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Effects of Dietary Mannan-Oligosaccharides and Phytase Supplementation Alone or in Combination on Growth Performance, Serum Metabolites, Cecal Microbiota Activity and Intestinal Morphology in Broiler Chickens

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Abstract

This study evaluated the effect of a combination of mannan-oligosaccharides (MOS) and microbial phytase (MP) on growth performance, some blood biochemical metabolites, intestinal morphology and cecal microbial population of ROSS 308 broiler chickens from d 0 to 35 of age. A total of 200 one-day-old broiler chickens (mixed sex) were randomly distributed into four treatments with five replicates and 10 birds per each. The dietary treatments included a basal diet, the basal diet supplemented with MOS (as 2 g/kg of diet), MP (as 100 g/t Phyzyme XP 5000), and the combination of MOS and MP (MOS+MP). Supplementation of MOS+MP improved feed conversion ratio and body weight gain of broilers compared with the control group ($P < 0.05$). Dietary treatments had no significant effect on carcass traits. In the intestinal morphometric indices, villus length was greater in the birds which received MP individually or in combination with MOS ($P < 0.05$). Supplementation of MP, MOS or MOS+MP increased serum concentration of calcium in the broilers compared with control treatment ($P < 0.05$). Broiler chickens fed diets containing MOS+MP had lower numbers of *E. coli*, but higher count of *Lactobacilli* rather than control group ($P < 0.05$). Based on these results, it is concluded that dietary supplementation of MP in combination with MOS improved growth performance and intestinal microbial ecosystem of broiler chickens.

Introduction

Antibiotics growth promoters have long been utilized as a feed additive to enhance the broiler's growth performance and control of intestinal health. Nowadays, following the ban of in-feed antibiotics and to maintain the balance of the gut microbial ecosystem and health status in broiler chickens, non-antibiotic approach requested (Roofchaei *et al.,* 2019). Several studies have shown the beneficial effects of supplements such as prebiotics as a good alternative for antibiotics in poultry nutrition (Sohail *et al.,* 2013; Ghasemian and Jahanian, 2016; Hajiaghapour and Rezaeipour, 2018). Mannan-oligosaccharides (MOS) are non-digestible carbohydrate fractions that potentially influence the bird by selectively stimulating the growth and activity of one or several microorganisms in the gastrointestinal tract (Gibson and Roberfroid, 1995). It is well documented that such supplements affect the hindgut microbiota activity and intestinal nutrient absorption which enhances the growth performance of broiler chickens (Sohail *et al.,* 2012). On the other hand, it is reported that the dietary inclusion of MOS improved the intestinal morphology and increased surface absorption (Mourão *et al.*, 2006; Pourabedin *et al.,* 2014). It is hypothesized that these modes of action may alter the enzyme activities such as exogenous phytase supplementation in the digestive tract of broiler chickens.

The poor gut availability of phytic acid phosphorous by poultry and its effects on diet cost, environmental pollution, and interference with macro-minerals and protein absorption has led to extensive studies toward improving phytate digestion

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(Dilger *et al.,* 2004). Recently, the use of exogenous microbial phytase (MP) to achieve the intestinal hydrolysis of dietary phytate increased in poultry production. Phytase supplementation improves alimentary tract health, nutrient utilization, and digestion and absorption of amino acids and minerals in the broiler chickens (Cowieson *et al.,* 2009). A lot of researches have been performed to investigate the influences of phytase on growth performance and nutrient digestibility in broiler chickens. However, there is little information regarding the effects of dietary MOS in combination with MP supplement on broiler chickens. Therefore, this study was aimed to investigate the effects of MOS supplementation alone or in combination with MP on growth performance, cecal microbiota activity and intestinal morphology of broiler chickens.

Materials and Methods

Broiler chickens and treatments

This study was conducted at a poultry farm located in Qaemshahr city, Mazandaran province, Iran. All broilers care and use procedures were approved by the Department of Animal and Poultry Science, Islamic Azad University of Qaemshahr Care and Use Committee. Two hundred one-day-old male and female broiler chickens (ROSS 308) were purchased from a commercial hatchery and randomly assigned into four treatments with five replicate pens per each. The dimensions of the pens were 1 × 1.7 m and a height of 0.7 m. A manual drinker and a separate feeder were provided individually for each pen. Birds had *ad libitum* access to feed and water throughout the experiment. The house temperature was 34° C in the first week and reduced gradually of 2° C per week until a temperature of 23° C was attained. The dietary treatments were a basal diet, the basal diet supplemented with MOS (as 2 g/kg of diet), MP (as 100 g/t Phyzyme XP 5000), and the combination of MOS and MP (MOS+MP). The basal diet (Table 1) was formulated to meet the nutrient requirements of broiler chickens according to the ROSS 308 recommendation. The MOS supplement was provided from Techno-MOS product (Biochem Co., Lohne, Germany), and MP was purchased from Danisco Animal Nutrition (Marlborough, UK).

Table 1. The ingredients and chemical composition of the basal diet.

Item	Starter	Grower	Finisher
	d 1 to 10	d 11 to 24	d 25 to 35
Ingredient (g/kg)			
Corn grain	554.0	576.0	617.1
Soybean meal (440 g CP/kg)	379.4	354.2	306.0
Soybean oil	18.2	29.1	40.0
Oyster shell	13.6	11.1	10.2
Dicalcium phosphate	18.1	16.2	14.7
Common salt	1.5	2.0	1.5
Sodium bicarbonate	3.5	2.7	2.5
Vitamin premix[A]	2.5	2.5	2.5
Mineral premix[B]	2.5	2.5	2.5
DL-Methionine	3.2	2.5	2.1
L-Lysine-HCl	2.0	1.0	0.8
Choline chloride	0.5	-	-
L-Threonine	1.0	0.2	0.1
Chemical composition			
Metabolizable energy (Kcal/kg)	2950	3050	3150
Crude protein (%)	21.95	20.91	19.08
Calcium (%)	1.02	0.87	0.79
Available Phosphorous (%)	0.49	0.46	0.40
Sodium (%)	0.20	0.18	0.16
Lysine (%)	1.39	1.25	1.11
Methionine + Cystine (%)	1.03	0.92	0.83
Threonine (%)	0.94	0.83	0.75

[1] Provides per kilogram of diet: (Starter:13,000 IU vitamin A; 5,000 IU vitamin D_3; 80 IU vitamin E; 3.2 mg menadion; 3.2 mg thiamine; 8.6 mg riboflavin; 65 mg niacin; 5.4 mg pyridoxine; 17 µg vitamin B_{12}; 20 mg pantothenic acid; 2.2 mg folic acid; 0.3 mg biotin; 1700 mg choline chloride; and 9.4 mg antioxidant.), (Grower: 11,000 IU vitamin A; 4,500 IU vitamin D_3; 65 IU vitamin E; 3 mg menadion; 2.5 mg thiamine; 6.5 mg riboflavin; 60 mg niacin; 4.3 mg pyridoxine; 17 µg vitamin B_{12}; 18 mg pantothenic acid; 1.9 mg folic acid; 0.25 mg biotin; 1600 mg choline chloride; and 8.85 mg antioxidant.), (Finisher: 10,000 IU vitamin A; 4,000 IU vitamin D_3; 55 IU vitamin E; 2.2 mg menadion; 2.2 mg thiamine; 5.4 mg riboflavin; 45 mg niacin; 3.2 mg pyridoxine; 11 µg vitamin B_{12}; 15 mg pantothenic acid; 1.6 mg folic acid; 0.2 mg biotin; 1500 mg choline chloride; and 8.25 mg antioxidant).

[2] Provides per kilogram of diet: 120 mg Mn; 110 mg Zn; 20 mg Fe; 16 mg Cu; 1.25 mg I; and 0.3 mg Se.

Growth performance and carcass traits

Birds were weighed on the beginning as well as the end of the experiment (1 and 35 days of age), and feed consumption per pen was recorded throughout the experiment. Initial body weights (d 1) were subtracted from the final body weight to achieve body weight gain. Feed intake was measured by subtracting residual feed from the offered feed. Feed conversion ratio (FCR) for each pen was calculated by dividing feed consumption to body weight gain. The FCR was modified for mortality and calculated on a per pen basis. At 35 days of age, five randomly selected birds from each treatment were weighed and killed by cervical dislocation. After evisceration and to determine the carcass indices, the weights of the carcass compartments including the breast, thigh, spleen, and liver were measured and obtained data were expressed as a percentage of live weight of the broiler chickens.

Serum metabolites

At 35 days of age, five birds per treatment were bled through the wing vein to determine the serum biochemical metabolites. Blood samples were rapidly centrifuged at 5000 rpm for 5 min at 23 °C and then sera were collected. The concentration of serum glucose, cholesterol, triglycerides and high-density lipoprotein- cholesterol (HDL-c) were determined using an auto-analyzer (Autolab, BT 3500, Auto-analyzer medical system, Rome, Italy). Liver enzymes activity indices including serum concentration of aspartate transferase (AST) and alanine transaminase (ALT) was measured by using commercial kits (Pars Azmon, Tehran, Iran). The color-metrically procedure was used to determination of serum total calcium and phosphorous (Rezaeipour *et al*, 2016).

Intestinal morphology and microbial population

At 35 days, one bird from each pen was selected randomly and killed by cervical dislocation for determining of morphometric indices and gut microbiota. Jejunal morphometric indices were measured according to Roofchaei *et al.* (2019). Briefly, the midpoint segment (about 2 cm) of the jejunum was cut and flushed clean with PBS (phosphate-buffered saline) to avoid damage to the tissues. The sample was shortly fixed in a Clark fixative solution and then was maintained in a 50 % ethanol solution. For measuring morphometric indexes, a 0.5 cm section was processed, embedded in paraffin, stained with eosin blue. The villus variables and associated crypts were counted by an optical microscope.

For the determination of cecal-associated microbiota composition, the contents of broiler ceca were used. A sample of the fresh digesta from the ceca of each bird was immediately transferred into sterile tubes. Then, the enumeration of the microbial population was done according to the method of Eftekhari *et al* (2015). Briefly, the homogenized samples were decimally diluted (from10^{-1} to 10^{-7} in sterilized physiological saline solution) and aliquots of 0.1 mL of each dilution sample were spread and cultured anaerobically on specific agar plates for enumeration of *Escherichia coli*, *Lactobacilli,* and total count bacteria.

Statistical analysis

Experimental data were analyzed using the ANOVA procedure of SAS (2001) software. Means were compared using the Tukey test. Statistical significance was determined at $P < 0.05$.

Results

Broiler feed intake did not differ between the dietary treatments (Table 2). However, broilers in the MOS+MP treatment had higher body weight gain compared with the control group ($P < 0.05$). Similarly, treatment MOS+MP improved feed conversion ratio compared with MP and control groups ($P < 0.05$). According to Table 2, experimental treatments had no significant effect on the carcass characteristics of broiler chickens.

Table 2. Effects of dietary treatments on body weight gain, feed intake, feed conversion ratio (FCR) and carcass characteristics (g/100 g body weight of bird) in broiler chickens.

Parameters	Treatments[1]				*SEM*	*P*-value
	Control	MP	MOS	MP + MOS		
Weight gain (g/bird/d)	56.21^{b}	57.03^{b}	58.10^{ab}	60.17^{a}	0.82	0.02
Feed intake (g/bird/d)	102.58	101.64	101.93	103.30	0.83	0.52
FCR	1.82^{a}	1.78^{ab}	1.75^{bc}	1.71^{c}	0.017	0.005
Breast	30.17	31.05	30.87	31.54	0.46	0.91
Thigh	18.22	17.57	17.98	19.80	0.61	0.07
Liver	2.16	2.01	2.03	2.15	0.08	0.46
Spleen	0.080	0.081	0.084	0.086	0.004	0.78

[a-c] Means within the same row with no common superscripts differ ($P < 0.05$).

[1]MP = microbial phytase; MOS = mannan-oligosaccharides; *SEM* = standard error of the means.

From the data in Table 3, the concentration of *E. coli* bacteria in the cecal region was significantly decreased by MOS+MP inclusion, while the concentration of *Lactobacilli* bacteria increased ($P <$

0.05). Total count bacteria did not affect by the experimental treatments. The effects of experimental diets on the jejunal morphometric indices are shown in Table 3. Except for villus length, the jejunal morphological traits did not alter by the dietary treatments. The results showed that broiler chickens fed MOS+MP diet had a greater villus length compared with control and MOS groups ($P < 0.05$).

Table 3. Effects of dietary treatments on the cecal microbial population and jejunal morphology in broiler chickens.

Parameters	Treatments[1]				*SEM*	*P*-value
	Control	MP	MOS	MP + MOS		
E. coli (log_{10} cfu/g)	6.75[a]	6.61[a]	6.22[ab]	5.76[b]	0.21	0.02
Lactobacilli (log_{10} cfu/g)	7.11[b]	6.91[b]	7.25[b]	7.72[a]	0.11	0.001
Total anaerobic count (log_{10} cfu/g)	9.63	9.54	9.51	9.13	0.14	0.10
Villus length (µm)	1101[c]	1178[ab]	1109[bc]	1195[a]	23.40	0.02
Villus width (µm)	132.7	133.2	130.4	132.3	3.11	0.92
Crypt depth (µm)	237.8	235.4	231.9	246.4	6.76	0.48
Villus length/Crypt depth	4.63	5.03	4.83	4.58	0.18	0.54

[a-c] Means within the same row with no common superscripts differ ($P < 0.05$).
[1] MP = microbial phytase; MOS = mannan-oligosaccharides; *SEM* = standard error of the means.

The effects of dietary treatments on some selected serum biochemical parameters of broiler chickens are shown in Table 4. Except for calcium concentration, experimental treatments had no significant effect on the serum metabolites. In the case of calcium concentration, the supplementation of MP, MOS, and MOS+MP in the diet increased serum concentration of calcium in broiler chickens compared with the control group ($P < 0.05$).

Table 4. Effects of dietary treatments on the blood metabolites and enzymes in broiler chickens [a].

Parameters	Treatments[1]				*SEM*	*P*-value
	Control	MP	MOS	MP + MOS		
Glucose (mg/dL)	216.0	216.8	218.4	212.4	8.86	0.93
Cholesterol (mg/dL)	123.8	121.0	123.3	125.4	2.91	0.75
Triglycerides (mg/dL)	62.61	65.01	66.25	62.27	3.76	0.85
HDL-c (mg/dL)	74.21	75.02	73.08	71.32	2.92	0.81
AST (IU/L)	223.8	224.2	219.8	213.6	2.97	0.07
ALT (IU/L)	2.51	2.42	2.70	2.18	0.27	0.61
Calcium(mg/dL)	9.65[b]	11.24[a]	10.88[a]	11.31[a]	0.26	0.001
Phosphorous(mg/dL)	5.68	6.43	5.93	6.17	0.28	0.27

[a-b] Means within the same row with no common superscripts differ ($P < 0.05$).
[1]MP = microbial phytase; MOS = mannan-oligosaccharides; HDL-c = high density lipoprotein cholesterol; AST = aspartate transaminase; ALT = alanine transaminase; *SEM* = standard error of the means.

Discussion

In the present study, the inclusion of MP+MOS improved the growth performance of broiler chickens. These results are in accordance with findings of Narasimha *et al.* (2013) who found that supplementation of phytase in combination with a dietary symbiotic improved growth performance of broiler chickens. On the other hand, the beneficial effect of the consumption of prebiotic supplementation such as fructooligosaccharides (FOS) on phytic acid degradation has been documented (Wang *et al.,* 2010). However, research findings on the influence of prebiotic in combination with phytase supplementation on the growth performance of broiler chickens are inconsistent with our results (Shang *et al.,* 2015). A possible explanation for these contradictory results may be due to the nature of the prebiotic compound. It is reported that an acidic gut pH is favorable for mineral solubility as well as for phytase activity (Selle *et al.,* 2009). Prebiotics such as MOS enhances the short-chain fatty acids ($SCFA_s$) production in the hindgut of the birds. Therefore, increased $SCFA_s$ in the intestinal digesta resulted in acidic gut pH. This mechanism may explain the better effects of MOS and MP concomitant use.

In the current study, the addition of MOS in combination with MP had a positive effect on the intestinal microbial population. A prebiotic supplement as a non-digestible feed ingredient is utilized by the gut microbial population. Prebiotics has a positive function because of selective stimulation of the proliferation or metabolic activity of a limited number of beneficial intestinal bacteria species, such as *Bifidobacteria* and *Lactobacillus spp.* (Gibson and Roberfroid, 1995). Most of the MOSsupplements are derived from yeast cell walls (*Saccharomyces cerevisiae*) and are rich in chemical compounds such as mannoproteins, mannan, and, glucan (Teng and Kim, 2018). It is well documented

that prebiotic supplements such as MOS have specific receptors for fimbriae of *Escherichia coli* (sensitive to mannose) and *Salmonella* spp., which leads to deletion of these microorganisms with the digesta flow instead of binding an intestinal receptor (Huyghebaert *et al.,* 2011). These effects, as mentioned earlier, improve the function of the phytase enzyme by modifying $SCFA_s$ production and altering the intestine acidity.

On the other hand, in the present experiment, MOS+MP increased the jejunal villus length of broiler chickens. It is well documented that any positive change in the microbial population through feed supplementation such as prebiotics and enzymes improves the morphological characteristics of the intestine (Pourabedin *et al.,* 2014). Bogucka *et al.* (2016) observed that *in ovo* injection of inulin, as a prebiotic supplement, enhanced villus length in post-hatch broiler chickens. Besides, it is well-known that the addition of dietary prebiotic could enhance intestinal mucin mRNA expression to produce more mucin, protecting intestinal epithelial cells in broiler chickens (Huang *et al.,* 2015). Therefore, by improving intestinal morphometric indices, prebiotic may further increase enzyme activities and nutrient digestion and absorption, leading to greater growth performance (Xu *et al.,* 2003).

In the present research, dietary supplementation of MOS in combination with MP increased serum concentration of calcium in broiler chickens. Several studies demonstrated the effect of phytase supplementation on the serum mineral concentration in broiler chickens (Jiang *et al.,* 2013; Momeneh *et al.,* 2018). In parallel, Momeneh *et al. (*2018) reported that dietary phytase increased serum concentration of calcium in broiler chickens. Likewise, Viveros *et al.* (2002) observed a decrease in serum calcium concentrations with phytase addition. The effect of the phytase on blood calcium concentration may be due to the breakdown of the structure of calcium-bound phytate units, resulting in a greater release of calcium and an increase in its absorption in the gastrointestinal tract of the bird (Rezaeipour *et al.,* 2016). The mechanism of the effect of the MOS on the plasma concentration of minerals and especially calcium has not been well understood. However, it is reported that prebiotic consumption could affect mineral absorption in mice (Wang *et al.,* 2010).In recent years, special focus has been given to the influence of microbial fermentation on mineral absorption in the large intestine. Therefore, the most important function of prebiotics is mainly associated with their fermentation by hindgut microbiota (Ziemer and Gibson, 1998), which converts prebiotics such as MOS into SCFAs. Furthermore, SCFAs decline intestine acidity and thus create an acidic environment more favorable for mineral solubility (Momeneh *et al.*, 2018).

Conclusion

Dietary supplementation of MP in combination with MOS improved growth performance and intestinal microbial ecosystem and jejunal villus length of broiler chickens. However, further studies are needed to explain the mechanisms associated with dietary MOS supplementation and the synergetic impacts of MP and MOS in broiler diets.

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