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Dietary Mannanoligosaccharide Supplementation Improves Growth Performance, Intestinal Integrity, Serum Immunity, and Antioxidant Capacity of Partridge Shank Chickens

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Herein, we assessed the impact of dietary addition of *konjac* mannanoligosaccharide (MOS) on the growth, intestinal morphology, serum immune status, and oxidative status in Partridge Shank chickens. For the experiment, one-day-old chicks ($n=192$) were randomized into six replicates ($n=8$/replicate) and fed four different diets: a basal diet containing 0 (Control group), 0.5, 1, or 1.5 g MOS per kg of diet (g/kg) for 50 d. Relative to the control, the group fed 0.5 g/kg MOS decreased feed consumption from 22nd to 50th d and 1st to 50th d ($P<0.05$). By adding MOS, the height of the intestinal villus and the villus height to crypt depth ratio were increased ($P<0.05$); 1.5 g/kg MOS was the best dosage for these parameters. Jejunal and ileal goblet cell density increased following MOS supplementation at 21 d ($P<0.01$) and 50 d in the jejunum ($P<0.05$), respectively. Moreover, adding MOS to the diet increased the contents of IgA and IgM at 21 d ($P<0.05$) and total antioxidant capacity ($P<0.05$) at 50 d in the serum but decreased malondialdehyde content ($P<0.01$) at 21 d in the group fed 0.5 and 1.5 g/kg MOS. The findings suggested that MOS supplementation could affect feed consumption, intestinal health, serous immunity, and antioxidant capacity of Partridge Shank chickens.

Key words: growth performance, immune function, intestinal integrity, mannanoligosaccharide, oxidative status, Partridge Shank chickens

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Introduction

Mannanoligosaccharide (MOS) can be utilized in many fields including modern poultry production as a kind of functional oligosaccharide and feed additive and is effective for antibody production (Toloei *et al*., 2010). MOS can be obtained from different sources. Extensive reports suggest that mannanase can hydrolyze the polysaccharides containing mannan to yield MOS; fungi, bacteria, and plants can be used to obtain mannanase (Dhawan and Kaur, 2007; Moreira and Filho, 2008; Monia *et al*., 2011; Chen *et al*., 2013; Ariandi *et al*., 2015; Li *et al*., 2018; Shaymaa *et al*., 2019; Li *et al*., 2020).

Adding MOS to the diet can improve the immunity and intestinal health of animals. It could help bolster body weight gain and enhance feed conversion (Parks *et al*., 2001). Commercial mannanoligosaccharides from yeast cell walls promote the specific proliferation of beneficial bacteria and inhibit pathogenic bacteria. Phanwipa *et al*., 2015 reported that commercial MOS from yeast cell walls could promote beneficial bacterial growth such as that of *Lactobacillus*. Moreover, it could also prevent pathogenic bacteria. Zhang *et al*. (2005) added yeast cell wall inclusion, a commonly utilized product, to the diet and found a reduction in the concentration of malondialdehyde (MDA). MDA is a lipid peroxidation end product in chickens; it can be found in raw and boiled muscles. Our recent study showed that MOS had an effect on Partridge Shank chicken immune functionality and oxidative status in the intestines (Zhou *et al*., 2019).

Over the last few years, MOS use has increased in broilers. However, the supply of MOS is currently not sufficient for meeting its demand. *Amorphophallus konjac* K. Koch is a perennial herb. It grows in mountainous and hilly areas in subtropical regions, primarily in southeastern Asia (Zhang *et al*., 2005). The roots and tubers of *Amorphophallus konjac* contain a kind of functional polysaccharide called *Konjac* glucomannan (KG) (Liu *et al*., 2015) and is a precursor to

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MOS. *Konjac* powder can be depolymerized by β-mannanase to get MOS with high antioxidant activity (Liu *et al*., 2015).

Thus, high-quality MOS can be produced from KG by optimizing enzymatic hydrolysis. However, barely anything is known regarding the effects of MOS on broilers including the locally important Partridge Shank chicken breed. We hypothesized that enzymatic MOS may present high bioactivity under *in vivo* conditions. Therefore, we characterized the impact of enzymatic MOS from KG on Partridge Shank chicken growth performance, intestinal integrity, serum immunity, and oxidative status.

Materials and Methods

Mannanoligosaccharide

Enzymatic hydrolysis was used to prepare MOS. KG prepared from *Amorphophallus konjac* powder was used as the raw material in the laboratory; the powder was purchased from a local market in Yunnan Province, China. *Aspergillus niger*-derived β-mannanase was selected as the main enzyme. The conditions for hydrolysis were: time of hydrolysis＝2 h, pH＝5.0, and environmental temperature of experiment＝50℃. After hydrolysis, the free-flowing enzymatic hydrolysate was subjected to inactivation by adding it in a beaker with boiling water for 10 minutes. Impurities were eliminated via ultrafiltration and MOS was separated. Lastly, spray drying (BUCHI, Flawil, Switzerland) was used to obtain solid MOS. MOS content reached more than 96% of the final sample.

Husbandry, Diets, and Experimental Design

The Nanjing Agricultural University Institutional Animal Care and Use Committee approved these animal studies.

From a commercial hatchery, 192 one-day-old broiler chickens (Partridge Shank chickens) of similar weight were procured. The chicks were then randomized into four dietary treatment groups. Each group consisted of six replicates (one cage per replicate; n＝8 chicks per cage). The treatments included the supplementation of 0, 0.5, 1, and 1.5 g/kg MOS to the basal diet. The study lasted for 50 d. Basal diet composition was determined per the recommendation of the Nutrient Requirements of Poultry (NRC, 1994) and are detailed in Table 1. Birds were raised from 1 to 50 days and had free access to mash feed and water in three-level cages in a temperature-controlled facility. In the first three days, the room temperature was adjusted to 32−34℃; it was decreased by 2−3℃ each week. Finally, the temperature was adjusted to 26℃. Natural light exposure was allowed during the day; the light intensity was set to ~ 10 lx during the night. At 21 d and 50 d of age, chickens were maintained under fasting conditions for 12 h and their body weights (BW) were recorded. The body weight gain was calculated by recording the feed intake of the replicate (cage). All the birds were weighed including the dead.

Table 1. **Basal diet composition (g/kg, as fed basis unless otherwise stated)**

Items	1−21 days	22−50 days
Ingredients		
Corn	576.1	622.7
Soybean meal	310	230
Corn gluten meal	32.9	60
Soybean oil	31.1	40
Limestone	12	14
Dicalcium phosphate	20	16
L-Lysine·HCl	3.4	3.5
DL-Methionine	1.5	0.8
Sodium chloride	3	3
Premix[1]	10	10
Calculated nutrient levels[2]		
Apparent metabolizable energy (MJ/kg)	12.56	13.19
Crude protein	211	196
Calcium	10.00	9.50
Available phosphorus	4.60	3.90
Lysine	12.00	10.50
Methionine	5.00	4.20
Methionine + cysteine	8.50	7.60
Analyzed composition[3]		
Crude protein	208	192
Ash	57.2	56.5

[1] On a per kg basis, this diet provided: vitamin A (transretinyl acetate), 10,000 IU; vitamin D_3 (cholecalciferol), 3,300 IU; Fe (from ferrous sulphate), 80 mg; thiamin, 2.2 mg; Cu (from copper sulphate), 8.0 mg; Mn (from manganese sulphate), 110 mg; Zn (from zinc oxide), 60 mg; vitamin E (all-rac-α-tocopherol), 30 IU; I (from calcium iodate), 1.1 mg; riboflavin, 8 mg; nicotinamide, 40 mg; choline chloride, 600 mg; Se (from sodium selenite), 0.3 mg; menadione, 1.3 mg; calcium pantothenate, 10 mg; pyridoxine·HCl, 4 mg; biotin, 0.04 mg; folic acid, 1 mg; vitamin B_{12} (cobalamin), 0.013 mg

[2] Based upon feed composition and nutrition in China (2012)

[3] Determined through triplicate sample analyses

Sample Collection

On days 21 and 50, all the birds were weighed after 12 h of food deprivation. In each pen, there were several chickens. When their weight reached the mean weight, one bird was picked for weighing. Later, blood samples (each of about 5 mL) were withdrawn from the wing vein. The samples were then centrifuged at 4,450×g for 15 min at 4℃ to obtain the serum. After blood collection, the animals were euthanized by cervical dislocation and then necropsied. Gastrointestinal tracts were rapidly removed. The jejunum and ileum were then removed from the mesentery and were stored in a cold steel tray. Mid-jejunum and mid-ileum samples of ~2 cm size were collected and flushed carefully and gently with cold PBS (pH 7.4). For further histological research, the samples were stored in 10% freshly chilled formalin solution.

Histological Measurement

The samples from the intestine were dehydrated and impurities were removed. Finally, paraffin was used to embed these samples. Samples of 5 μm thickness were then cut and deparaffinized using xylene. Further, the samples were rehydrated and stained with hematoxylin and eosin. A light microscope (Nikon, Tokyo, Japan) was used to view the villus and crypts from ten well-oriented villi of every sample. The height of villi and crypt depth were measured by a

computer-assisted morphometric system. The samples were re-stained using Alcian Blue and periodic acid-Schiff stain to calculate the goblet cell number (Luna, 1968; Horn *et al*., 2009). Specifically, the samples were deparaffinized, hydrated, and stained with the Alcian Blue solution for 30 min (1 g Alcian Blue, 3 mL/L acetic acid, 97 mL dH_2O, pH 2.5). Next, the samples were rinsed with tap water for 10 min followed by a 15-min oxidation step in the presence of periodic acid. They were rinsed for 5 min with lukewarm tap water and subsequently stained with periodic acid-Schiff stain for 30 min. The mucin-containing cells were counted using a light microscope. These cells were selected from five villi of every segment and were averaged. The goblet cell density was calculated by dividing the average goblet count by the average villus length; the resultant values were reported as goblet cells per 100 μm of villus length. The chemicals used for staining were bought from Sigma-Aldrich (MO, USA).

Serum Immune and Antioxidant Parameter Measurements

To analyze total antioxidant capacity (T-AOC) and the levels of superoxide dismutase (SOD) and MDA, commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, Jiangsu, China) were used based on provided directions. The hydroxylamine approach was utilized for measuring T-SOD activity (Oyanagui, 1984). The concentration of MDA was measured by the barbiturate thiosulfate assay (Placer, 1966). T-AOC was measured by the ferric-reducing approach (Benzie and Strain, 1996), which indicates the strength of antioxidant capacity. Chicken-specific ELISA (enzyme-linked immunosorbent assay) kits (Nanjing Jiancheng Bioengineering Institute) were used to calculate the immunoglobulin M (IgM), IgG, and IgA levels in the serum samples. Total protein levels in individual samples were used for normalization between samples.

Statistical Analysis

SPSS v. 19.0 (SPSS Inc., IL, USA) was used to analyze the data. One-way ANOVA was chosen to identify statistical differences. The pen (cage) was used as the experimental unit. Tukey's multiple range tests were used to detect the differences among treatments. $P<0.05$ served as the significance threshold. Data were the means alongside their pooled standard errors.

Results

Growth Performance

Relative to controls, adding MOS to the basal diets of chickens showed similar body weight (BW) per bird over the 50-d study ($P>0.05$) (Table 2). However, the addition of 0.5 g/kg MOS decreased feed consumption from 22 to 50 d and 1 to 50 d versus control ($P<0.05$).

Histological Findings

Supplementation with 1 and 1.5 g/kg MOS bolstered the villus height in jejunum and ileum ($P<0.05$) at 21 d versus the control (Table 3). Additionally, ileal crypt depth ($P<0.05$) was bolstered by adding 1 g/kg MOS to the diet. At 50 d, compared with the control, MOS supplementation elevated villus height in the jejunum in the group supplemented with 1.5 g/kg MOS; elevated villus height was observed in the ileum in the group supplemented with 1 and 1.5 g/kg ($P<0.05$). The villus height to crypt depth ratios in both jejunum ($P<0.05$) and ileum ($P<0.05$) were also higher after 1.5 g/kg MOS supplementation. However, the crypt depth was decreased in the jejunum with 1 and 1.5 g/kg MOS supplementation ($P<0.05$). Ileal and jejunal goblet cell density was increased in 21 d by MOS addition ($P<0.05$). Simultaneously, cell density in the jejunum was increased after adding 1 and 1.5 g/kg MOS ($P<0.05$) at 50 d compared to the control. However, ileal goblet cell density was unaffected by the addition of MOS ($P>0.05$) at 50 d.

Immunoglobulins in Serum

On day 21, relative to the controls, the contents of serum IgA and IgM significantly increased by adding MOS to the diet irrespective of the dosage ($P<0.05$) (Table 4). However, the contents of IgG at 21 d and that of IgA, IgG, and IgM at 50 d did not change with the inclusion of MOS ($P>0.05$).

Oxidative Status of Serum

Chickens consuming a diet supplemented with 0.5 and 1.5 g/kg MOS exhibited decreased MDA contents in the serum ($P<0.01$) compared to the control (Table 5). Moreover, the

Table 2. **Partridge Shank chicken growth performance after being fed diets with or without MOS supplementation[1]**

Items	MOS (g/kg diet)				SEM[2]	*P*-value
	0	0.5	1	1.5		
Body Weight (g)						
21 d	400	386	391	395	0.03	0.285
50 d	1674	1646	1656	1653	0.01	0.852
Feed consumption (g)						
1 to 21 d	568	544	561	541	0.01	0.170
22 to 50 d	2551[b]	2399[a]	2590[b]	2522[b]	0.02	0.016
1 to 50 d	3117[b]	2942[a]	3150[b]	3063[ab]	0.03	0.014

[a–b] Means within a row with different superscripts are different at $P<0.05$

[1] MOS, mannanoligosaccharide

[2] SEM, Standard error of means ($n=6$)

Table 3. **Intestinal mucosal morphology and goblet cell number in Partridge Shank chickens fed diets containing varying levels of MOS[1]**

Items	MOS (g/kg diet)				SEM[2]	*P*-value
	0	0.5	1	1.5		
21 d						
Jejunum						
Villus height (μm)	1099.00^{a}	1107.51^{a}	1334.01^{b}	1602.05^{c}	78.84	<0.001
Crypt depth (μm)	298.30	302.62	301.02	315.69	3.25	0.250
Villus height:crypt depth (μm: μm)	3.65^{a}	3.66^{a}	4.44^{ab}	5.01^{b}	0.24	0.083
Ileum						
Villus height (μm)	827.01^{a}	828.11^{a}	868.50^{b}	902.23^{c}	13.94	<0.001
Crypt depth (μm)	285.44^{ab}	281.93^{a}	296.67^{c}	292.40^{bc}	2.30	0.019
Villus height:crypt depth (μm: μm)	2.83^{a}	2.94^{ab}	2.93^{ab}	3.08^{b}	0.03	0.150
50 d						
Jejunum						
Villus height (μm)	1663.00^{a}	1668.02^{a}	1669.41^{a}	1707.81^{b}	6.91	0.003
Crypt depth (μm)	290.32^{b}	288.55^{b}	278.92^{a}	271.07^{a}	3.03	0.008
Villus height:crypt depth (μm: μm)	5.73^{a}	5.78^{ab}	5.99^{b}	6.29^{c}	0.09	0.005
Ileum						
Villus height (μm)	1078.01^{a}	1081.11^{a}	1095.00^{b}	1106.14^{b}	4.26	<0.001
Crypt depth (μm)	235.44^{ab}	236.93^{b}	233.67^{ab}	228.40^{a}	1.44	0.133
Villus height:crypt depth (μm: μm)	4.58^{a}	4.57^{a}	4.68^{a}	4.84^{b}	0.04	0.024
Goblet cell number (n per 100 μm of villus)						
21 d						
Jejunum	9.49	9.80	9.96	10.50	0.14	<0.001
Ileum	9.66^{a}	9.89^{a}	10.59^{b}	11.26^{c}	0.24	<0.001
50 d						
Jejunum	11.23^{a}	11.27^{a}	11.34^{b}	11.36^{b}	0.02	0.021
Ileum	11.36^{a}	11.34^{a}	11.38^{a}	11.47^{b}	0.02	0.126

$^{a-c}$ Means within a row with different superscripts are different at $P<0.05$
[1] MOS, mannanoligosaccharide
[2] SEM, Standard error of means ($n=6$)

T-AOC of the serum was elevated at 50 d by MOS inclusion ($P<0.05$). However, the T-SOD activity of the serum was comparable across the treatments ($P>0.05$) at both 21 and 50 d.

Discussion

Table 2 shows that the addition of MOS to the diet did not impact BW. However, less feed consumption was observed by adding 0.5 g/kg MOS. Compared with other studies on broilers, the growth performance in this study had a slightly different trend. Nursoy *et al*. (2004) and Yalçin *et al*. (2008) found that supplemented yeast-derived MOS failed to impact the feed intake in laying hens. Yang *et al*. (2008) fed 1 or 2 g/kg of MOS for 1−5 weeks but observed no differences in the weight gain, intake of feed, or feed conversion efficiency compared to the control. Additionally, our recent study showed that MOS had no impact on feed intake and feed conversion ratio. However, in the current study, feed consumption was affected by MOS; the reason may stem from the source of MOS. Indeed, the MOS used here may enhance the secretion of digestive enzymes of chickens and thereby improve the digestion of feed.

It is very important to maintain the microarchitecture of the intestine because it can affect the growth performance of the chicken (Cheng *et al*., 2019). As a prebiotic, MOS can promote the development of villus and improve intestinal function and health (Spring *et al*., 2000; Baurhoo *et al*., 2007). In the current study, the addition of MOS increased the intestinal villus height; similar results were observed with the crypt depth and villus height to crypt depth ratio. These results proved that MOS impacted the chicken intestinal morphology. Similarly, Cheng *et al*. (2019) found that adding MOS increased the villus height and villus height to crypt depth ratio in the small intestine. In fish, Lu *et al*. (2020) found that MOS supplementation protected the intestinal histological morphology. Goblet cells secrete cysteine-rich products such as mucin 2 (MUC2) and trefoil factor 2 (TFF2). These cells also secrete the resistin-like molecule β that can maintain the integrity of intestinal mucosa (McGuckin *et al*., 2009). Herein, the MOS addition enhanced the intestinal goblet cell density. This was consistent with an increase in the expression of *MUC2* mRNA that serves as a physical barrier between the lumen and the epithelium and offers sites for the binding of Ig molecules such as sIgA (Lamont, 1992; Linden *et al*., 2008; Chen *et al*., 2017). Our results were partially consistent with the findings of Park *et al*. (2019) on white Pekin ducks and of Jahanian *et al*. (2016) on broilers. However, other studies have shown that MOS does not alter

Table 4. **Immunoglobulin levels in the serum of Partridge Shank chickens given diets containing varying levels of MOS[1] (μg/mg protein)**

Items[2]	MOS (g/kg diet)				SEM[3]	*P*-value
	0	0.5	1	1.5		
21 d						
IgA	1.48[a]	1.85[b]	1.92[b]	1.98[b]	0.07	0.029
IgG	2.11	2.02	2.17	2.01	0.09	0.937
IgM	1.46[a]	1.96[b]	1.77[b]	1.88[b]	0.06	0.004
50 d						
IgA	1.36[ab]	1.41[ab]	1.10[a]	1.75[b]	0.09	0.085
IgG	1.72	1.61	1.33	1.92	0.11	0.265
IgM	1.40	1.47	1.40	1.80	0.08	0.092

[a–b] Means within a row with different superscripts are different at $P<0.05$.
[1] MOS, mannanoligosaccharide
[2] IgG, immunoglobulin G; IgM, immunoglobulin M; IgA, immunoglobulin A
[3] SEM, standard error of means ($n=6$)

Table 5. **Antioxidant status in the serum of Partridge Shank chickens fed diets containing varying levels of MOS[1]**

Items[2]	MOS (g/kg diet)				SEM[3]	*P*-value
	0	0.5	1	1.5		
21 d						
SOD (U/mL)	255.17	269.76	260.12	254.12	9.93	0.616
MDA (nmol/mL)	4.28[b]	2.22[a]	3.66[b]	2.34[a]	0.21	<0.001
T-AOC (U/mL)	0.64	0.64	0.67	0.84	0.05	0.195
50 d						
SOD (U/mL)	327.80	322.56	324.10	339.35	9.96	0.942
MDA (nmol/mL)	4.47	4.12	4.03	4.86	0.20	0.190
T-AOC (U/mL)	0.54[a]	0.92[b]	0.99[b]	0.73[ab]	0.06	0.020

[a–b] Means within a row with different superscripts are different at $P<0.05$.
[1] MOS, mannanoligosaccharide
[2] MDA, malondialdehyde; T-SOD, total superoxide dismutase; T-AOC, Total antioxidant capacity
[3] SEM, standard error means ($n=6$)

intestinal goblet cell numbers For example, Lourenco *et al*. (2015) found that MOS did not affect the number of goblet cell numbers in broilers. This discrepancy may be linked to the dietary composition, MOS dosage, and physiological status.

Three immunoglobulins participate in immune system function in chickens – IgM, IgG, and IgA (Ulmer-Franco *et al*., 2012). It has been reported previously that dietary MOS can regulate antibody and Ig secretion. Our recent study showed that IgM and IgG in the intestine were increased by adding MOS in Partridge Shank chickens (Zhou *et al*., 2019). In pigs, dietary MOS increased the serum concentrations of IgA and IgG (Duan *et al*., 2016). We found that MOS supplementation increased the concentrations of IgA and IgM in the serum. This finding is in line with that of Attia *et al*. (2017) who reported that MOS supplementation elevated IgA and IgM contents in the broilers. In the present study, the concentrations of immunoglobulins were increased. This suggests that the synthesis of immunoglobulins could be stimulated by adding MOS; this is hypothesized because MOS has been proposed to provide alternative binding sites for pathogenic bacteria (Mosan and Paul, 1995). Increased Ig synthesis may additionally account for improved gut morphology. Overall, the results showed that our MOS preparations could improve the function of broiler immune systems.

Cells produce reactive oxygen species (ROS) during normal metabolic activities. However, when the ROS levels extend beyond the handling capacity of antioxidants, DNA damage may occur with proteins and endogenous lipids (Yu, 1994). Excessive ROS generation is closely linked to cancer, inflammation, autoimmunity, cardiovascular disease, and endocrine diseases (Dong *et al*., 2020). SOD is considered a primary antioxidant enzyme and functions as an oxygen-free radical scavenger (McCord, 1979). As the main end-product, MDA is caused by ROS and the content of MDA is usually used as a marker of lipid peroxidation (Ayala *et al*., 2014). T-AOC is a biomarker of antioxidant potential and redox synergistic interactions. Herein, dietary MOS bolstered the oxidative status of chickens by reducing

MDA accumulation and increasing T-AOC activity in the serum. Our recent study also showed that MOS decreases the MDA content in the intestine (Zhou *et al*., 2019). Similarly, Bozkurt *et al*. (2012) found that adding MOS to the laying hens could increase the SOD activity of the liver and decrease the MDA concentration in eggs and liver. These findings were also in line with the finding of Cheng *et al*. (2018) that MDA content in the breast muscle of broilers could be decreased by adding MOS to the diet under heat stress. Several studies have shown that MOS improves the growth performance because it helps the gastrointestinal tract mature and get more nutrients (Zdunczyk *et al*., 2005; Solis de los Santos *et al*., 2007; Safari *et al*., 2014). Some small molecules are adsorbed and utilized by the intestine; these molecules may have a positive effect on the synthesis of antioxidant molecules.

In conclusion, our enzymatic MOS can affect feed consumption and improve the intestinal health, immune function, and antioxidant capacity of the serum in Partridge Shank chickens.

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Conflicts of Interest

The authors declare no conflict of interest.

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