Bulgarian Journal of Agricultural Science, 16 (No 5) 2010, 643-650
Agricultural Academy

EFFECTS OF *SACCHAROMYCES CEREVISIAE* AND/OR MANNANOLIGOSACCHARIDE ON PERFORMANCE, BLOOD PARAMETERS AND INTESTINAL MICROBIOTA OF BROILER CHICKS

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Abstract

KOC, F., H. SAMLI, A. OKUR, M. OZDUVEN, H. AKYUREK and N. SENKOYLU, 2010. Effects of *Saccharomyces cerevisiae* and/or mannanoligosaccharide on performance, blood parameters and intestinal microbiota of broiler chicks. *Bulg. J. Agric. Sci.,* 16: 643-650

The experiment was carried out to evaluate the effects of *Saccharomyces cerevisiae* and mannanoligosaccharide in single or in combination on growth performance, blood parameters and intestinal microbiota in 120, one-d-old, Ross 308 male broiler chicks raised from 1 to 21 days of age. There were 4 dietary treatments; (i) control, (ii) MOS (2 kg/ton feed), (iii) yeast (2 kg/ton feed) and (iv) yeast (2 kg/ton feed) + MOS (2 kg/ton feed).

Weight gain and feed intake of the broilers were significantly influenced by the addition of *Saccharomyces cerevisiae* with/without MOS.

The results suggest that supplementation of *S. cerevisiae* w/wo MOS to diets for growing broilers might enhance counts of LAB and yeast in the gut. In addition, the *S. cerevisiae* supplementation w/wo MOS to diets might have a negative effect on *E. coli*

Key words: Saccharomyces cerevisiae, mannanoligosaccharide, blood morphology, intestinal microbiota, broiler

Introduction

The use of antibiotics as growth promoters was completely banned in 1999 by the European Union (EU) (European Commission, 2001). This was due to increases in microbial resistance to antibiotics and residues in chicken meat products which might be harmful to consumers. Currently, in many parts of the world, feed additives, such as probiotics, prebiotics, are being experimented to alleviate the problems associated with the withdrawal of antibiotics from feed.

Probiotic microorganisms (non-pathogenic bacteria and/or yeast) are one of the alternatives for growth promoters in poultry. Functions of supplemental dietary microbial products in the digestive system are not known exactly, but some suggested mechanisms are; 1) they provide nutrient, 2) they aid digesting foods, and 3) they inhibit harmful bacteria in the gut (Owings et al., 1990). Gastrointestinal normal flora plays an important role in the health and performance

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of poultry (Thongsong et al., 2008).

One such alternative is the addition of yeast and yeast products to poultry diets. The inclusion of non-pathogenic yeast, *Saccharomyces cerevisiae*, in the diet has been shown to improve bird performance and decrease mortality (Miles and Bootwalla, 1991; Madrigal et al., 1993; Bradley et al., 1994; Santin et al., 2001).

Mannanoligosaccharides (MOS), which are derived from the cell wall of *Saccharomyces cerevisiae*, are an alternative to antibiotic growth promoters. MOS have shown promising effects, such as decreasing pathogenic microflora of the gut, stimulating a strong immune response, and elevating the strength of the intestinal mucosa in studies with poultry (Spring, 1999a; Spring, 1999b; Spring et al., 2000; Iji et al., 2001). By balancing the intestinal microflora and stimulating the immune response, MOS have been shown an increase at the growth of broilers (Hooge, 2004). The bacterial populations in the gut of birds were altered when MOS were added to their diets (Spring et al., 2000; Kocher et al., 2005; Yang et al., 2007; Yang et al., 2008b).

MOS supplementation to broiler diets improves growth performance in terms of body weight gain and feed conversion (Hooge, 2004; Rosen, 2007). By contrast, there are some researches demonstrating that growth performance was not significantly influenced by the addition of MOS (Yalcinkaya et al., 2008; Yang et al., 2008a).

Experiments conducted with MOS revealed inconsistent performance results in broilers. Moreover, the effects of *Saccharomyces cerevisiae* and MOS on intestinal microbiota have not been extensively investigated.

Therefore, the objective of the present study was to determine the effects of *Saccharomyces cerevisiae* and mannanoligosaccharide supplementation in single or in combination on growth performance, blood parameters and intestinal microbiota in commercial broilers.

Materials and Methods

Animals and housing

The present experiment was carried out with 120 one-day-old male Ross 308 broilers purchased from a local hatchery and randomly transferred to compact-type three-tier cages, five chicks per cage. Battery cages were equipped with wire mesh, dropping trays, nipple drinkers and trough feeders.

The experiment was set up in a completely randomized design where 30 birds were randomly assigned to each of four treatments with six replicates. The battery cages were placed in an environmentally controlled room with windows. The experiment lasted for 21 days and the chicks were fed the experimental diets throughout the experimental period. Chicks had free access to feed and water. The lighting regime was 23h/d. Birds were weighed by pen at 1, 7, 14 and 21 days of age.

Diets

Viable yeast 2 kg/ton feed (BeneSacc, manufactured and supplied by GLOBAL NUTRITECH 41600 Kandira, Kocaeli-Turkey) containing *Saccharomyces cerevisiae* NCYC R618 with 4 billion cfu/g and a mannanoligosaccharide mixture 2 kg/ton feed (ExcelMOS, manufactured and supplied by GLOBAL NUTRITECH 41600 Kandira, Kocaeli-Turkey) containing mannanoligosaccharides and beta glucans derived from the cell wall of a selected strain of *Saccharomyces cerevisiae* were used.

Dietary treatments were: (i) basal diet (as a control), (ii) basal diet + yeast 2 kg/ton feed, (iii) basal diet + MOS 2 kg/ton feed and (iv) basal diet + yeast 2 kg/ton feed+ MOS 2 kg/ton feed. Experimental diets were formulated by using ration-formulation software (UFFDA, University of Georgia, 1992, Athens, GA) to be isocaloric and isonitrogenous following National Research Council recommendations (NRC 1994). The basal diet consisted mainly of corn, soybean meal, wheat, full fat soybean and fish meal, from

a local feed market. Experimental diets were formulated to contain 22% crude protein and 3050 kcal/kg, and other essential nutrients (Table 1). Birds were fed the experimental diets *ad libitum* in mash form. Feed intake was recorded weekly. The feed conversion ratio (FCR) was calculated as grams of feed consumed per chick divided by grams of weight gain per chick.

Intestinal Microbiology

Birds were killed by cervical dislocation while feeding normally. The abdominal cavity was opened, and all digest contents of ileum and cecum were immediately collected under aseptic conditions into sterile glass bags and put on ice, until they were transported to the laboratory for enumeration of microbial populations.

Table 1
Chemical composition of the basal diet (as fed)

Ingredients, g/kg	
Corn	483.3
Soybean meal	272.8
Wheat	120
Full fat soybean	50
Fish meal	10
Sunflower oil	18.5
Limestone	15.8
MCP	15.2
Salt	3.2
DL-Methionine	2.8
L-Lysine HCl	5.9
Premix[1]	2.5
Nutrient content[2]	
ME, kcal/kg	3050
Crude protein, %	22
Ether extract, %	5.17
Crude fiber, %	2.7
Lysine, %	1.65
Met + Cys, %	0.97
Methionine, %	0.61
Calcium, %	1
Non phytate P, %	0.5

[1]Provided the following per kg of diet: vitamin A (retinyl acetate), 14,000 IU; vitamin D3, 5,000 IU; vitamin E, 50 mg; vitamin K3, 4 mg; vitamin B1, 3 mg; vitamin B2, 8 mg; vitamin B6, 4 mg; vitamin B12, 16 µg; niacin, 20 mg; iron, 80 mg; folic acid, 2

[2]Based on NRC (1994) values for feed ingredients

MRS agar (MERCK, 1.10660) was used for lactic acid bacteria (LAB) and malt extract agar (MERCK, 1.05398) was used for yeast, as the incubation medium. LAB and yeast counts of the ileum or cecum contents were obtained at 30°C degrees following 3 days incubation period. *E. coli* was grown on VRB agar (MERCK, 1.01406) aerobically at 37°C for 24-48 hours.

The bacterial colonies were enumerated, and the average number of live bacteria was calculated based on per gram of original ileal and cecal contents. All quantitative data were converted into logarithmic colony forming units (cfu/g).

Preparation of Blood Smears and Image Analysis

The air dried blood smears were fixed in methanol and counterstained with Giemsa's azur eosin methylene blue solution (MERCK, 1.09204) for 30 minutes. Then, preparations were washed with distilled water and left to dry. Afterwards, blood smears were observed under a microscope (BX 51, Olympus, Japan) at 40 x magnification. Erythrocyte length (EL) and erythrocyte width (EW) were determined using an image processing and analysis system (Motic Images Plus 2.0).

Statistical Analysis

Results were submitted to one-way analysis of variance (ANOVA) using general linear model procedure of a software program (Statistica, 1999). Differences among the means were tested by Duncan's multiple range tests.

Results

The impacts of MOS and *Saccharomyces cerevisiae* supplementation on performance of male broiler chicks are shown in Table 2. Weight gain ($P<0.01$) and feed intake ($P<0.05$) of the broilers in this study were significantly influenced by the addition of *Saccharomyces cerevisiae* with/without MOS. Although it was not significant, a numerical decrease in FCR was observed in the all groups compared to the control group. The lowest FCR was detected in the MOS+ *Saccharomyces cerevisiae* supplemented groups ($P>0.05$).

Table 3 shows the effects of MOS and *Saccharomyces cerevisiae* supplementation on digestive organ weights (g/100 g body weight). No significant differences were found, except for gizzard and duodenum weights. *Saccharomyces cerevisiae* and MOS+ *Saccharomyces cerevisiae* supplemented treatments significantly decreased the gizzard and duodenum weights ($P<0.05$).

Blood parameters are presented in Table 4. Erythrocyte length (EL) was significantly influenced by *Saccharomyces cerevisiae* fed groups ($P<0.05$). The erythrocyte width (EW) was significantly increased for the MOS treatments ($P<0.05$).

The effects of dietary treatments on ileal microbiota (log cfu/g ileal content) are shown in Table 5. In ileal

Table 2

Effects of MOS and *Saccharomyces cerevisiae* supplementation on broiler performance (0-21 d)

Treatments	Weight Gain, g	Feed Intake, g	FCR
Control	510.7 [b]	598.9 [b]	1.173
MOS	504.0 [b]	584.8 [b]	1.16
Saccharomyces cerevisiae	574.0 [a]	655.2 [ab]	1.142
Saccharomyces cerevisiae + MOS	620.5 [a]	689.4 [a]	1.111
SEM	12.61	16.78	0.029
P levels	<0.001	0.085	0.856

[a, b] Means in the same column with different superscripts differ significantly ($P < 0.05$).

Table 3
Effects of MOS and *Saccharomyces cerevisiae* supplementation on digestive organ weights (21 d) (g/100 g Body Weight)

Treatments	Proventriculus	Gizzard	Duodenum	Jejunum	Ileum
Control	0.69	2.79 [ab]	1.49 [a]	2.3	1.53
MOS	0.65	3.11 [a]	1.50 [a]	2.16	1.52
Saccharomyces cer	0.59	2.54 [b]	1.26 [ab]	2.2	1.51
Saccharomyces cer	0.6	2.56 [ab]	1.17 [b]	2.09	1.48
SEM	0.022	0.103	0.054	0.043	0.037
P levels	0.3	0.151	0.069	0.43	0.972

[a, b] Means in the same column with different superscripts differ significantly ($P < 0.05$).

Table 4
Effects of dietary treatments on erythrocyte length (EL) and erythrocyte width (EW) of broiler (21 d)

Treatments	EL µm	EW µm
Control	13.01 [b]	9.56 [b]
MOS	13.05 [b]	10.03 [a]
Saccharomyces cerevisiae	13.99 [a]	9.79 [ab]
Saccharomyces cerevisiae + MOS	13.22 [b]	9.67 [ab]
SEM	0.102	0.06
P levels	0.014	0.094

[a, b] Means in the same column with different superscripts differ significantly ($P < 0.05$).

Table 5
Effects of dietary treatments on ileal microbiota (log cfu/g ileal content)

Treatments	LAB	Yeast	*E. coli*
Control	2.631 [d]	3.291 [c]	2.017 [a]
MOS	4.366 [c]	2.938 [d]	1.600 [ab]
Saccharomyces cerevisiae	5.095 [b]	6.416 [a]	0.800 [b]
Saccharomyces cerevisiae +	5.683 [a]	5.993 [b]	0.000 [c]
SEM	0.268	0.36	0.229
P levels	<0.001	<0.001	<0.001

[a-d] Means in the same column with different superscripts differ significantly ($P < 0.001$).

digesta, LAB counts were consistently increased (P<0.001), whereas *E. coli* numbers were consistently decreased compared to control groups (P<0.001).

Table 6 reveals the effects of dietary treatments on cecal microbiota (log cfu/g cecal content). In cecal

Table 6
Effects of dietary treatments on cecal microbiota (log cfu/g cecal content)

Treatments	LAB	Yeast	*E. coli*
Control	4.990 [b]	4.795 [c]	7.095 [a]
MOS	4.855 [c]	4.781 [c]	6.673 [b]
Saccharomyces cerevisiae	4.954 [bc]	5.534 [b]	5.868 [c]
Saccharomyces cerevisiae + MOS	6.937 [a]	5.864 [a]	5.031 [d]
SEM	0.2	0.115	0.185
P levels	<0.001	<0.001	<0.001

[a-d] Means in the same column with different superscripts differ significantly ($P < 0.001$).

digesta, LAB counts were significantly increased for the birds fed with MOS + *Saccharomyces cerevisiae*, whereas *E. coli* were significantly decreased compared to control groups (P<0.001). An increase in the population of yeast in ileal and cecal digesta were observed for *Saccharomyces cerevisiae* w/wo MOS groups (P<0.001).

Discussion

The present study revealed that the addition of *Saccharomyces cerevisiae* w/wo MOS improved the weight gain and feed intake (Table 2). Besides, FCR is tended to decrease by *Saccharomyces cerevisiae* with MOS. These results are in line with the findings of Miles and Bootwalla, 1991; Madrigal et al., 1993; Bradley et al., 1994; Santin et al., 2001. *Saccharomyces cerevisiae* in the diet has been shown an improvement at the bird performance and decreased mortality. This improvement may be related with the balanced microbial population in the gastrointestinal tract which has an important role in the health and performance of the broilers (Thongsong et al., 2008).

In our study, no significant differences were found in organ weights, except for gizzard and duodenum weights. Supplementation of *Saccharomyces cerevisiae* w/wo MOS significantly (Table 3, P<0.05) decreased gizzard and duodenum weights compared to the MOS group.

Blood erythrocytes are elongated and elliptical shape of chickens (Ji et al., 2007). Erythrocyte length and erythrocyte width were affected significantly by *Saccharomyces cerevisiae* treatments (Table 4). The results indicated that the erythrocyte length was significantly increased (P<0.01). However, the erythrocyte width was increased by MOS group (P<0.05). Further studies are needed to understand this possible relationship between erythrocyte size and MOS supplementation. Since the erythrocyte is the most important carrier of oxygen and CO_2, its surface area to size ratio is a determining factor in the exchange of these gases with the tissues. Thus, a small corpuscle offers the possibility of a greater rate of exchange than a larger one. Likewise an elliptical body is more efficient than a spherical one of the same volume. Avian erythrocytes are efficient in both of these respects (Gregory, 2002).

The results of the present study showed that the supplementation of *Saccharomyces cerevisiae* in single or in combination with MOS positively influenced the ileal microbiota. Counts of LAB and yeast were significantly higher (P<0.001) in *Saccharomyces cerevisiae* w/wo MOS groups compared with the control and MOS groups (Table 5). *Saccharomyces cerevisiae* with MOS groups were the most efficient group in the LAB count in ileal digesta. Also, addition of the *Saccharomyces cerevisiae* with MOS significantly reduced *E. coli* count (P<0.001). Fecal coliform bacteria normally host the intestinal tract of warm blooded animals (Hartel et al., 2000).

Saccharomyces cerevisiae and MOS combination supplementation substantially increased the population of lactic acid bacteria and yeast in the cecum content. The population of *E. coli* was significantly decreased by the addition of *Saccharomyces cerevisiae* and MOS combination in cecum ($P<0.001$).

Savage and Zakrzewska (1996) reported that the removal of potential pathogens from the intestinal tract of growing animals may provide a more favorable environment for the digestion, absorption, and metabolism of growth-enhancing nutrients.

MOS selectively prevent pathogen colonization of the gastrointestinal tract by offering competitive binding sites for undesirable microorganisms including Salmonella and Escherichia coli (Newman, 1994). Multiple strains of *E. coli* and Salmonella agglutinated to MOS in vitro (Spring et al., 2000). The MOS is not enzymatically digested in the small intestine; therefore, bacteria bound to MOS likely exit the intestine without attaching to the epithelium (Spring et al., 2000). Though; in the present study, *Saccharomyces cerevisiae* with MOS had more beneficial effects compared to the other treatments, especially to control and MOS groups.

Conclusion

The present study demonstrated that supplementation of *Saccharomyces cerevisiae* w/wo MOS might improve growth performance and enhance the growth of lactic acid-fermenting bacteria and yeast in the gut. In addition to these, the *Saccharomyces cerevisiae* supplementation w/wo MOS to diets might have a negative effect on *E. coli.*

References

Bradley, G. L., T. F. Savage and K. I. **Timm,** 1994. The effects of supplementing diets with *Saccharomyces cerevisiae* var. boulardii on male poultry performance and ileal morphology. *Poult. Sci.,* **73:** 1766-1770.

European Commision [Internet]. 2001. 2nd opinion on anti-microbial resistance [cited 2009 Feb 11]. Available from: http://ec.europa.eu/food/fs/sc/ssc/out203_en.pdf

Gregory, T. R., 2002. The c-Value Enigma, A Thesis Presented to The Faculty of Graduate Studies of The University of Guelph, Doctor of Philosophy, Chapter 3, pp. 224. http://www.genomesize.com/rgregory/thesis/TRG-TOC.pdf

Hartel, P. G., W. I. Segars, J. D. Summer, J. V. Collins, A.T. Phillips and E. Whittle, 2000. Survival of fecal coliforms in fresh and stacked broiler litter. *J. Appl. Poult. Res.,* **9:** 505-512.

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

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

Ji, H. S., J. Y. Lee and S. J. **Lee,** 2007. *In-vitro* study on the hemorheological characteristics of chicken blood in microcirculation. *Korea-Australia Rheology J.,* **19** (2): 89-95.

Kocher, A., S. A. Denev, I. Dinev, I. Nikiforov and C. Scheidemann, 2005. Effects of mannanoligosaccharides on composition of the cecal microflora and performance of broiler chickens. Paper presented at: 4 BOKU-Symposium Tierernahrung; Tierernahrung ohne antibiotische Leistingsforderer; Universitat fur Bodenkunde Wien, Wien, pp. 216-220.

Madrigal, S. A., S. E. Watkins, J. T. Skinner, M. H. Adams, A. L. Waldroup and D. W. **Waldroup,** 1993. Effect of an active yeast culture on performance of broilers. *Poult. Sci.,* **72** (Suppl 1), **87** (Abstr.).

Miles, R. D. and S. M. Bootwalla, 1991. Direct-Fed Microbials in Animal Production- A review of the Literature. National Feed Ingredients Association, West Des Moines, IA. Direct-fed microbials in animal production ''avian''. pp. 117-146.

Newman, K., 1994. Biotechnology in the Feed Industry. Proc. Alltech's 10th Annu. Symp.. T. P. Lyons

and K. A. Jacques, (eds.) Nottingham Univ. Press, Nottingham, UK. Mannan-Oligosaccharides: Natural Polymers with Significant Impact on the Gastrointestinal Microflora and the Immune System. Pp. 167-174.

NRC, 1994. Nutrient Requirements of Poultry, 9th rev. ed. Washington D. C., *National Academy Press.*

Owings, W. J., D. L. Reynolds and R. J. Hasiak, 1990. Influence of dietary supplementation with Streptococcus faecium M-74 on broiler body weight, feed conversion, carcass characteristics and intestinal microbial colonization. *Poult. Sci.,* **69** (8): 1257-1264.

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

Santin, E., A. Maiorka, M. Macari, M. Grecco, J. C. Sanchez, T. M. Okada and A. M. Myasaka, 2001. Performance and intestinal mucosa development of broiler chickens fed diets containing *Saccharomyces cerevisiae* cell wall. *J. Appl. Poult. Res.,* **10:** 236-244.

Savage, T. F. and E. I. **Zakrzewska,** 1996. The performance of male turkeys fed a starter diet containing a mannanoligosaccharide (Bio-Mos) from day-old to eight weeks of age. In: Biotechnology in the Feed Industry. Proc. Alltech's 12th Annu. Symp.. T. P. Lyons and K. A. Jacques. (eds.) Nottingham Univ. Press, Nottingham, UK. pp. 47-54.

Spring, P., 1999a. Modes of action of dietary mannanoligosaccharide as a growth enhancer. *Zootech. Int.,* **22:** 34-36.

Spring, P., 1999b. Mannanoligosaccharides as an alternative to antibiotic use in Europe. *Zootech. Int.,* **22:** 38-41.

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

Statistica for the Windows Operating System. 1999. Stat Soft, Inc., Tulsa, OK.

Thongsong, B., S. Kalandakanond-Thongsong and V. Chavananikul, 2008. Effects of the addition of probiotic containing both bacteria and yeast or an antibiotic on performance parameters, mortality rate and antibiotic residue in broilers. *The Thai J. Vet. Med.,* **38** (1): 17-26.

Yalcinkaya, I., T. Gungor, M. Basalan and E. Erdem, 2008. Mannanoligosaccharides (MOS) from *Saccharomyces cerevisiae* in broilers: Effects on Performance and Blood Biochemistry. *Turk. J. Vet. Anim. Sci.,* **32** (1): 43-48.

Yang, Y., P. A. Iji, A. Kocher, L. L. Mikkelsen and M. Choct, 2007. Effects of mannanoligosaccharide on growth performance, the development of gut microflora and gut function of broiler chickens raised on new litter. *J. Appl. Poult. Res.,* **16:** 280-288.

Yang, Y., P. A. Iji, A. Kocher, E. Thomson, L. L. Mikkelsen and M. Choct, 2008a. Effects of mannanoligosaccharide in broiler chicken diets on growth performance, energy utilisation, nutrient digestibility and intestinal microflora. *Br. Poult. Sci.,* **49** (2): 186-194.

Yang, Y., P. A. Iji, A. Kocher, L. L. Mikkelsen and M. Choct, 2008b. Effects of dietary mannanoligosaccharide on growth performance, nutrient digestibility and gut development of broilers given different cereal-based diets. *J. Anim. Physiol. Anim. Nutr.,* **92**: 650-659.

Received December, 22, 2008; accepted for printing November, 2, 2009.