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The Influence of Mannan Oligosaccharides and Beta Glucan Supplementation on Growth Performance, Blood Constituents, and Cecal Parameters of Broiler Chickens

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ABSTRACT

Growth promoters in poultry feed have been under severe attention since antibiotics were banned for use in animal diets by the European Union. Thus, it has been important for poultry researchers to find alternatives to antibiotic growth promoters (AGPs) to boost the health and production performance of poultry. This research was conducted to evaluate the effects of adding ALTIMOS® (cell wall of *Saccharomyces cerevisiae*; mannan oligosaccharides [MOS] + beta-glucan [BG]) to broiler diets on productive performance, blood parameters, intestine histopathology, and cecum microbiota of broiler chicken. A total of 252 one-day-old Ross chicks were randomly selected and divided into seven treatments, with six replicates of each treatment. The treatments were the control group (0% feed additives), and groups that received 0.05, 0.125, 0.250, 0.500, 1.0, and 2.0 g MOS+BG /kg basal diet for 35 days feeding trial. The results showed that during most trial periods, the group fed the basal diet supplemented with 1.0 g MOS+BG/kg had the highest body weight and weight gain, as well as the lowest feed consumption and best feed conversion ratio, compared to other treated groups. Moreover, this group had the best productive performance in the accumulative period. The inclusion of MOS+BG at 1.0 g/kg diet showed no significant effect on carcass percent compared to the control group. In addition, the inclusion of MOS+BG at 1.0 g/kg diet resulted in the lowest count of *Escherichia coli* and *Enterococcus* in the cecum, the highest *Lactobacillus* bacteria count among all experimental treatments, and a higher yeast count compared to the control group. The group fed 1.0 g MOS+BG/kg ration had the lowest blood cholesterol, whereas there were no significant differences among all experimental groups in the measured liver functions. Notably, the Hemoglobin percentage in the group fed MOS+BG at 1.0 g/kg feed was the highest. In the group fed 1.0 and 2.0 g MOS+BG/kg, the intestinal villi length was longer, and the histopathology revealed mild alteration. Overall, the supplementation of 1.0 g MOS+BG/kg diet improved growth performance, blood constituents, and cecum's beneficial bacteria counts of broilers.

Keywords: Beta-glucan, Blood constitute, Broiler chicken, Cecal parameter, Growth Performance, Mannan Oligosaccharide

INTRODUCTION

In poultry production, the main target is improving the broiler chickens' performance. To obtain optimal development, broiler chickens must receive diets that meet their needs to be healthy and ensure maximum output. Antibiotics have been used for decades to boost avian immune responses as well as growth promoters (AGP). As known, the supplementation of growth-promoting antibiotics in the animal diet has been banned by the European Union (EU) since 2006 (Anadón, 2006). Long-term use of antibiotics in poultry feed affects mainly the consumer due to the development of drug-resistant bacteria (Sweeney et al., 2018). Therefore, antibiotics are no longer used as promoters for growth in chickens' diets due to the harmful consequences on poultry or human health (Kovitvadhi et al., 2019). Prebiotics and probiotics have been used as alternative feed additives instead of antibiotics to improve intestinal health and chickens' performance (Karar et al., 2023). Potential prebiotics derived from the outer cell wall of yeast are now known as mannan-oligosaccharides (MOS). Mannan-oligosaccharides are used as an energy source by beneficial microorganisms such as *lactobacilli* and *bifidobacteria* (Leblebicier and Aydoğan, 2018). Broilers' growth parameters were enhanced by MOS. In the previous study, there was an increase in body weight, body weight gain, and feed conversion ratio when the broiler chickens were supplemented with 1.0 g MOS+ beta-glucan (BG)/kg of ration (Tufail et al., 2019). Otherwise, the chicks fed prebiotics such as MOS (0.2% of diet), had a lower level of cholesterol and creatinine compared to the control chickens (Biswas et al., 2019). In addition, MOS can reduce intestinal pathogenic microbes and it may improve the health of mucous membranes (Mahfuz et al., 2019). Therefore, this study was undertaken to assess the performance,

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blood biochemical parameters, relative organ weights, and cecal microbial content of broiler chickens fed different levels of a prebiotic compound (MOS+BG) as a growth promoter.

MATERIALS AND METHODS

Ethical approval

The ethical statement for all treated chickens was performed following the protocol of the Animal Use Committee of the Agriculture Research Centre, Ministry of Agriculture and Land Reclamation, Giza, Egypt.

Experimental design, management, and diets

The present study was conducted at the Regional Center for Food and Feed (RCFF), Agricultural Research Center (ARC), Giza, Egypt. A total of 252, one-day-old, straight-run Ross broiler chicks with an average initial body weight of 40.00 ± 1.00 g were adapted for three days. On the fourth day of age, with an average 100 g body weight/chick, they were randomly assigned to seven treated groups of six replicates of each with six chicks per replicate, using a completely randomized design. The treated groups are the control (basal diet), and groups that received 0.05, 0.125, 0.250, 0.500, 1.0, and 2.0 g of ALTIMOS® (24.5% MOS+27.7% BG, as yeast cell wall)/kg basal diet. Chicks were housed in wire-bottomed stainless-steel cages in a closed room with exhaust fans to keep normal ventilation. During the experimental period (35 days), feed and water were available *ad libitum*. In the first week, the temperature was adjusted at 31 ± 0.5˚C, and the relative humidity was approximately 60-70%. Then, the temperature decreased by 2˚C per week and the relative humidity reduced from the second week to a final temperature and relative humidity of 24 ± 0.5˚C and 50-60%, respectively, at the fifth week of the age.

Broiler chicks were subjected to continuous light for 24 hours daily during the first week of the experiment. From the second week up to the end of the trial, the daily light schedule was changed to 23 hours of light and 1 hour of darkness per day. According to the vaccination program followed by most Egyptian broiler chicken farms, all experimental broiler chicks were vaccinated against common diseases as shown in Table 1. According to the National Research Council (NRC, 1994)**,** diets were designed to meet the nutritional requirements of broiler chickens during production periods (Table 2).

Table 1. Vaccination program of broiler chickens in the present study

Age (day)	Vaccines	Method used
7	IB	Eye drop
10	H_5N_3	Subcutaneously injected into the lower back part of the neck
14	Infectious bursal disease (Gumboro D78)	Drinking water
24	Infectious bursal disease (Gumboro D78)	Drinking water

Corporation and country made of vaccines: CEVA company, France

Table 2. Composition and calculated chemical analysis of basal diets during the starting, growing, and finishing periods of broiler chickens

Ingredients (kg)	Starting (Day 1-14)	Growing (Day 15-28)	Finishing (Day 29-35)
Yellow corn (7.5% CP)	54.099	57.740	63.400
Soybean meal (46% CP)	34.380	28.480	23.920
Corn gluten meal (60% CP)	4.360	5.010	4.512
Soybean oil	3.179	4.705	4.390
Di-calcium phosphate	1.620	1.842	1.676
Limestone	0.936	0.701	0.697
Vitamin Mixture[1]	0.200	0.200	0.200
Mineral Mixture[2]	0.200	0.200	0.200
NaCl	0.400	0.400	0.400
Lysine-HCl	0.314	0.420	0.348
Methionine	0.237	0.227	0.182
Choline chloride	0.075	0.075	0.075
Total	100.000	100.000	100.000
Calculated values			
Metabolizable energy (KCal/kg)	2999.401	3160.462	3203.099
Crude protein (%)	23.003	21.074	18.989
Calcium (%)	0.960	0.900	0.850
Available phosphorus (%)	0.450	0.480	0.440
Lysine (%)	1.360	1.300	1.130
Methionine (%)	0.610	0.580	0.510
Methionine + cysteine (%)	0.980	0.940	0.850

[1]Vitamin mixture (IU or mg/kg diet): 12000 IU Vitamin A, 2000 IU Vitamin D3, 10 mg Vitamin E, 5 mg Vitamin K3, 3 mg Vitamin B1; 6 mg Vitamin B2; 5 mg Vitamin B6, 0.03 mg Vitamin B12, 40 mg nicotinic acid amine, 10 mg D-Ca-pantothenate, 0.075 mg folic acid, 375 mg choline, 80 mg.
[2]Mineral mixture (mg/kg diet): 60 mg Manganese, 80 mg Iron, 8 mg Copper, 0.5 mg Iodine, 0.2 mg Cobalt, 0.15 mg Selenium.

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Measurements and methods of interpreting results

Productive performance and relative organ weights

Body weight (BW), body weight gain (BWG), feed intake (FI), and the mortality rate of broilers were recorded weekly for each replicate during all periods of growth. The feed conversion ratio (FCR) was calculated by dividing feed intake by body weight gain. At 35 days of age, six chicks were randomly selected from each treated group weighed, slaughtered, blood filtered, feathered, and then eviscerated. The dressing, front part, part, liver, gizzard, heart, spleen, bursa of Fabricius, and abdominal fat were weighed, and the relative weight was calculated.

Microbiological, blood biochemical constituents, and histopathological study

The cecal contents were taken from the six chicks of each group that were slaughtered at 35 days of age, for cecal contents, and bacterial counting, including *Escherichia coli* (*E. coli)*, *Enterococcus*, *Lactobacillus*, Yeast, and *Salmonella* as colony-forming units, Cfu/g (Collin et al., 1995). As well as, at 34 days of age, blood samples were collected from the wing vein of six individuals of each treated group into 2 ml sterile vials and allowed to clot for 4 hours followed by serum separation using a centrifuge (10 minutes, 2000 rpm) before being stored at -20°C for later analysis. Serum measurements were made using commercially available kits (Biosystem S.A., Costa Brava, 30, Barcelona, Spain) following the manufacturer's instructions. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) as indicators of liver function, urea, and creatinine as signs of kidney function, as well as cholesterol and triglycerides were measured. Also, at the same time (34 days of age), other blood samples were aliquoted into 2 ml sterile vials with anti-coagulant and centrifuged for 10 min at 4000 rpm to measure hematological parameters including red blood cell count (RBCs), Hemoglobin content (Hb), white blood cells (WBCs), lymphocytes (L), neutrophil, monocytes, and eosinophils according to Sysmex software of automated hematology analyzer for animal, XT-2000iV analyzer (software version, 00-11, Sysmex, Kobe, Japan).

In all groups, the intestinal tissue samples were collected from the six chicks of each group that were slaughtered at 35 days of age and were preserved in 10% neutral buffered formalin for 72 hours. Then fixed tissue was processed using a paraffin embedding technique and cut into 4 µm thick sections using a microtome (Leica 2135, Germany), and stained by hematoxylin and eosin stain. The stained tissue was examined under a light microscope and photographed with an Olympus XC30 (Tokyo, Japan) digital camera. Intestinal villi length, width, and crypt depth of six intestinal villi were measured in captured images at 40x magnification using TS view software for morphometric analysis. Six images were analyzed to calculate an average for each chick (Mohamed et al., 2020).

Economic efficiency

The administration variables for broiler meat output in each group were assumed to be constant, but weight gain and feed consumption for each experimental group have been calculated to evaluate the economic efficiency of feeding (EEF). Then the following formula has been used to calculate the economic efficiency of feeding/Egyptian pound (EGP):

$$EEF = \frac{Net\ revenue\ (EGP)}{total\ cost\ \ (EGP)} \times 100$$

Statistical analysis

Data were analyzed by the least square procedure of the general linear model (GLM) of SAS software (SAS, 2010)**.** The separation of mean was done using Duncan's New Multiple Range Test (Duncan, 1955)**.** The fixed effects model used in the analysis was :$Y_{ij} = \mu + T_i + \varepsilon_{ij}$ Where Y_{ij} is the value of the respective variable, µ is the overall mean of the respective variable, T_i is the effect due to the i^{th} treatments where i = 1, 2 …and 7 (1 = Control, 2 = 0.05g MOS+BG, 3 = 0.125g MOS+BG, 4 = 0.250g MOS+BG, 5 = 0.5 MOS+BG, 6 = 1 g MOS+BG and 7 = 2g MOS+BG), ε_{ij} is a random error associated with the ij^{th} observation and is assumed to be independently and normally distributed. The significance level was set at ($p \leq 0.05$).

RESULTS

Productive performance

Performance parameters influenced by prebiotic supplementation (MOS+BG) are mentioned in Table 3. There were significant differences ($p \leq 0.05$) among treated groups during all periods from 0 to 35 d in body weight (BW), body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR).

The findings showed that the group fed a basal ration provided by 1.0 g MOS + BG/kg ration had the highest BW and BWG in growing and accumulative periods among all experimental groups ($p < 0.05$). The group fed MOS + BG (0.25 g/kg) had the highest BW and BWG in the starting period, whereas there was no significant difference ($p > 0.05$) between BW and BWG of MOS + BG (1.0g/kg) group and MOS + BG (0.25 g/kg) group. At the finishing period, MOS + BG (1.0 g/kg) group had the highest BW among all experimental groups and a higher BWG than that of the control group. As observed in every period, MOS + BG (0.5 g/kg) group had higher BW and BWG than that of the control group. Moreover, MOS+ BG (0.125 g/kg) group and MOS + BG (2.0 g/kg) group had significantly higher ($p < 0.05$)

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BWG as compared with the control group throughout the entire period. MOS+ BG (1.0 g/kg) group had the highest FI among all experimental groups at the growing and the entire period whereas it had a higher FI than the control group at the starting and finishing periods. Throughout the entire period, MOS + BG (0.125 g/kg), (0.5 g/kg), and (2.0 g/kg) groups had higher ($p < 0.05$) FI than the control group (Table 3). Similarly, MOS + BG (1.0 g/kg) group had the best FCR at growing, finishing, and accumulative periods. As noticed, MOS + BG (0.05 g/kg) group had the highest Feed conversion ratio (FCR) value throughout the entire period. It is worth noting that, MOS + BG 0.125 g/kg, and 0.5 g/kg groups had higher mortality rates than the control group in the accumulative period ($p < 0.05$).

Carcass characteristics

MOS + BG (1.0 g/kg) group had significantly ($p < 0.05$) similar relative carcass and liver weights as that of the control group. Moreover, MOS + BG (1.0 g/kg) group had the highest relative bursa, spleen, and heart weights followed by 0.05 g/kg group (Table 4). There were no significant differences among the relative spleen weights of the control group and MOS + BG (0.05 g/kg and 0.125 g/kg) groups; they had the lowest relative spleen weights compared with the remaining groups ($p < 0.05$). It was observed that there were no significant differences ($p > 0.05$) among relative liver, gizzard, and abdominal fat weights among all experimental groups (Table 4).

Table 3. Effects of diets supplemented by different levels of mannan oligosaccharides and beta-glucan (g/kg) on the performance of broiler chickens during 35 days of age

Productive parameters	Control	MOS+BG						SEM
		(0.05)	(0.125)	(0.250)	(0.5)	(1.0)	(2.0)	
Day 0-14								
Body weight (g)	477^{c}	485^{bc}	462^{d}	496^{a}	489^{b}	492^{ab}	480^{c}	6.00
Body weight gain (g)	377^{c}	385^{bc}	362^{d}	396^{a}	389^{b}	392^{ab}	380^{c}	6.00
Feed intake (g)	437^{a}	431^{b}	411^{c}	439^{a}	441^{a}	441^{a}	437^{a}	5.00
Feed conversion ratio (FCR)	1.16^{a}	1.12^{b}	1.13^{b}	1.10^{c}	1.13^{b}	1.12^{b}	1.15^{a}	0.01
Mortality	0^{b}	0^{b}	0^{b}	0.16^{a}	0^{b}	0.16^{a}	0.16^{a}	0.05
Day 15-28								
Body weight (g)	1744^{c}	1706^{d}	1731^{c}	1792^{b}	1730^{c}	1825^{a}	1778^{b}	17.00
Body weight gain (g)	1268^{c}	1221^{e}	1268^{c}	1295^{b}	1241^{d}	1333^{a}	1298^{b}	15.00
Feed intake (g)	1715^{c}	1686^{d}	1756^{b}	1798^{a}	1695^{d}	1796^{a}	1762^{b}	18.00
Feed conversion ratio (FCR)	1.35^{b}	1.38^{a}	1.38^{a}	1.39^{a}	1.36^{ab}	1.35^{b}	1.36^{ab}	0.02
Mortality	0.16^{b}	0.16^{b}	0.66^{a}	0.16^{b}	0.50^{a}	0.16^{b}	0^{b}	0.1
Day 29-35								
Body weight (g)	2321^{e}	2310^{e}	2359^{d}	2423^{b}	2382^{c}	2454^{a}	2369^{cd}	16.00
Body weight gain (g)	577^{d}	604^{c}	629^{b}	631^{b}	652^{a}	629^{b}	592^{cd}	15.00
Feed intake (g)	1153^{c}	1197^{b}	1193^{b}	1207^{ab}	1239^{a}	1185^{b}	1166^{bc}	24.00
Feed conversion ratio (FCR)	2.01^{a}	1.98^{b}	1.90^{cd}	1.92^{c}	1.90^{cd}	1.89^{d}	1.98^{b}	0.02
Mortality	0.16^{ab}	0.16^{ab}	0.33^{a}	0^{b}	0.16^{ab}	0^{b}	0.16^{ab}	0.09
Day 0-35								
Body weight gain (g)	2221^{e}	2210^{e}	2259^{d}	2323^{b}	2282^{c}	2354^{a}	2269^{cd}	16.00
Feed intake (g)	3305^{c}	3314^{c}	3361^{b}	3444^{a}	3374^{b}	3422^{a}	3364^{b}	27.00
Feed conversion ratio (FCR)	1.48^{b}	1.50^{a}	1.49^{ab}	1.47^{b}	1.47^{b}	1.45^{c}	1.48^{b}	0.01
Mortality	0.33^{c}	0.33^{c}	1.00^{a}	0.33^{c}	0.66^{b}	0.33^{c}	0.33^{c}	0.15

[a-e] Means, within a row with different superscripts, are significantly different ($p < 0.05$). SEM: Standard error of the means.

Table 4. Effects of diets supplemented by different levels of mannan oligosaccharides and beta-glucan (g/kg) on the relative organ weights of broiler chickens during 35 days of age

Treatment	Carcass	Liver	Bursa	Spleen	Gizzard	Heart	Abdominal fat
Control	77.03^{ab}	1.97	0.17^{e}	0.14^{c}	1.28	0.53^{b}	0.99
MOS+BG (0.050)	76.63^{b}	2.04	0.18^{e}	0.16^{c}	1.53	0.55^{ab}	1.01
MOS+BG (0.125)	76.78^{ab}	2.06	0.22^{d}	0.17^{c}	1.36	0.54^{b}	0.94
MOS+BG (0.250)	78.70^{a}	2.05	0.25^{c}	0.25^{b}	1.39	0.65^{ab}	0.97
MOS+BG (0.500)	77.40^{ab}	2.13	0.34^{a}	0.32^{a}	1.34	0.68^{ab}	1.14
MOS+BG (1.000)	76.71^{ab}	2.12	0.36^{a}	0.35^{a}	1.43	0.72^{a}	0.97
MOS+BG (2.000)	77.12^{ab}	2.01	0.28^{b}	0.25^{b}	1.40	0.67^{ab}	0.98
SEM	0.75	0.07	0.02	0.02	0.08	0.03	0.09

[a-c] Means, within a column with different superscripts, are significantly different ($p < 0.05$). SEM: Standard error of the means.

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Cecal microbiota

Regarding cecal microbiota presented in Table (5), there were significant increases ($p < 0.05$) in lactic acid bacteria in all experimental groups than that of the control group, except in the MOS + BG (2.0 g/kg) group which had the same value as that of the control group. It was observed that MOS + BG (0.5 g/kg) and MOS + BG (1.0 g/kg) groups had significantly lower *Escherichia coli* and *Enterococcus* counts than those of the control group ($p < 0.05$). All experimental treatments increased the yeast count significantly ($p < 0.05$) compared with the control group and the highest was obtained from MOS + BG (0.05 g/kg) group. Also, *Salmonell*a was not detected in all groups in the experiment (Table 5).

Table 5. Effects of diets supplemented by different levels of mannan oligosaccharides and beta-glucan (g/kg) on cecum's microbiota (Cfu/g) of broiler chickens during 35 days of age

MOS+BG / Parameters	Control	(0.05)	(0.125)	(0.250)	(0.5)	(1.0)	(2.0)
Lactic acid bacteria (Cfu/g)	12×10^4	$>10^5$	$>10^5$	$>10^5$	$>10^5$	$>10^5$	29×10^4
Escherichia coli (Cfu/g)	16×10^4	10×10^4	18×10^4	7×10^4	6×10^3	43×10^2	25×10^4
Enterococcus count (Cfu/g)	37×10^6	27×10^4	25×10^4	20×10^5	9×10^4	12×10^4	8×10^5
Total yeast count (Cfu/g)	20×10	27×10^4	49×10^3	28×10^2	33×10^2	39×10^3	39×10^3
Salmonella	ND	ND	ND	ND	ND	ND	ND

ND: Non-detected.

Blood constituents

As represented in Table 6, the group fed a 1.0 g MOS+BG/kg diet had a significant ($p < 0.05$) reduction in cholesterol, and urea levels compared to other groups. The data in Table 7 show the blood count (CBC) findings of broiler chickens fed a basal diet supplemented with different levels of MOS + BG. In comparison to the other groups, the group fed a 1.0 g MOS/kg had the greatest ($p < 0.05$) percent level of Hemoglobin (Hgb).

With increasing MOS+BG levels in the diet, the percentage of neutrophils gradually increased, while the percentage of monocytes gradually decreased. Moreover, there was no significance ($p > 0.05$) in the percentage of lymphocytes, RBCs, WBCs, and eosinophils among all experimental groups. There were no significant differences between groups that fed 0.5 and 1.0 g MOS+BG/kg diet in the percentage of neutrophils and monocytes ($p > 0.05$).

Table 6. Effects of diets supplemented by different levels of mannan oligosaccharides and beta-glucan (g/kg) on blood serum constituents of broiler chickens during 35 days of age

Treatment	Cholesterol (mg/dl)	Triglycerides (mg/dL)	Urea (mg/dl)	Creatinine (mg/dl)	AST (U/L)	ALT (U/L)
Control	130.20^{bc}	132.43^{a}	453.67^{ab}	76.00^{a}	4.23	0.2
MOS+BG (0.05)	132.80^{bc}	97.57^{b}	413.00^{ab}	74.00^{a}	4.4	0.2
MOS+BG (0.125)	152.03^{b}	75.27^{d}	316.00^{b}	51.67^{c}	4.06	0.2
MOS+BG (0.250)	181.43^{a}	130.27^{a}	415.67^{ab}	53.67^{c}	4.43	0.2
MOS+BG (0.5)	140.13^{bc}	96.87^{bc}	387.67^{ab}	57.33^{b}	4.50	0.2
MOS+BG (1.0)	117.73^{c}	91.87^{c}	311.33^{b}	57.00^{c}	4.06	0.2
MOS+BG (2.0)	123.73^{c}	90.40^{c}	443.33^{ab}	55.67^{c}	3.83	0.2
SEM	7.93	5.34	27.6	5.8	0.14	---

$^{a-c}$ Means, within a column with different superscript letters, are significantly different ($p < 0.05$). MOS: Mannan oligosaccharides, AST: Aspartate transaminase, ALT: Alanine aminotransferase, SEM: Standard error of the means.

Table 7. Effects of diets supplemented by different levels of mannan oligosaccharides and beta-glucan (g/kg) on blood serum count of broiler chickens during 35 days of age

MOS+BG / Variable	Control	(0.05)	(0.125)	(0.250)	(0.5)	(1.0)	(2.0)	SEM
Hgb (%)	8.43^{c}	8.50^{c}	8.62^{b}	8.57^{bc}	8.53^{bc}	9.70^{a}	8.55^{bc}	0.11
RBCs (Cells/µL)	2.88	2.55	2.60	2.52	2.80	2.57	2.63	0.17
WBCs (19-30×103/mm^3)	28.67	27.33	25.67	22.00	28.67	24.33	24.67	1.58
Lymphocytes (%)	63.00	64.00	65.00	61.67	66.33	63.00	65.00	1.75
Neutrophil (%)	25.33^{e}	26.67^{d}	27.33^{c}	27.33^{c}	28.33^{b}	28.00^{b}	30.33^{a}	0.06
Monocytes (%)	7.33^{a}	7.00^{b}	7.00^{b}	6.67^{c}	6.33^{d}	6.33^{d}	4.33^{e}	0.10
Eosinophils (%)	2.33	2.33	2.33	2.33	2.00	2.33	2.00	0.03

$^{a-e}$ Means, within a row, with different superscript letters are significant ($p < 0.05$). MOS+BG: Mannan oligosaccharides [MOS] + beta-glucan [BG], Hgb: Hemoglobin, RBCs: Red blood cells, WBCs: White blood cells, SEM: Standard error of the means.

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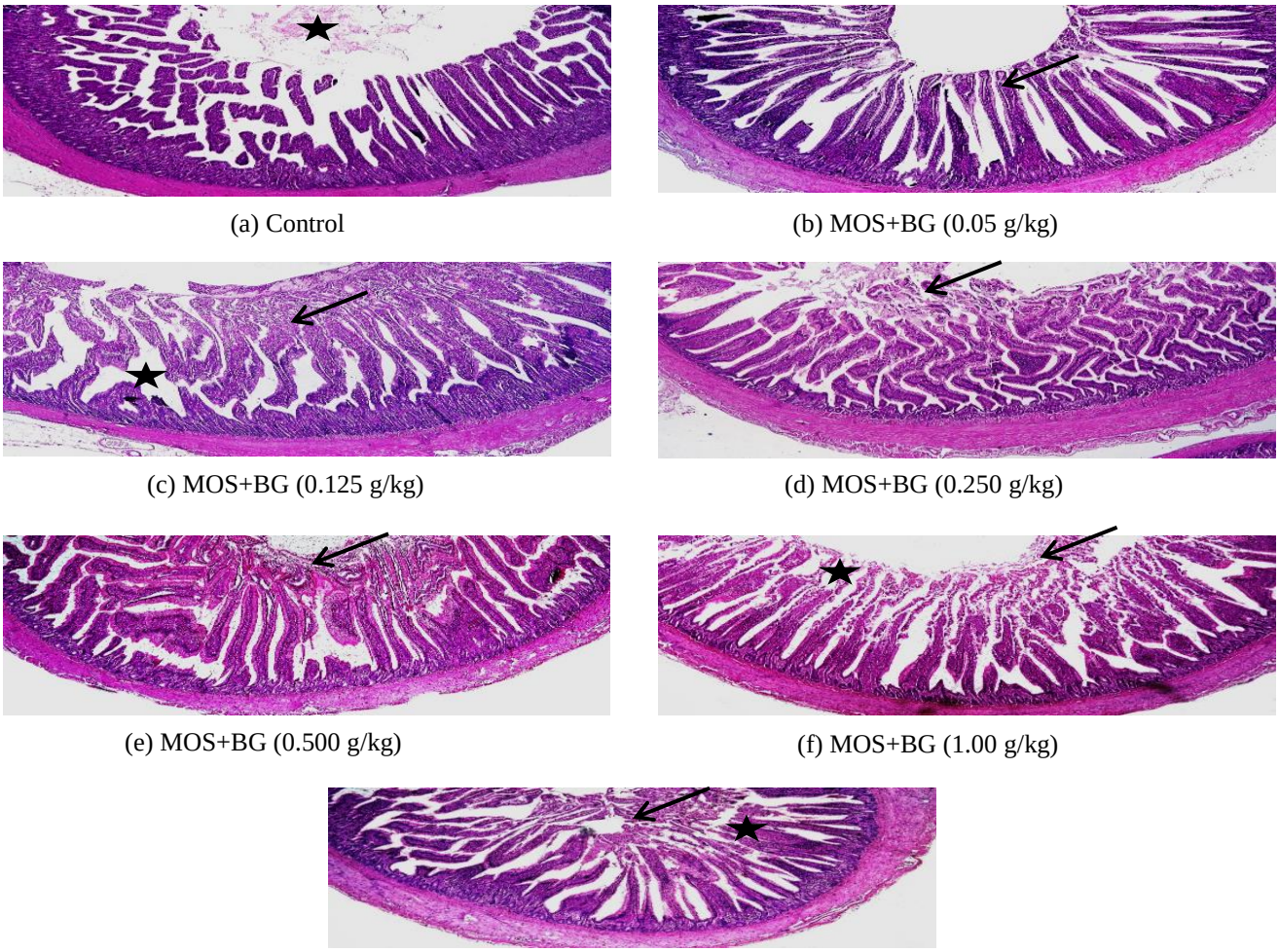
Intestinal histomorphometry and histopathology

A slight shortening of intestinal villi in the intestine of the control group chicks was observed under the microscope. In addition, mucous exudates, a few goblets cell hyperplasia in the lumen, and limited inflammatory cell infiltration in the lamina propria and submucosa (Figure 1a). In a group supplemented with 0.05 g MOS+BG/kg, the intestine had long intestinal villi with regression of the lesions observed (Figure 1b). In the group that fed 0.125 g MOS+BG/kg, the intestine had a mild histopathological change with a few mononuclear cell infiltrations and mucous exudates in the lumen (Figure 1c). Regarding the group treated with 0.25 g MOS+BG/kg, the intestine had a mild histopathological alteration with moderate goblet cell hyperplasia (Figure 1d). The intestine microscopy revealed severe goblet cell hyperplasia in the group supplemented with 0.5 g MOS+BG/kg (Figure 1e). In either group that fed a basal diet supplemented with 1.0 g MOS+BG/kg, or that supplemented with 2.0 g MOS+BG/kg, long intestinal villi, and mild epithelial hyperplasia were recorded (Figure 1f, g). The longest significant villi length was recorded in the 2.0 g MOS+BG/kg group followed by groups of 0.05 g MOS+BG/kg, 0.5 g MOS+BG/kg group, and 1.0 g MOS+BG/kg group. The highest crypt depth was recorded in the control group, and the lowest crypt depth appeared in the intestines of chicks that were fed 0.25 g MOS+BG/kg (Table 8).

Economic efficiency

According to Table 9, although the MOS+BG (1.0 g/kg) group showed higher ($p < 0.05$) cost/kg BW (31.365 EGP) than that of the control group (the lowest cost/kg BW; 30.135 EGP) among all experimental groups, it had the best value of net revenue and economic efficiency compared to other experimental groups ($p < 0.05$). The data revealed that the lowest cost/kg BW among all MOS+BG supplemented groups has resulted from the group fed 0.05 g/kg MOS+BG.



(a) Control (b) MOS+BG (0.05 g/kg)

(c) MOS+BG (0.125 g/kg) (d) MOS+BG (0.250 g/kg)

(e) MOS+BG (0.500 g/kg) (f) MOS+BG (1.00 g/kg)

(g) MOS+BG (2.00 g/kg)

Figure 1. The intestine of broiler chickens at 35 days of age. The mucous exudates in the lumen of the control group (**a**, star), long intestinal villi in MOS+BG (0.05 g/kg) group (**b,** arrow), luminal mucous exudates (**c**, star) with mild goblet cell hyperplasia in MOS+BG (0.125 g/kg) group (arrow), moderate goblet cell hyperplasia in MOS+BG (0.250 g/kg) group (**d**, arrow), severe goblet cell hyperplasia in MOS+BG (0.500 g/kg) group (**e**, arrow), long intestinal villi (**f** and **g,** star), and mild epithelial hyperplasia (**f** and **g** arrow), in MOS+BG (1.00 and 2.00 g/kg) group are shown. Haematoxylin and eosin staining, X40.

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Table 8. Effects of diets supplemented by different levels of mannan oligosaccharides and beta-glucan (g/kg) on intestine morphology of broiler chickens during 35 days of age

Treatment	Length (µm)	Width (µm)	Depth (µm)
Control	1089.53^{b}	159.05	279.44^{e}
MOS+BG (0.05)	1389.57^{cd}	187.37	221.36^{cd}
MOS+BG (0.125)	1029.08^{b}	174.26	240.18^{de}
MOS+BG (0.250)	818.43^{a}	153.45	120.71^{a}
MOS+BG (0.5)	1460.16^{bc}	182.56	153.67^{ab}
MOS+BG (1.0)	1341.31^{c}	210.68	251.5 ^{de}
MOS+BG (2.0)	1568.8^{d}	284.35	179.11^{bc}
SEM	40.63	10.45	9.41

$^{a-e.}$ Means, within a column, with different superscript letters differs significantly ($p < 0.05$). MOS+BG: Mannan oligosaccharides [MOS] + beta-glucan [BG], and SEM: Standard error mean.

Table 9. The economic efficiency of broiler chickens fed diets provided by different levels of mannan oligosaccharides and beta-glucan

Treatment	FI (g/chick)	Feed Cost L.E/chick	*Total Cost L.E/chick	BW (g/chick)	**Total Revenue L.E/chick	Net Revenue L.E/chick	EE	Relative E. E (%)
Control	3305	23.135	30.135	2321	88.198	58.063	1.93	100
MOS+BG (0.05)	3314	23.218	30.218	2310	87.780	57.562	1.90	99
MOS+BG (0.125)	3360	23.570	30.570	2359	89.642	59.072	1.93	100
MOS+BG (0.250)	3444	24.211	31.211	2423	92.074	60.863	1.95	101
MOS+BG (0.5)	3375	23.828	30.828	2382	90.516	59.689	1.94	100
MOS+BG (1.0)	3422	24.365	31.365	2454	93.252	61.887	1.97	102
MOS+BG (2.0)	3665	24.363	31.363	2369	90.022	58.659	1.87	97

* Including chick price which was 7 L.E, **assuming the price of 1 kg live weight was 38 L.E, ***assuming the economic efficiency of the control was 100, FI: Feed intake, BW: Body weight, EE: Economic efficiency

DISCUSSION

Productive performance

Throughout the experimental period, the higher body weight and body weight gain with the best feed conversion ratio were observed in the group that was given a 1.0 g MOS+BG/kg diet. This might be due to the MOS mechanism, which causes a reduction in the load of harmful bacteria and a rise in the production of helpful bacteria, leading to the creation of a healthy intestinal environment, which is reflected in the improvement of performance parameters as a result of better nutrients absorption in the gut. Furthermore, according to Sadeghi et al. (2013), the intestine-beneficial bacteria in the colon, such as *Lactobacillus* and *Bifidobacterium* spp. were developed by MOS+BG at 1.0 g/kg. The villi's surface area increased, and so did intestinal digestion and nutrient absorption (Chand et al., 2016).

The current study is in agreement with previous research (Kamran et al., 2013) which reported that broiler chickens given a basal diet supplemented with 1.0 g MOS+BG/kg showed a substantial improvement in BW, weight gain, and FCR. According to the dosage of MOS+BG in several previous studies, the finest level of MOS+BG for optimal growth performance is almost 1.0 g/kg diet (Abdel-Hafeez et al., 2017; Rehman et al., 2020). The results regarding BW and weight gain were in line with the findings of Ozpinar et al. (2010), who reported that the supplementation of 1.5 g MOS+BG /kg basal diet of the broiler chickens' ration increased the chickens' growth performance.

Carcass characteristics

The significant increase of lymphoid organs percentages (bursa and spleen) in this study due to MOS + BG supplementation, agreed with the results demonstrated by ELnaggar and Abdelkhalek (2017); they reported an increase in spleen relative weight significantly for hens supplemented with high levels of MOS (0.25 and 0.5 g of MOS /kg diet) compared to other experimental groups. Also, the indicated results in the current study, in contrast with results by Muhammad et al. (2020) who indicated no significant effect in relative weights of bursa and spleen between the broiler chickens fed MOS at either 0.5 g/kg or 1.0 g/kg diet, and that fed a basal diet, at 42 days of age. It was shown that carcass, and liver relative weights in broilers fed diets supplemented with MOS at 1.0 g/kg diet were not affected regarding the control group, which is following the results of the previous studies (Rehman et al., 2020; Karar et al., 2023). In disagreement with the present results, Habib et al. (2020) reported an increase in abdominal fat and a decline in the liver size of broiler chickens that were fed the diet supplemented with 4 g/kg MOS.

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Cecum microbial content

The groups that fed a basal diet with all different levels of MOS+BG exhibited a rise in yeast and *Lactobacillus* count while simultaneously showing a drop in *Enterococcus* count. According to Huyghebaert et al. (2011), MOS supplementation may increase the population of lactic acid bacteria in broilers' digestive tracts, improving their resistance to pathogenic bacteria like *E. coli* and *Enterococcus*. Additionally, *salmonella* spp. and *E. coli* fimbriae, which are sensitive to mannose, have unique receptors for the MOS supplements, which cause them to be removed with the digestive flow rather than adhering to intestinal receptors when the pathogen exposure is high. Also, MOS can reduce the number of pathogenic bacteria in the hindgut (Castillo et al., 2008).

According to the results of the current study, the gut microbial population's enhancement has a positive impact on growth performance. The present study's findings are consistent with those of Afrouziye et al. (2014), and Mostafa et al. (2015), who found an increase in *lactobacillus* and *bifidobacterial* spp. and a decrease in the number of *E. coli* in the cecum of broiler chickens fed a diet supplemented with MOS, compared to broilers on a basal diet. According to several studies, broilers fed a basal diet supplemented with MOS had lower cecum *E. coli* counts than those fed a basal diet without any supplements (Mostafa et al., 2015).

Blood parameters

The groups fed a basal diet supplemented with 1.0 g MOS+BG/kg diet and 2.0 g MOS+BG/kg diet had the lowest cholesterol levels. Biswas et al. (2019) found that the level of cholesterol decreased in broilers given a 0.2% MOS group compared to all treatment groups. The most important mechanism of prebiotics to reduce blood cholesterol levels is undoubtedly reduced intestinal lipid absorption by binding bile acids, which affects cholesterol excretion and hepatic production for new bile acids (Kumar et al., 2022).

In contrast with the present results, it was reported that the different quantities of MOS (0.5, 1.0, and 1.5 g/kg ration) showed no significant differences among the levels of blood biochemical indicators (Muhammad et al., 2020). Furthermore, there were no significant changes in AST and ALT levels across the experimental groups. These findings contradict the findings of Jameel et al. (2014) and Helal et al. (2015), who reported a substantial reduction in AST and ALT levels in chickens fed a prebiotic diet compared with chicks fed a basal diet. In contrast to the current results, Biswas et al. (2019) observed that 0.2% MOS significantly increased the AST of 42-day broiler chickens.

In the present study, creatinine levels were considerably lower in most of the experimental treated groups compared to the control group. This is consistent with the findings of Helal et al. (2015) and Muhammad et al. (2020), who reported that there was a decrease in creatinine in chicks fed a prebiotic diet compared to chicks fed a basal diet. The results indicated that the groups (0.125 g MOS+BG/kg diet) and (1.0 g MOS+BG/kg diet) had considerably lower blood urea levels than other experimental groups. These findings disagreed with those of Biswas et al. (2019), who reported a significant rise in blood uric acid concentration in broiler chickens, and with Muhammad et al. (2020) who showed no differences in the level of urea among all broiler chickens' groups. The hematological findings in this investigation revealed no significant difference in RBCs, WBCs, and lymphocytes across all treatment groups that are in agreement with Muhammad et al. (2020), who reported insignificant differences in RBCs, WBCs, and lymphocytes among broilers fed diets supplemented with MOS at levels of 0.5 and 1.0 g/kg basal diet.

Among all experimental groups, the current results confirmed that the Hemoglobin was considerably the highest in the treated group with a 1.0 g MOS+BG/kg diet. Furthermore, Hgb levels were considerably higher in all MOS experimental treated groups when compared to the control group, except the 0.05 g MOS+BG/kg treated group, which exhibited no significant change but was numerically higher than the control. The findings of the present study contradict previous research by Muhammad et al. (2020) who found no effect of MOS supplementation at 0.5, 1.0, and 1.5 g to the basal diet on Hgb levels of broilers compared to the control group.

Intestinal histopathology

Mannan supplementation substantially improved intestinal villi length and lowered crypt depth in the current investigation. Similarly, Karimian and Rezaeipour (2020) revealed that the dietary MOS 2.0 g/kg diet enhanced the height of the villus and reduced crypt depth in the broiler chicken's small intestine. However, some studies found no difference in the length of intestinal villi owing to MOS in diet (Baurhoo et al., 2009) and a rise in crypt depth (Oliveira et al., 2008). The rise in intestinal villi may have occurred as a result of higher *Lactobacillus* counts in the intestine of treated groups, which promote a healthy intestinal environment (Baurhoo et al., 2009). The surface area was increased by long villi and shallow crypts, which improves nutrient absorption (Yang et al., 2009). Stressors that impair the immune response can cause inflammation and damage to the host tissue (Berghman, 2016). In the current investigation, a little histological change in the intestine was seen in the control group; however, the MOS-supplemented groups showed fewer such alterations. These results are consistent with a prior study that found that MOS and live yeast supplementation reduced inflammation (Tarradas et al., 2020).

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Economic efficiency

According to the findings, the control group had the lowest feed costs of all experimental groups, and the group fed a ration with 0.05 g of MOS /kg of ration had lower costs than groups that fed higher MOS dosages. Except for MOS+BG (0.05 g/kg), all groups treated with MOS+BG had greater net revenue values as compared to the control. However, chickens were fed diets supplemented with MOS at 1.0 g/kg was the best. In Egypt, Mostafa et al. (2015) found no appreciable changes in the feed cost of 1.0 kg BWG across broiler chickens given diets supplemented with 0.5, 1.0, or 1.5 g of Bio-Mos/kg diet. The economic impact of using Bio-Mos in broiler diets is only of very limited interest to academics. So long as economic criteria are not compromised, a greater MOS dosage rate may be examined for improved outcomes. The increased body weight growth, enhanced feed conversion, and affordable MOS might be reasons for these improvements.

CONCLUSION

Conclusively, Mannan oligosaccharides plus beta-glucan supplementation, notably at level 1.0 g/kg feed, improved blood parameters without harming intestinal morphology and histology in broiler chickens. It is recommended to conduct further studies regarding the use of mannan oligosaccharides plus beta-glucan in broiler diets.

DECLARATIONS

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Authors' contributions

The study design, data collection, data analysis, writing, and manuscript review were all contributed equally by all authors. Additionally, the statistical results and the final edition of the manuscript were endorsed and agreed upon by all authors.

Competing interests

There are no stated conflicts of interest by the authors.

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Ethical considerations

All authors reviewed the manuscript for ethical issues such as plagiarism, consent to publish, misconduct, forgery and/or falsification of data, duplicate publication and/or submission, and redundancy.

Availability of data and materials

All data from the current study are available by request from the authors.

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