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 21d (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
MOS. *Konjac* powder can be depolymerized by β-man-
nannanase 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 bio-
activity under *in vivo* conditions. Therefore, we character-
ized 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 pre-
pared 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°C. After hydrolysis, the free-flowing enzymatic hydroly-
sate was subjected to inactivation by adding it in a beaker
with boiling water for 10 minutes. Impurities were elimi-
nated 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 treat-
ments 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°C; it was decreased
by 2–3°C each week. Finally, the temperature was adjusted
to 26°C. 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 re-
corded. The body weight gain was calculated by recording
the feed intake of the replicate (cage). All the birds were
weighed including the dead.

**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°C to obtain the
serum. After blood collection, the animals were euthanized
by cervical dislocation and then necropsied. Gastrointesti-
nal tracts were rapidly removed. The jejunum and ileum were
then removed from the mesentery and were stored in a cold
steel tray. Mid-jejenum 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 im-
purities 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 re-
hydrated 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

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

| Items               | 1–21 days | 22–50 days |
|---------------------|-----------|------------|
| Ingredients         |           |            |
| Pretix 1            | 10        | 10         |
| Dicalcium phosphate | 5.00      | 4.20       |
| Soybean meal        | 310       | 230        |
| Corn                | 576.1     | 622.7      |
| Corn gluten meal    | 32.9      | 60         |
| Corn gluten meal    | 32.9      | 60         |
| Corn gluten meal    | 32.9      | 60         |
| 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       |
| Premix 1            | 10        | 10         |
| Calculated nutrient levels 2 | | |
| 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       |
| Analysis of compo-    | 208       | 192        |
| nent 3             | 208       | 192        |
| Ash                 | 57.2      | 56.5       |

1. On a per kg basis, this diet provided: vitamin A (transretinyl acetate), 10,000 IU; vitamin D₃ (cholecalciferol), 3,300 IU; Fe (from ferrous sulphate), 80 mg; thiamin, 2.2 mg; Cu (from copper sulphate), 8.0 mg; Mn (from manganous sulphate), 110 mg; Zn (from zine oxide), 60 mg; vitamin E (all-rac-α-tocopherol), 301 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 B₁₂ (cobalamin), 0.013 mg
2. Based upon feed composition and nutrition in China (2012)
3. Determined through triplicate sample analyses
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 dH2O, 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 (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

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

| Items             | MOS (g/kg diet) | SEM² | \( P \)-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        | 2551b| 2399a| 2590b| 2522b| 0.02 | 0.016 |
| 1 to 50 d         | 3117b| 2942a| 3150b| 3063ab| 0.03 | 0.014 |

*a, b* Means within a row with different superscripts are different at \( P < 0.05 \)

²MOS, mannanoligosaccharide

³SEM, 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 \( \beta \) 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

| Items                  | MOS (g/kg diet) | SEM\(^2\) | \( P\)-value |
|------------------------|-----------------|-----------|-------------|
| 21 d                   |                 |           |             |
| Jejunum                |                 |           |             |
| Villus height (\(\mu m\)) | 1099.00\(^a\)  | 1107.51\(^a\) | 1334.01\(^b\) | 1602.05\(^c\) | 78.84 | <0.001 |
| Crypt depth (\(\mu m\)) | 298.30          | 302.62    | 301.02      | 315.69      | 3.25  | 0.250  |
| Villus height: crypt depth (\(\mu m\): \(\mu m\)) | 3.65\(^a\)  | 3.66\(^a\) | 4.44\(^b\) | 5.01\(^b\) | 0.24  | 0.083  |
| Ileum                  |                 |           |             |
| Villus height (\(\mu m\)) | 827.01\(^a\)  | 828.11\(^a\) | 868.50\(^b\) | 902.23\(^c\) | 13.94 | <0.001 |
| Crypt depth (\(\mu m\)) | 285.44\(^ab\) | 281.93\(^a\) | 296.67\(^c\) | 292.40\(^ce\) | 2.30  | 0.019  |
| Villus height: crypt depth (\(\mu m\): \(\mu m\)) | 2.83\(^a\)  | 2.94\(^ab\) | 2.93\(^b\) | 3.08\(^b\) | 0.03  | 0.150  |
| 50 d                   |                 |           |             |
| Jejunum                |                 |           |             |
| Villus height (\(\mu m\)) | 1663.00\(^a\) | 1668.02\(^a\) | 1669.41\(^a\) | 1707.81\(^b\) | 6.91  | 0.003  |
| Crypt depth (\(\mu m\)) | 290.32          | 288.55    | 278.92      | 271.07      | 3.03  | 0.008  |
| Villus height: crypt depth (\(\mu m\): \(\mu m\)) | 5.73\(^a\)  | 5.78\(^ab\) | 5.99\(^b\) | 6.29\(^b\) | 0.09  | 0.005  |
| Ileum                  |                 |           |             |
| Villus height (\(\mu m\)) | 1078.01\(^a\) | 1081.11\(^a\) | 1095.00\(^b\) | 1106.14\(^b\) | 4.26  | <0.001 |
| Crypt depth (\(\mu m\)) | 235.44\(^ab\) | 236.93\(^b\) | 233.67\(^b\) | 228.40\(^a\) | 1.44  | 0.133  |
| Villus height: crypt depth (\(\mu m\): \(\mu m\)) | 4.58\(^a\)  | 4.57\(^a\) | 4.68\(^a\) | 4.84\(^b\) | 0.04  | 0.024  |
| Goblet cell number (n per 100 \(\mu m\) 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 \((n=6)\)
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...

### Table 4. Immunoglobulin levels in the serum of Partridge Shank chickens given diets containing varying levels of MOS

| Items² | MOS (g/kg diet) | SEM³ | P-value |
|---|---|---|---|
| | 0 | 0.5 | 1 | 1.5 |
| 21 d | | | | |
| IgA | 1.48<sup>a</sup> | 1.85<sup>b</sup> | 1.92<sup>b</sup> | 1.98<sup>b</sup> | 0.07 | 0.029 |
| IgG | 2.11 | 2.02 | 2.17 | 2.01 | 0.09 | 0.937 |
| IgM | 1.46<sup>a</sup> | 1.96<sup>b</sup> | 1.77<sup>b</sup> | 1.88<sup>b</sup> | 0.06 | 0.004 |
| 50 d | | | | |
| IgA | 1.36<sup>ab</sup> | 1.41<sup>ab</sup> | 1.10<sup>a</sup> | 1.75<sup>b</sup> | 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 |

<sup>a-b</sup> Means within a row with different superscripts are different at <i>P</i> < 0.05.

1 MOS, mannanoligosaccharide
2 IgG, immunoglobulin G; IgM, immunoglobulin M; IgA, immunoglobulin A
3 SEM, standard error of means (<i>n</i> = 6)

### Table 5. Antioxidant status in the serum of Partridge Shank chickens fed diets containing varying levels of MOS

| Items² | MOS (g/kg diet) | SEM³ | 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<sup>b</sup> | 2.22<sup>a</sup> | 3.66<sup>b</sup> | 2.34<sup>a</sup> | 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<sup>a</sup> | 0.92<sup>b</sup> | 0.99<sup>b</sup> | 0.73<sup>ab</sup> | 0.06 | 0.020 |

<sup>a-b</sup> Means within a row with different superscripts are different at <i>P</i> < 0.05.

1 MOS, mannanoligosaccharide
2 MDA, malondialdehyde; T-SOD, total superoxide dismutase; T-AOC, Total antioxidant capacity
3 SEM, standard error means (<i>n</i> = 6)
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.

References

Ariandi, Yopi, Anja Meryandini. Enzymatic Hydrolysis of Copra Meal by Mannanase from Streptomyces sp. BF3.1 for The Production of Mannooligosaccharides. HAYATI Journal of Biosciences, 22: 79–86. 2015.
Attia YA, H Al-Khalafia, MS Ibrahim, AE A Al-Hamid, MA Al-Harthi and A El-Naggar. Blood hematological and biochemical constituents, antioxidant enzymes, immunity and lymphoid organs of broiler chicks supplemented with propolis, bee pollen and mannan oligosaccharides continuously or intermittently. Poultry Science, 96: 4182–4192. 2017.
Ayala A, Muñoz MF and Argüelles S. Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonalal. Oxidative Medicine and Cellular Longevity, 2014: 1–31. 2014.
Baurhoo B, L Phillip and CA Ruiz-Feria. Effects of purified lignin and mannan oligosaccharides on intestinal integrity and microbial populations in the ceca and litter of broiler chickens. Poultry Science, 86: 1070–1078. 2007.
Benzie F and Strain JJ. The Ferric Reducing Ability of Plasma (FRAP) as a Measure of “Antioxidant Power”. The FRAP Assay. Analytical Biochemistry, 239: 70–76. 1996.
Bozkurt M, Tokuçoğlu Ö, Küçükyılmaz K, Akşit H, Çabuk M, UğurÇatli A, Seyrek K and Çinar M. Effects of dietary mannan oligosaccharide and herbal essential oil blend supplementation on performance and oxidative stability of eggs and liver in laying hens. Italian Journal of Animal Science, 11: 223–229. 2012.
Chen JF, Liu DS, Shi B, Wang H, Cheng YQ and Zhang WJ. Optimization of hydrolysis conditions for the production of glucomanno-oligosaccharides from konjac using β-mannanase by response surface methodology. Carbohydrate Polymers, 93: 81–88. 2013.
Chen YP, Cheng YF, Li XH, Yang WL, Wen C, Zhuang S and Zhou YM. Effects of threonine supplementation on the growth performance, immunity, oxidative status, intestinal integrity, and barrier function of broilers at the early age. Poultry Science, 96: 405–413. 2017.
Cheng YF, Chen YP, Chen R, Su Y, Zhang RQ, He QF, Wang K, Wen C and Zhou YM. Dietary mannan oligosaccharide ameliorates cyclic heat stress-induced damages on intestinal oxidative status and barrier integrity of broilers. Poultry Science, 98: 4767–4776. 2019.
Cheng YF, Du MF, Xu Q, Chen YP, Wen C and Zhou YM. Dietary mannan oligosaccharide improves growth performance, muscle oxidative status, and meat quality in broilers under cyclic heat stress. Journal of Thermal Biology, 75: 106–111. 2018.
Dhawan S and Kaur J. Microbial mannanses: an overview of production and applications. Critical Reviews in Biotechnology, 27: 197–216. 2007.
Dong HL, Zheng LM, Yu PJ, Jiang Q, Wu Y, Huang CX and Yin BS. Characterization and application of lignin-carbohydrate complexes from lignocellulosic materials as antioxidants for scavenging in vitro and in vivo reactive oxygen species. ACS Sustainable Chemistry & Engineering, 8: 256–266. 2020.
Duan XD, Chen DW, Zheng P, Tian G, Wang JP, Mao XB, Yu J, He J, Li B, Huang QZ, Ao ZG and Yu B. Effects of dietary mannan oligosaccharide supplementation on performance and immune response of sows and their offspring. Animal Feed Science and Technology, 218: 17–25. 2016.
Horn NL, SS Donkin, TJ. Applegate and O Adeola. Intestinal mucin dynamics: Response of broiler chicks and White Pekin ducklings to dietary threonine. Poultry Science, 88: 1906–1914. 2009.
Jahanian E, Mahdavi AH, Asgary S and Jahanian R. Effect of dietary supplementation of mannanoligosaccharides on growth performance, ileal microbial counts, and jejunal morphology inbroiler chicks exposed to aflatoxins. Livestock Science, 190: 123–130. 2016.
Lamont JT. Mucus: the front line of intestinal mucosal defense. Annals of New York Academy of Sciences, 664: 190–201. 1992.
Li XY, Yi P, Liu J, Yan QJ and Jiang ZQ. High-level expression of an engineered β-mannanase (mRmMan5A) in Pichia pastoris for manno-oligosaccharide production using steam explosion pretreated palm kernel cake. Bioresource Technology, 256: 30–37. 2018.
Li XY, Liu HJ, Shi YQ, Yan QJ, You X and Jiang ZQ. Preparation, characterization, and prebiotic activity of manno-oligosaccharides produced from cassia gum by a glycoside hydrolase family 134 β-mannanase. Food Chemistry, 309: 125790. 2019.
Linden SK, P Sutton, NG Karlsson, V Korolik and MA McGuckin. Mucins in the mucosal barrier to infection. Mucosal Immunology, 1: 183–197. 2008.
Liu JH, Xu QH, Zhang JJ, Zhou XX, Lyu F, Zhao PC and Ding YT. Preparation, composition analysis and antioxidant activities of konjacoligo-glucomanann. Carbohydrate Polymers, 130: 398–404. 2015.
Lourenco M, L Kuritza, R Hayashi, L Miglino, J Durau, L Pickler

Journal of Poultry Science, 58 (3)
and E Santin. Effect of a mannanoligosaccharide-supplemented diet on intestinal mucosa T lymphocyte populations in chickens challenged with *Salmonella Enteritidis*. Journal of Applied Poultry Research, 24: 15–22. 2015.

Lu ZY, Feng L, Jiang WD, Wu P, Liu Y, Kuang SY, Tang L and Zhou XQ. Mannanoligosaccharides improved growth performance and antioxidant capacity in the intestine of on-growing grass carp (*Ctenopharyngodonidella*). Aquaculture Reports, 17: 100313. 2020.

Luna LG. Manual of histologic staining methods of the armed forces institute of pathology. McGraw-Hill, New York, NY. 1968.

Mccord JM. Superoxide: superoxide dismutase and oxygen toxicity. Reviews of Biochemistry and Toxicology, 1: 109–124. 1979.

McGuckin MA, R Eri, LA Simms, TH Florin and G Radford-Smith. Intestinal barrier dysfunction in inflammatory bowel diseases. Inflammmatory Bowel Diseases, 15: 100–113. 2009.

Monia B, Fatma C, Fatma B, Ilyes D, Raoudha EG and Semia EC. Monia B, Fatma C, Fatma B, Ilyes D, Raoudha EG and Semia EC. Production of manno-oligosaccharides from locust bean gum using immobilized *Penicillium occitanicum*mannanase. Journal of Molecular Catalysis B: Enzymatic, 73: 111–115. 2011.

Moreira LR and Filho EX. An overview of mannan structure and mannan-degrading enzyme systems. Applied Microbiology and Biotechnology, 79: 165–178. 2008.

Mosan PF and Paul F. Oligosaccharide feed additives. In:Biotechnology in Animal Feeds and Feeding (Wallence RJ and Chessen CH. Effects of yeast (*Saccharomyces cerevisiae*) cell components on growth performance, meat quality, and ileal mucosa development of broiler chicks. Poultry Science, 84: 1015–1021. 2005.

Yang Y, P Lji, A Kocher, E Thomason, L Mikkelsen and M Choct. Effects of mannanoligosaccharide in broiler chicken diets on growth performance, energy utilisation, nutrient digestibility and intestinal microflora. British Poultry Science, 49: 186–194. 2008.

Yu BP. Cellular defenses against damage from reactive oxygen species. Physiological Reviews, 74: 139–162. 1994.

Zhou et al. Mannanoligosaccharide Supplementation in Chickens 153

and E Santin. Effect of a mannanoligosaccharide-supplemented diet on intestinal mucosa T lymphocyte populations in chickens challenged with *Salmonella Enteritidis*. Journal of Applied Poultry Research, 24: 15–22. 2015.

Lu ZY, Feng L, Jiang WD, Wu P, Liu Y, Kuang SY, Tang L and Zhou XQ. Mannanoligosaccharides improved growth performance and antioxidant capacity in the intestine of on-growing grass carp (*Ctenopharyngodonidella*). Aquaculture Reports, 17: 100313. 2020.

Luna LG. Manual of histologic staining methods of the armed forces institute of pathology. McGraw-Hill, New York, NY. 1968.

Mccord JM. Superoxide: superoxide dismutase and oxygen toxicity. Reviews of Biochemistry and Toxicology, 1: 109–124. 1979.

McGuckin MA, R Eri, LA Simms, TH Florin and G Radford-Smith. Intestinal barrier dysfunction in inflammatory bowel diseases. Inflammmatory Bowel Diseases, 15: 100–113. 2009.

Monia B, Fatma C, Fatma B, Ilyes D, Raoudha EG and Semia EC. Monia B, Fatma C, Fatma B, Ilyes D, Raoudha EG and Semia EC. Production of manno-oligosaccharides from locust bean gum using immobilized *Penicillium occitanicum*mannanase. Journal of Molecular Catalysis B: Enzymatic, 73: 111–115. 2011.

Moreira LR and Filho EX. An overview of mannan structure and mannan-degrading enzyme systems. Applied Microbiology and Biotechnology, 79: 165–178. 2008.

Mosan PF and Paul F. Oligosaccharide feed additives. In:Biotechnology in Animal Feeds and Feeding (Wallence RJ and Chessen CH. Effects of yeast (*Saccharomyces cerevisiae*) cell components on growth performance, meat quality, and ileal mucosa development of broiler chicks. Poultry Science, 84: 1015–1021. 2005.

Yang Y, P Lji, A Kocher, E Thomason, L Mikkelsen and M Choct. Effects of mannanoligosaccharide in broiler chicken diets on growth performance, energy utilisation, nutrient digestibility and intestinal microflora. British Poultry Science, 49: 186–194. 2008.

Yu BP. Cellular defenses against damage from reactive oxygen species. Physiological Reviews, 74: 139–162. 1994.

Zdunczyk Z, Juskiewicz J, Iankowski J, Biedrzycka E and Koncicki A. Metabolic response of the gastrointestinal tract of turkeys to diets with different levels of mannan-oligosaccharide. Poultry Science, 84: 903–909. 2005.

Zhang AW, Lee BD, Lee SK, Lee KW, An GH, Song KB and Lee CH. Effects of yeast (*Saccharomyces cerevisiae*) cell components on growth performance, meat quality, and ileal mucosa development of broiler chicks. Poultry Science, 84: 1015–1021. 2005.

Zhang Y, Xie B and Gan X. Advance in the applications of konjac glucomann and its derivatives. Carbohydrate Polymers, 60: 27–31. 2005.

Zhou MY, Tao YH, Lai CH, Huang CX, Zhou YM and Yong Q. Effects of Mannanoligosaccharide Supplementation on the Growth Performance, Immunity, and Oxidative Status of Paradise Shank Chickens. Animals, 9: 817. 2019.