004; Hooge, 2004) most beneficial additives are most effective under stress and disease conditions. Supplementation of poultry diets with MOS results in improved production in terms of body weight gain and feed conversion (Parks et al., 2001), partly due to its hypothesized nutrient sparing effect but primarily due to its influence on nutrient utilization in the gastrointestinal tract (Kumprecht et al., 1997; Savage et al., 1997; Sonmez and Eren, 1999). In addition, previous reports suggest that MOS supplementation resulted in significant improvement in antibody responses in broiler and layers (Cotter et al., 2000; Raju and Devegowda, 2002). Most of the experiments have investigated the effects of MOS on performance and gut microflora, but there is no information about the effects of these products on blood parameters such as cholesterol. On the other hand almost all of the experiments in this field were done at the commercial or challenging condition where the effects of MOS are so clear. The hypothesis tested in this study was that mannanoligosaccharides (MOS) from yeast cell walls even so are able to improve broiler performance, decrease cholesterol concentrations of plasma and stimulate immune response in broiler chicks under strict controlled conditions.

MATERIALS AND METHODS

Sixty four 1-day old male Ross 308 broilers were obtained from a commercial local hatchery and grown over a 42 days experimental period. The chicks were housed in thermostatically controlled batteries with raised wire floors in an environmentally controlled building. At hatch, chicks were weighed and randomly allotted to 4 pens of 4 chicks per treatment so that each pen of chicks had a similar initial weight and weight distribution. Throughout the study, the birds were brooded following standard temperature regimens, which gradually decreased from 32 to 24°C, and under a 23L: 1D cycle. The 4 experimental diets included control diet and control diet with addition of graded levels (0.05, 0.1 and 0.15%) of TechnoMOS (Biochem®) as a commercial source of mannan oligosaccharide. The ingredient composition and nutrient content of the diets are shown in Table 1. Body weights and feed intakes were measured at the end of Weeks 3, 5 and 7. Weight gain, feed conversion ratio and mortality were calculated for each pen replicate. No mortality occurred in any of the experimental groups.

Immune measurements

At the 21 and 35 days of age 0.1 ml/kg body weight of 0.5% sheep red blood cell was injected into brachial vein of two chicks per replicate. Seven days after each injection, blood was collected in nonheparinized tubes by puncturing the brachial vein. Serum was isolated and stored at −20°C. Individual serum samples were analyzed for antibody responses against SRBC by ELISA technique using commercial kits, and the plates were read at 405 nm on an ELISA reader. Two chicks per pen were randomly chosen to evaluate a cutaneous basophil hypersensitivity test to phytohaemagglutinin-P (PHA-P) on day 36. At 36 days of age, the right toe web thickness was measured with a constant tension caliper before injection of 100 μg of PHA-P suspended in 0.10 ml of sterile PBS. Twenty-four hours after the injection, the toe web was measured again. Relative swelling and toe thickness were indicators of the cellular immune response. A saline control was not used due to minor differences observed between the PHA-P and the saline-injected toe web (Corrier et al., 1990).

Carcass analysis and blood parameter

At the end of experimental period, 2 chicks with weight closest to the mean average of each replicate were selected randomly and blood samples were collected in heparinized tubes by puncturing the brachial vein to measuring the cholesterol, triglyceride and HDL, then these chicks were slaughtered by neck cutter and the weights of breast, thigh, abdominal fat, gizzard, duodenum, jejunum and ileum, also the length of duodenum, jejunum and ileum were measured.

Cecal microbial populations

At 42 days, 2 chicks from each pen replicate were slaughtered by neck cutter for the extraction of cecal contents. The cecal contents from ceca of each bird were pooled together for serial dilution. Microbial populations were determined by serial dilution (10^{-4} to 10^{-7}) of cecal samples in anaerobic diluents before inoculation onto Petri dishes of sterile agar as described by Bryant and Burkey (1953). *Lactobacilli* were grown on Rogosa SL agar, and *E. coli* were grown on EMB agar. Plates for *Lactobacillus* was incubated anaerobically (73% N: 20% CO2: 7% H2) at 37°C. *E. coli* were incubated aerobically at 37°C. Plates were counted between 24 and 48 h after inoculation. Colony forming units (CFU) were defined as being distinct colonies measuring at least 1 mm in diameter.

Statistical analysis

Data were analyzed by one way ANOVA using the general linear model (GLM) procedure of SAS software (SAS Institute, 2003) with pen as the experimental unit for performance parameters and bird as the experimental unit for microbiological parameters. Differences between treatment means were tested using Duncan multiple comparison test, and statistical significance was declared at a probability of $p< 0.05$. Microbiological concentrations were subject to log10 transformation before analysis.

RESULTS

Dietary treatments did not alter growth performance or feed conversion ratio (Table 2). MOS had no significant effect on relative weight of breast, thigh, abdominal fat and gizzard. Neither relative weight nor relative length of duodenum, jejunum and ileum were affected by addition of MOS (Table 3). The effect of MOS on immune response was significant and MOS increased linearly cutaneous basophil hypersensitivity test to phytohaemagglutinin-P ($p<0.05$) but immune response against the SRBC was not significant and immune response increased numerically by addition of MOS compared to the basal diet (Table 4). There was no significant effect of MOS on plasma triglyceride and HDL concentration at 42 days of age (Table 4). Supplementation of the basal diet with MOS significantly reduced the plasma cholesterol concentration at 42 days

Table 1. Nutritional composition of basal diets %.

Ingredients	0 - 21 days of age	22 - 42 days of age
Wheat grain	10	10
Corn grain	39.3	49.28
Soybean meal (44%)	41.63	31.01
Soybean oil	5	6.07
Dicalcium phosphate	2.07	1.73
Oyster shell	0.86	0.76
Common salt	0.36	0.36
Vitamin mix[1]	0.25	0.25
Mineral mix[1]	0.25	0.25
DL-Met	0.22	0.22
L-Lysine	0.06	0.07
Calculated analysis		
CP (%)	23	19.2
ME (kcal/kg)	3010	3200
Digestible Lys (%)	1.19	0.96
Digestible Met (%)	0.52	0.49
Digestible Met + cystine (%)	0.86	0.77

[1]Vitamin and mineral mix supplied the following per kilogram of diet: vitamin A, 11,000 IU; vitamin D3, 1,800 IU; vitamin E, 11 mg; vitamin K3, 2 mg; vitamin B2, 5.7 mg; vitamin B6, 2 mg; vitamin B12, 0.024 mg; nicotinic acid, 28 mg; folic acid 0.5 mg; pantothenic acid, 12 mg; choline chloride, 250 mg; Mn, 100 mg; Zn, 65 mg; Cu, 5 mg; Se, 0.22 mg; I, 0.5 mg; and Co, 0.5 mg.

Table 2. Effects of mannanoligosaccharide on body weight and feed conversion of broiler chicks.

Age	Treatments				SEM[1]	*p* value
	Control	MOS				
		0.05%	0.1%	0.15%		
BW (g)						
Day 21	784.00	814.88	791.31	830.63	9.54	0.30
Day 35	1974.38	1947.50	1995.00	2029.38	22.35	0.66
Day 42	2485.31	2436.25	2497.19	2517.69	15.97	0.34
Feed conversion						
Day 21	1.300	1.290	1.320	1.270	0.017	0.72
Day 35	1.586	1.555	1.558	1.552	0.014	0.84
Day 42	1.674	1.659	1.652	1.643	0.012	0.86

[1-]Standard error of mean.

of age ($p<0.05$). There was no effect of dietary treatment on cecal lactobacilli and *E.* coli populations.

DISCUSSION AND CONCLUSION

Body weight gain and feed conversion ratio were not affected by MOS. In studies with broilers fed with MOS, virginiamycin, a combination of MOS and virginiamycin, or an antibiotic free diet, Waldroup et al. (2003) reported no improvement in growth performance and feed efficiency. However, based on a metanalysis of 44 research trials with broilers, Hooge (2004) concluded that birds fed MOS showed improved growth performance and feed efficiency compared with antibiotic free diet. It is reported that MOS and most beneficial additives (Hooge, 2004) are most effective under disease and stress conditions, such as extremes of ambient temperature, crowding, and poor management, which are invariably present in commercial broiler production. The present study was conducted under good hygienic conditions (new experimental facility, strict biosecurity measures,

Table 3. Effect of MOS on relative weight of different parts of gut and relative length of duodenum, jejunum and ileum (g/100 g BW).

Treatments	%Weight								%Length		
	Carcass	Breast	Thigh	Abdominal fat	Gizzard	Duodenum	Jejunum	Ileum	Duodenum	Jejunum	Ileum
Control	72.975	24.913	19.890	1.335	1.141	0.376	0.939	0.822	1.039	2.884	3.191
0.05% MOS	72.269	25.228	19.540	1.258	1.049	0.346	0.914	0.705	1.125	2.926	3.029
0.1% MOS	72.488	25.628	19.440	1.468	1.109	0.388	1.004	0.798	1.157	2.779	2.779
0.15% MOS	72.974	25.140	19.412	1.761	1.122	0.382	1.034	0.841	1.121	2.897	2.973
SEM	0.272	0.195	0.11	0.078	0.027	0.009	0.02	0.019	0.022	0.055	0.046
p value	0.8	0.67	0.43	0.09	0.7	0.47	0.11	0.3	0.26	0.83	0.21

Table 4. Effect of MOS on cutaneous basophil hypersensitivity test to PHA-P, immune response against SRBC and on blood cholesterol, triglyceride and HDL with cecal microbial populations.

Treatments	Toe web thickness (mm)	SRBC 28 day	SRBC 42 d	Triglyceride (mg/dl)	HDL(mg/dl)	Cholesterol (mg/dl)	*Lactobacilli* log10 cfu/g of DM	*Escherichia coli* log10 cfu/g of DM
Control	0.995^{b}	4.13	7.75	97.25	80.625	135.13^{a}	9.11	6.04
0.05 % MOS	1.685^{ab}	5.38	8.125	122.13	82.625	118.00^{ab}	8.92	5.93
0.1 % MOS	1.890^{a}	4.88	7.75	120.75	77.125	112.50	8.52	6.15
0.15 % MOS	2.045^{a}	4.88	8.125	83.38	79.25	108.75^{b}	8.51	6.34
SEM	0.157	0.285	0.362	7.607	2.122	3.634	0.21	0.11
P Value	0.06	0.53	0.97	0.2	0.86	0.02	0.16	0.1

no access to feces, clean litter, good ventilation, and low stocking density), thus implying minimum bacterial challenge. Under such conditions, the chicks may not have required any feed additive for maximum productive response. Relative weight of different parts of gut and relative length of small intestine were not influenced by supplementation of MOS. Ferket et al. (2002) reported that intestinal weight and crypt depth were similar when turkeys were fed MOS or an antibiotic-free diet; however, muscularis thickness was significantly reduced. Increases in gut mass are associated with inflammation following bacterial infection (Walton, 1988), and this notion is supported by the observation that germ-free birds have thinner muscularis mucosa than conventional birds (Gordon and Bruckner-Kardoss, 1961). The reason for *the lack of an effect of the feed additives on gut parameters may be that under the* conditions of this experiment the pathogen load in the gut was low. Controlling the growth of intestinal microflora is important for improving the well being of the host. Many bacteria compete with the host for nutrients within the gastrointestinal tract, elicit an immune response that causes a reduction in appetite and an increase in muscle catabolism to maintain the immune response, cause disease, and reduce nutrient absorption in the intestine (Bedford, 2000). There was no significant effect of MOS on ceca population in the condition of this experiment. 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 by supplementing of MOS. 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. For example, Coon et al. (1990) reported any effects of oligosaccharide on cecal microbe populations with soybean meal diet, because soybean meal contains approximately

6% raffinose plus stachyose, the lack of response in cecal microbial numbers may have resulted because the dietary soybean meal already provided a large amount of indigestible oligosaccharides. Regardless of the strict controlled conditions, results of this experiment showed that MOS can improve the immune response of broiler chicks. Results of research have shown that MOS has immunomodulatory properties (MacDonald, 1995; Savage et al., 1996; Cotter, 1997; Cotter et al., 2002). The yeast cell wall has powerful antigenic stimulating properties, and it is well established that this property is a characteristic of the mannan chain (Ballou, 1970) . MOS had no effect on plasma triglyceride and HDL concentration but significantly reduced cholesterol. Results of many experiments have shown that prebiotics can reduce plasma cholesterol by different ways.

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