African Journal of Biotechnology Vol. 10(32), pp. 6172-6176, 4 July, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB10.2103
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Influence of dietary mannanoligosaccharides on histological parameters of the jejunal mucosa and growth performance of broiler chickens

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Accepted 15 April, 2011

The trial involved 480 Hubbard Classic broiler chicks which were from either mannanoligosaccharide (MOS) fed breeder flock (Bio-Mos, Alltech Inc. USA at level of 1 kg/t) or control fed breeder flock (without MOS). Three groups with four replicates per treatment were formed: control fed breeders/control fed broilers (C/C); MOS fed breeders/control fed broilers (BM/C) and MOS fed breeders/MOS fed broilers (BM/BM). All chicks were fed the same basal diet, except for the inclusion of Bio-Mos (1, 0.75 and 0.5 kg/t in the starter, grower and finisher diet, respectively). The results showed a significant improvement ($p<0.05$) in the body weight gain with the addition of Bio-Mos in broiler feed. Feed conversion ratio was improved by 0.03 points, but the difference was not significant ($P>0.05$). The gut morphology examination showed that chick origin (chicks that originated from Bio-Mos fed breeders or control fed breeders) did not influence the morphological parameters of the jejunum in the broiler chickens, but addition of Bio-Mos directly to the broiler feed had a significant influence on the gut morphology and played an important role in processes of digestion and absorption, leading to improved performance.

Key words: Broiler, mannanoligosaccharides, growth, jejunum, histology.

INTRODUCTION

The gastrointestinal tract has different ways of adapting or reacting morphologically to changing conditions such as alteration in the diet (Huisman et al., 1990; Van der Klis et al., 1993) or altered composition of the intestinal microflora (Koninkx et al., 1988). The intestine has an inherent ability to create and maintain regional differences with regards to mucosal structure (Ferraris et al., 1992). The intestine can change its surface by growing in length, and/or by increasing or decreasing the height of its villi. Shortening and fusion of villi will result in loss of surface area for digestion and absorption of food (Van Dijk et al., 2002). Many benefits associated with the presence of oligosaccharides in the diet have been identified. In chicken nutrition, mannanoligosaccharide has been largely shown to reduce disease risk, possibly through a reduction in proliferation of pathogenic species (Spring et al., 2000; Peuranen et al., 2006). According to Lange (2007), adding Bio-Mos to broiler breeder diets improves breeder and broiler performance and immune status. Shashidhara and Devegowda (2003) reported that maternal antibody titres in progeny were positively influenced by mannanoligosaccharide (MOS) supplementation in breeder diets. Beyond the maintenance of health, they have been shown to improve the growth performance of poultry (Hooge, 2004; Rosen, 2007) and to have an important influence on gut morphology of broiler chickens (Uščebrka et al., 2005; Yang et al., 2007).

The objective of this study was to examine the effects

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Table 1. Composition of the basal diets.

Ingredient	Starter (from 1 to 21 days)	Grower (from 22 to 35 days)	Finisher (from 36 to 42 days)
Corn (%)	50.0	57.5	64.0
Soybean meal (44% CP)	22.5	16.5	16.0
Full fat soybeans (%)	22.0	20.0	14.0
Vegetable oil (%)	1.2	2.0	2.0
Limestone (%)	0.9	1.0	1.1
Dicalcium phosphate (%)	1.8	1.6	1.5
Salt (%)	0.3	0.3	0.3
DL-methionine (%)	0.2	0.1	0.1
L- Lysine HCl (%)	0.1	0.0	0.0
Premix[1] (%)	1.0	1.0	1.0
Nutrients and energy level (calculated)			
Crude protein (%)	22.92	20.04	18.11
ME (MJ/kg)	13	13.4	13.4
Lysine (%)	1.37	1.09	0.94
Methionine (%)	0.55	0.51	0.39
Cystine (%)	0.34	0.30	0.27
Tryptophan (%)	0.32	0.27	0.24
Calcium (%)	0.93	0.92	0.90
Total P (%)	0.71	0.66	0.63
Available P (%)	0.45	0.40	0.35

[1]Vitamin + mineral mixture provided per kg of diet: 12000 IU of vitamin A; 5000 IU of vitamin D_3; 50 mg of vitamin E; 4 mg of vitamin K3; 4 mg of vitamin B1; 10 mg of vitamin B2; 6 mg of vitamin B6; 60 mg of nicotinamide; 2 mg of folic acid; 0.02 mg of vitamin B12; 0.2 mg of biotin; 400 mg of choline chloride; 15 mg of pantothenic acid; 2 mg of Co, 2 mg of I, 40 mg of Fe, 100 mg of Zn, 20 mg of Cu; 120 mg of Mn, 0.3 mg of Se.

of dietary supplementation of Bio-Mos (Alltech Inc., USA) in broiler diets that originated from Bio-Mos fed breeders as compared to negative control group (control fed breeders/control fed broilers) on the growth performance and jejunal morphology of broiler chicks.

MATERIALS AND METHODS

Trial design

A total of 24000 broiler breeders (Hubbard classic) were included in the first phase of the trial designed as a 2 x 4 test (2 groups with 4 replicates). Each replicate consisted of 3000 broiler breeders. The dietary treatments for broiler breeder stock comprised a standard control feed versus a Bio-Mos supplemented feed (BIO-MOS, Alltech Inc. USA) at a level of 1 kg/t from 24 weeks of age. At 50 weeks of age of the broiler breeders, eggs from both groups were collected and set into the same incubator for the broiler trial.

The broiler trial was set up on an experimental farm of the Faculty of Agriculture, University of Novi Sad, Serbia. The trial was designed as 3 x 4 test (3 groups with 4 replicates). Each replicate included 40 broilers in a floor pen. Stocking density was 16 birds/m^2. The lighting regime was 23 h light + 1 h dark. Air temperature was adjusted in accordance to the physiological demands during the trial. Dietary treatments for broilers were disigned as: C/C: Control feed for breeders (c) + control feed for progeny (negative control); BM/C: Bio-Mos supplemented feed for breeders + control feed for progeny; BM/BM, Bio-Mos supplemented feed for breeders + Bio-Mos supplemented feed for progeny. Bio-Mos was supplemented in the broiler feed at levels of 1 kg/t for starter, 0.75 kg/t for grower and 0.5 g/t for finisher periods. Feed and water supply were provided *ad libitum.* Feed was prepared according to the requirement of the hybrid Hubbard Classic and the basic composition is presented in Table 1.

Body weight and feed intake were monitored by pen at weekly intervals and mortality was recorded daily. Birds that died were measured and their body weights were used to adjust the feed conversion ratio accordingly.

Gut measurements

Every seven days starting from week 1 until the end of the trial (42 days), 8 birds from each experimental group were slaughtered and the small intestines were removed. A part of jejunum of 1 cm length was fixed in Bouin solution, and after histological procedure, samples were cut on slices of 5 µm and stained with hematoxylin-eosin. Crypt depth and villus height were determined using light microscope and software for image analysis (IM 1000, Leica Microsystem, Germany). Apparent villus surface area was estimated by trigonometry as described by Iji et al. (2001). A minimum of 15 measurements were made for each parameter per chicken in order to get a representative sample.

Statistical analysis

Data were analyzed using analysis of variance (ANOVA), followed by a LSD post hoc test for the separate means, using StatSoft

Table 2. Effect of dietary mannanoligosaccharides on body weight and feed conversion ratio (FCR) of broilers.

Age (in week)	Treatment[1]			Statistic	
	C/C	BM/C	BM/BM	SEM	P-value
Body weight					
1	146	139	147	3.645	NS
2	376	380	381	4.022	NS
3	770	768	781	6.123	NS
4	1246[b]	1240[b]	1283[a]	15.474	*
5	1728[b]	1718[b]	1785[a]	14.493	*
6	2100[b]	2119[b]	2223[a]	19.476	*
FCR					
3	1.41	1.41	1.42	0.008	NS
6	1.90	1.89	1.87	0.013	NS

[a,b]Means within raw with no common superscript differ significantly ($p<0.05$); NS, not significant, *$p<0.05$; [1]C/C, Control feed for breeder flock (c) + control feed for progeny (negative control); BM/C, Bio-Mos supplemented feed for breeder flock + control feed for progeny; BM/BM, Bio-Mos supplemented feed for breeder + Bio-Mos supplemented feed for progeny; SEM, standard error of means.

software (STATISTICA 7, 2005). Results were considered significant at $p<0.05$.

RESULTS

Addition of MOS in the feed of the experimental groups showed a significant effect on the body weight of the chickens (Table 2). The results showed that adding Bio-Mos to the feed of broiler chickens significantly improved the body weight at 6 weeks of age as compared to the groups without Bio-Mos. Feed conversion ratio (FCR) was unaffected by the chick origin or by the feeding treatment. The morphometry of the broiler jejunum is shown in Table 3.

At the early age of chicks (7 days), there was a significant decrease in the crypt depth between BM/BM group and BM/C group, but there were no significant differences in the jejunal parameters at 14 days of age. As the birds grew up, the differences between Bio-Mos fed group and control fed groups became more obvious. At 21 days of age, a significant increase in villus/crypt ratio was observed in the BM/BM group as compared with the C/C group. Significant increase of the villus/crypt ratio was also noticed at day 28 in BM/BM group as compared with the BM/C group. At 35 and 42 days of age, significant differences were recorded in all the jejunal parameters, except crypt depth, between BM/BM group and the other two groups (BM/C and C/C). Bio-Mos showed a significant positive effect on the morphological characteristics of the jejunal mucosa of the birds and this effect was clearly shown in the last three weeks of fattening.

DISCUSSION

The growth promoting effect of Bio-Mos on body weight in this work is in line with the results of Mateo et al. (2000), Hooge (2004) and Perić et al. (2005). Positive effect on body weight can be related to improvement in jejunum morphology parameters (increased villus height, better villus/crypt ratio and enlarged villus surface area) in the last two weeks of fattening.

In our trial, comparing the three groups at different ages, the usage of Bio-Mos in broiler breeder diets did not influence the morphological parameters of the jejunum in broiler chickens (the offspring's of the former) but Bio-Mos supplemented directly in the broiler diets significantly influenced the jejunal morphology. The addition of Bio-Mos to the broiler diets, resulted in a decrease of crypt depth in week one. Our results are in agreement with the report of Yang et al. (2008) who pointed out that in their trial, in the 1st week of broiler's age, villus height was not statistically different between Bio-Mos fed broilers as compared to the unsupplemented group, but differences between groups were established in the crypt depth. Significant differences in the jejunal parameters were found from the 21 days of age until the end of the trial. Our results are not in accordance with the results of Sun et al. (2005) who stated that age of birds plays an important role in the changes in intestinal morphology, whereas micronutrients may have an effect on intestinal structure development after 35 days of age. In an experiment with Bio-Mos, Iji et al. (2001) observed that Bio-Mos significantly increased jejunal villi height. Addition of MOS to the feed of 14 days old poults had significant effects on jejunal villi/crypt ratio as compared to the control group (Ferket et al., 2002). These changes were represented by elongated villi and a higher villus/crypt ratio, which was indicative of a lower rate of enterocyte-cell migration from the crypt to the villus. It was suggested that Bio-Mos could reduce both the damage to enterocytes and the need for cell renewal in

Table 3. Effect of dietary mannanoligosaccharides in broiler diets on the jejunum morphology of broilers.

Age (in days)	Treatment[1]			Statistic	
	C/C	BM/C	BM/BM	SEM	P-value
Day 7					
Villus height (µm)	401.79	452.01	403.60	9.35	NS
Crypt depth (µm)	131.28^{ab}	145.06^{a}	122.38^{b}	6.12	*
Villus/crypt ratio	3.02	3.14	3.32	0.09	NS
Area (mm^2)	0.04	0.04	0.03	0.002	NS
Day 14					
Villus height (µm)	561.34	568.79	523.66	21.20	NS
Crypt depth (µm)	224.30	202.11	212.64	6.20	NS
Villus/crypt ratio	2.50	2.80	2.48	0.083	NS
Area (mm^2)	0.06	0.05	0.05	0.002	NS
Day 21					
Villus height (µm)	547.45	724.30	749.19	48.69	NS
Crypt depth (µm)	250.92	259.48	230.70	9.21	NS
Villus/crypt ratio	2.14^{a}	2.79^{ab}	3.26^{b}	0.180	*
Area (mm^2)	0.07	0.10	0.08	0.009	NS
Day 28					
Villus height (µm)	864.73	832.88	939.94	39.18	NS
Crypt depth (µm)	247.66	256.22	254.55	8.45	NS
Villus/crypt ratio	3.49^{ab}	3.23^{a}	3.78^{b}	0.104	*
Area (mm^2)	0.09	0.09	0.10	0.006	NS
Day 35					
Villus height (µm)	1075.20^{a}	1190.65^{ab}	1335.58^{b}	51.93	*
Crypt depth (µm)	329.62	335.44	307.40	11.29	NS
Villus/crypt ratio	3.33^{a}	3.60^{a}	4.32^{b}	0.185	*
Area (mm^2)	0.14^{a}	0.15^{a}	0.22^{b}	0.015	*
Day 42					
Villus height (µm)	1502.81^{a}	1556.56^{a}	1872.05^{b}	66.03	*
Crypt depth (µm)	434.51	421.04	440.84	13.13	NS
Villus/crypt ratio	3.47^{a}	3.71^{a}	4.28^{b}	0.135	*
Area (mm^2)	0.26^{a}	0.27^{a}	0.35^{b}	0.017	*

^{a,b}Means within raw with no common superscript differ significantly ($p<0.05$); NS, not significant, *$p<0.05$; [1]C/C, Control feed for breeder flock (c) + control feed for progeny (negative control); BM/C, Bio-Mos supplemented feed for breeder flock + control feed for progeny; BM/BM, Bio-Mos supplemented feed for breeder + Bio-Mos supplemented feed for progeny; SEM, standard error of means.

the gut. A high cell turnover occurs at the epithelium of the small intestine of chickens (Sklan, 2001), which is accompanied by an extremely high rate of metabolism, involving 23 to 36% of the whole body energy expenditure (Summers, 1991). Bio-Mos has also been shown to promote the production of lactic acid in the digestive tract, by stimulating growth of lactic-acid producing bacteria (Rekiel et al., 2007). Mannose-type oligosaccharides may directly protect the mucosa by acting as alternative sites of binding for pathogenic bacteria (Monsan and Paul, 1995).

These results point out that Bio-Mo has a significant influence on jejunal morphology and plays an important role in processes of digestion and absorption, which leads to improved performance.

ACKNOWLEDGEMENT

This work was supported in part by the Ministry of

Science of the Republic of Serbia (Project No. 31033).

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