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RESEARCH

Effect of using mannan-oligosaccharides and *β*-glucans as feed additives on growth, biochemical parameters, and immunity of *Salmonella enterica* serovar Enteritidis-challenged turkey

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Abstract
This study aimed to detect the prevalence, antimicrobial susceptibility, and molecular characterization of some virulence determinants in Salmonella recovered from 100 diseased turkeys in Dakahlia Governorate, Egypt. Twelve Salmonella isolates were recovered from the diseased turkeys with an overall prevalence of 12%. The recovered isolates were serotyped into 4 serovars: (*S. enterica* serovar Enteritidis, *S. enterica* serovar Typhimurium, *S. enterica* serovar Infantis and *S. enterica* serovar Heidelberg). Additionally, a 21-d trial investigated the effects of dietary Mannan-oligosaccharides (MOS) and β-glucans as feed additives on growth performance parameters, intestinal morphology, and blood biochemical, immunological parameters, and fecal shedding of *S. enterica* serovar Enteritidis-challenged turkey. A total of 300 One-day-old turkey poults were allocated into five treatment groups (100 poults for each) in a completely randomized design: group 1: a negative control group (non-challenged, non-supplemented), group 2: a positive control group (*S. enterica* serovar Enteritidis-challenged at 7th day, non-supplemented), group 3 and 4: two challenged groups supplemented with a mixture of MOS and *β*-glucan at 1 & 2 g/kg diet, respectively, and T5: a challenged group and treated with Florfenicol antibiotic on the 8th day of the experiment at a dose of 1 ml /liter drinking water for five consecutive days. The in vivo experimental results showed that birds in the positive control group showed reduced growth performance compared with the negative control group. Supplementation with MOS and *β*-glucan, particularly at 2 g/kg ration, significantly improved body weight gain, and feed conversion ratio, in addition to reduced clinical signs and mortality compared with the positive control group. Overall, MOS and *β*-glucan at 2 g/kg restored growth performance close to negative control and showed better feed efficiency than the antibiotic-treated group. MOS and *β*-glucan supplementation, particularly at 2 g/kg, significantly increased globulin, thyroid hormones, and immunoglobulins (IgA, IgG, and IgM), and IL1β, and interferon-gamma (IFN-γ) compared with the control groups. Histopathological examination revealed normal liver and spleen structures in the negative control group, while the positive control group showed marked degenerative and inflammatory changes in the liver and lymphoid depletion in the spleen. Supplementation with MOS and *β*-glucan, particularly at 2 g/kg, markedly improved hepatic and splenic histological structures with mild inflammatory changes, approaching the normal architecture. The antibiotic-treated group also exhibited nearly normal histological features. Bacteriological examinations revealed lower Salmonella shedding in the MOS and β-glucans 2 g/kg group in comparison with the positive control and the MOS and β-glucans 1 g/kg groups. In conclusion, dietary addition of MOS and β-glucans 2 g/kg in turkey diets improved growth performance, immune status, and hepatic and splenic histological structures in addition to lowering Salmonella fecal shedding.

Keywords Turkey · Mannan-oligosaccharides · *β*-glucan · *Salmonella enterica* serovar Enteritidis · Performance · Immunity

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Introduction

In recent years, the poultry industry has faced numerous challenges due to the widespread occurrence of infectious diseases that adversely affect growth performance and health status of commercial birds (Rafieian-Naeini et al. 2025). Salmonellosis is one of the most significant bacterial infections that lead to substantial economic losses in poultry production worldwide (Aljumaah et al. 2020). On a larger scale, turkey producers face great losses from strict trade bans, the forced destruction of infected flocks and costly recalls of contaminated meat products (Gaucher et al. 2010). It represents an important public health concern due to its zoonotic nature. It is a foodborne disease transmitted to humans through contaminated meat processing and consumption (Abudabos et al. 2017). Young birds are particularly susceptible to Salmonella infection owing to their rapid growth and immature immune system (Abudabos et al. 2019). The infections in turkeys with Salmonella are common worldwide, producing significant economic losses due to higher mortality rates at an early age, decline in egg production, poor quality of birds, in addition to higher costs needed for treatments and control measures. Salmonella infection in young poults showed mortalities ranged from 10 to 20%, and in severe cases it may reach to 80% or more. Clinical signs such as omphalitis, caseous cecal core and necrotic foci in liver were recorded (Hafez and Shehata 2024). Once Salmonella enters the host, it rapidly colonizes the intestinal tract, causing intestinal inflammation, impaired nutrient absorption, reduced growth performance, and increased mortality rates in infected birds (Chang et al. 20202019; Shaji et al. 2023). Antibiotic growth promoters (AGPs) have been utilized for decades in poultry feed to increase growth and enhance immunity (Osman et al. 2024). The prolonged use of AGPs in the poultry diet can cause the emergence of antibiotic resistant pathogens and drug residues, which primarily impact the consumer (Sweeney et al. 2018). Finding alternatives to enhance gut health and reduce foodborne pathogens in poultry production has been intensively studied mostly due to the restricted or banned use of AGPs in animal feed (De Oliveira and Hosotani 2023). Prebiotics is one alternative that could potentially compete with antibiotic growth promoters and support the growth of poultry industry (Abd El-Hack et al. 2022). Prebiotics are non-digestible feed components that selectively stimulate the growth and activity of beneficial gut microorganisms, thereby improving intestinal health and host immunity (Gibson et al. 2004). Among the most widely studied prebiotics are mannan-oligosaccharides (MOS) and β-glucans, which are derived from the outer cell wall of yeast (*Saccharomyces cerevisiae*) (Awaad et al. 2011).

MOS and β-glucans exert several beneficial effects on the host as MOS can bind to pathogenic bacteria such as Salmonella spp. and E. coli, preventing their attachment to intestinal epithelial cells and thereby reducing bacterial colonization in the gut (Teng et al. 2021). Additionally, β-glucans are recognized as immunomodulatory compounds that stimulate innate immune responses by activating macrophages and other immune cells (Pourabedin and Zhao 2015; Meijerink et al. 2021). Furthermore, prebiotics have been reported to enhance animal growth by improving nutrient utilization, increasing enzyme activity and maintaining digestive function (Liu et al. 2018), controlling inflammation (Song et al. 2019), and providing antioxidant activity (Wang et al. 2021). Prebiotics can also promote short-chain fatty acid (SCFA) synthesis, down-regulating *Salmonella* invasion in GIT epithelial cells (Van Immerseel et al. 2006).

Although numerous studies have evaluated the effects of MOS or β-glucan individually in poultry diets, there is still limited information regarding the combined supplementation of these compounds in turkeys under Salmonella challenge conditions, particularly concerning intestinal morphology and immune responses. Therefore, investigations are needed to better understand the potential synergistic effects of these prebiotics in improving gut health and resistance to enteric pathogens. Therefore, the objective of the current study was to assess the effects of a prebiotic mixture (MOS and β-glucan) on performance parameters, intestinal morphology, and blood biochemical and immunological parameters in turkey following oral Salmonella challenge.

Materials and methods

Field study (bacteriology)

Sample collection

One hundred diseased turkeys (age: 4 to 12 weeks) were collected from 10 turkey flocks (10 birds/flock) in Dakahlia Governorate, Egypt. The selected flocks were subjected to clinical and postmortem examinations, and the reported signs were diarrhea, depression, poor weight gain, inappetence, ruffled feathers, pericarditis, enlarged and congested liver and spleen, cheesy cecal cores, perihepatitis, and enteritis. The mortality rates ranged from 15 to 40%. Samples from internal organs: spleen, liver, cecum, and heart were collected from each bird and pooled together as one sample for each bird individually. The collected samples were labelled and transported as soon as possible to the Reference Laboratory for Veterinary Quality control on Poultry

production (RLQP) (Dakahlia and Sharkia laboratories) for further examinations.

Salmonella isolation and identification

The collected samples were examined for Salmonella isolation according to (ISO 2017). Salmonella isolates were subjected to serological identification for somatic and flagellar antigens according to the procedures of the White-Kauffmann-Le Minor scheme (Grimont and Weill 2007).

Antimicrobial susceptibility

The in vitro susceptibility testing of the confirmed isolates was performed using the disc diffusion method on Mueller–Hinton agar plates (Oxoid, UK). Eleven antimicrobial agents were selected: amoxicillin (AMX, 10 µg), ampicillin/sulbactam (SAM, 20 µg), doxycycline (DO, 30 µg), oxytetracycline (OT, 30 µg), neomycin (N, 30 µg), erythromycin (E, 25 µg), streptomycin (S, 10 µg), sulfamethoxazole- trimethoprim (SXT, 25 µg), florfenicol (FFC, 30 µg), norfloxacin (NOR, 10 µg) (CLSI 2020). Minimum inhibitory concentration (MIC) of colistin sulphate susceptibility was assessed using broth microdilution test according to the guide lines of (CLSI 2020). The multidrug resistance index (MDRI) was calculated using a/b formula, where (a) is the total number of antimicrobial agents exhibiting resistance in each isolate and (b) is the total number of the examined antimicrobial agents (Krumperman 1983). The isolates that showed resistance to at least one antimicrobial agent in 3 or more antimicrobial classes were categorized as multidrug resistant (MDR) (Awad et al. 2023).

Molecular characterization of Salmonella virulence determinants

The genomic DNA was extracted from 5 selected Salmonella isolates (exhibiting higher multidrug resistance) using the QIAamp DNA Mini kit (Qiagen, Germany, GmbH) with some modifications from the manufacturer's instructions. In brief, 200 µl of the Salmonella suspension was incubated with 200 µl of lysis buffer and 20 µl of proteinase K for 10 min at 56 °C. After that, 200 µl of ethanol (100%) was added to the lysate and the samples were washed and centrifuged according to the manufacturer's recommendations. Nucleic acid was eluted using 100 µl of elution buffer.

The used oligonucleotide virulence Primers were provided by Metabion (Germany):

1. *inv*A gene (F: GTGAAATTATCGCCACGTTCGGGCA A& R: TCATCGCACCGTCAAAGGAACC) (284 bp) (Oliveira et al. 2003).
2. *fim*H (F: GTGCCAATTCCTCTTACCGTT& R: TGG AATAATCGTACCGTTGCG) (164 bp) (Hojati et al. 2015).

PCR amplification (Uniplex PCR): primers were utilized in a 25-µl reaction containing 12.5 µl of EmeraldAmp Max PCR Master Mix (Takara, Japan), 5.5 µl of water, 1 µl of each primer of 20 pmol concentration, and 5 µl of DNA template. The reaction was conducted in an Applied Biosystem 2720 thermal cycler.

The PCR products were separated by electrophoresis on agarose gel (1.5%) (Applichem, Germany, GmbH) in 1x TBE buffer at room temperature using gradients of 5 V/cm. The gel analysis was performed as follows, 20 µl of the PCR products were loaded in each gel slot. Gelpilot 100 bp plus Ladder (Qiagen, Germany, GmbH) and Gene ruler 100 bp ladder (Fermentas, Thermo, Germany) were used to determine the fragment sizes. The gel was photographed using the gel documentation system (Alpha Innotech, Biometra), and the data were analyzed using computer software.

Animal study: efficacy of mannan and β-glucan as feed additives on controlling MDR Salmonella in turkeys

Animal ethics

The experimental procedures were approved by the Ethical Committee in the Faculty of Veterinary Medicine, Zagazig University, Egypt, performed in accordance with the Guide for the Care and Use of Laboratory Animals, and were approved under approval number ZU-IACUC/2/F/152/2025.

Salmonella challenge strains

A higher MDR *S. enterica* serovar Enteritidis isolate, which carried 2 virulent genes (*inv*A and *fim*H), was selected from this study and used for the challenge purposes. Turkey poults were challenged orally with *S. enterica* serovar Enteritidis at a dose of 10^5 CFU/ml in the 7th day of age (Rahimi et al. 2019).

Application of MOS and β-glucan feed additives

The Yeast Cell Wall (MOS and β-glucans, ABMOS®) was sourced from AB Chem (Egypt), and the product of Chinese origin. The product contains 20% MOS and 25% β-glucan.

Experimental management and design

One-day-old turkey poults (supplied from a commercial hatchery in Sharkia Governorates, Egypt) ($n=300$) were

maintained at age-appropriate temperatures and supplied with water and feed ad libitum all over the experimental period (21 days). Birds were fed diets free from antibiotic growth promotors. The experimental turkey poults were tested to be free from Salmonella by cloacal swabbing, which was plated on specific agar plates (ISO 2017). Turkeys were kept in floor pens (chopped wheat straw, 7–15 cm length) with dimensions of 1 m ×1 m. Turkey poults were tested to be free from Salmonella by cloacal swabbing, which was plated on specific agar plates (ISO 2017). Turkeys were distributed into 5 experimental groups (6 replicates/group, 10 poults/replicate). Group 1: control negative group (non-challenged, non-supplemented), group 2: positive control group (challenged with *S. enterica* serovar Enteritidison the 7th day of the experiment, non-supplemented), group 3 (challenged with *S. enterica* serovar Enteritidis, supplemented with 1gm of MOS and β-glucan mixture/kg diet), Group 4: challenged with *S. enterica* serovar Enteritidis, supplemented with 2 gm MOS and β-glucan mixture/kg diet), and group 5: challenged with *S. enterica* serovar Enteritidis, treated with Florfenicol antibiotic on the 8th day of the experiment at a dose of 1 ml/liter drinking water (Floricol 10%, Pharma Swede-Egypt, 10th of Ramadan City, Egypt) for 5 successive days. The experiment ended at the 21 days of experiment (14 days post challenge). The turkey diets were formulated according to (NRC 1994) and the proximate composition of the diets is shown in Table 1.

Table 1 Proximate chemical composition of the basal diets as a fed basis (%)

Ingredients	%
Yellow corn, 7.5%	41.80
Soybean meal, 48%	49.00
Corn gluten, 60%	2.525
Soybean oil	1.80
Calcium carbonate	1.50
Calcium dibasic phosphate,18%	2.00
Common salt	0.15
Premix[1]	0.30
DL- Methionine, 98%	0.20
L- Lysine, Hcl, 78%	0.20
Choline 60 veg	0.07
L- Threonine, 98.5%	0.10
Phytase	0.005
Sodium bicarbonate	0.25
Antimycotoxin	0.10
Chemical analysis (%)	
ME poultry (kcal/kg)	2815
Crude protein	28.03
Methionine	0.58
Lysine	1.67
Calcium	1.21
Available Phosphorus	0.62

1 Premix per kg of diet: vitamin E–10 mg; vitamin A–1500 IU; vitamin D3–200 IU; vitamin K3–0.5 mg; thiamine–1.9 mg; riboflavin–3.7 mg; pantothenic acid–10 mg; folic acid–0.55 mg; niacin–35 mg; cobalamin–0.01 mg; pyridoxine–3.5 mg; biotin–0.15 mg; Se–0.15 mg; Fe–80 mg; Mn–60 mg; Zn–40 mg; Cu–8 mg; I–0.35 mg

Growth performance

The feeding trial lasted for 3 weeks. Growth performance parameters were calculated and recorded on the 1 st day, and at the end of each week to determine their initial body weight (IBW), body weights (BW), feed intake (FI), and body weight gain (BWG). Feed conversion ratio (FCR) was calculated as the ratio of feed intake (g) to body weight gain (g) (Wanger et al. 1983).

Sample collection

Twelve birds from each treatment (two birds/replicate) were randomly selected for sample collection at the end of the study (d 21). Birds were euthanized via cervical dislocation, following the recommendations of the Veterinary Medical Association guidelines (Association 2013). Blood samples ($n=12$) were collected and centrifuged at 3000 rpm for 15 min to generate serum, which was stored at −20 °C until it was utilized for biochemical tests. Around 2 cm sections from the liver and the spleen ($n=6$) were collected and placed in 10% neutral buffered formalin for further histomorphology.

Blood biochemical analysis

Liver function markers, including aspartate aminotransferase (AST) and alanine aminotransferase (ALT), as well as kidney function indicators such as uric acid and creatinine, were measured in the collected serum according to the methods described by Abdi-Hachesoo et al. (2011).

Serum total protein and albumin concentrations were determined according to the methods described by Grant and Kachmer (1987) and Doum et al. (1981), respectively. Serum globulin levels were calculated by subtracting albumin from total protein values (Doumas et al. 1981).

Serum concentrations of triiodothyronine (T3) and thyroxine (T4) were determined using chicken ELISA kits (Cat. No. MBS269454 and MBS265796, respectively; MyBioSource, San Diego, CA, USA). Serum levels of immunoglobulins IgG, IgA, and IgM were determined using chicken-specific ELISA quantification kits (Bethyl Laboratories Inc., Montgomery, TX, USA).

Interferon γ (INF-γ) and interleukin 1β (IL-1β) levels were measured using ELISA assay kits (MyBioSource, San Diego, CA, USA) (Cat. No. MBS700243 and MBS2024496, respectively).

Histopathological technique

Specimens from liver and spleen of turkeys were fixed in neutral buffered formalin 10% for 24 h. Ascending grades of alcohol were used as dehydrating agents followed by clearing agents as xylene I then xylene II. After that we impregnated organs in paraffin wax to obtain paraffin blocks. 5-micron thickness paraffin sections were obtained by using microtome (Leica RM 2155, London, UK). Staining of sections were performed using hematoxylin and eosin (H&E) stain (Abd El-Aziz et al. 2025).

Bacteriological examinations

The spontaneously dead turkey poults and the humanely euthanized birds by cervical dislocation at the end of the experiment were necropsied and subjected to postmortem examinations as reported in the approved protocol of the Ethical Committee. Samples from liver, cecum, and spleen were collected aseptically and subjected to *S. enterica* serovar Enteritidis isolation and identification (ISO 2017). The representative colonies recovered from XLD agar plates were confirmed serologically by the slide agglutination test using the poly (O and H) and the serotype-specific antisera.

Salmonella fecal shedding: cloacal swabs were collected aseptically from all birds at 7time points: −6, −3, 0, 3, 5, 7, and 14 days post challenge (dpc). Each swab was suspended in 1 ml sterile saline solution, then subjected to serial dilution. From each dilution, 0.1 ml was plated on selective XLD agar plates and incubated at 37 °C for 24 h. The typical black colonies with red centers were counted (CFU/ml) and subjected to confirmation using slide agglutination with poly O, poly H, and serotype-specific antisera for *S. enterica* serovar Enteritidis.

Table 2 Salmonella serotypes recovered from Dakahlia Governorate

Serotype	Salmonella isolates
S. enterica serovar Enteritidis	5
S. enterica serovar Typhimurium	4
S. enterica serovar Infantis	2
S. enterica serovar Heidelberg	1
Total	12/100 (12%)

Statistical analysis

Data were analyzed using a one-way analysis of variance (ANOVA) via the General Linear Model (GLM) procedure of SPSS (SPSS Inc., Chicago, Illinois, USA). Prior to the ANOVA, the Shapiro-Wilk test was applied to verify data normality, and Levene's test was used to confirm the homogeneity of variances. The pen served as the experimental unit for all evaluated parameters (growth performance, blood profiles, and health status indicators). Differences between treatment means were compared using Tukey's post-hoc test at a 5% probability level. Data variance was expressed as pooled standard error of the mean (SEM), and the significance level was set at $P<0.05$."

Results

Prevalence and serovars of Salmonella

Out of the 100 examined diseased turkeys collected from Dakahlia Governorate, 12 Salmonella isolates were detected with an overall prevalence of (12%).

The serotyping of the recovered Salmonella showed the detection of Four serovars: *S. enterica* serovar Enteritidis (5), *S. enterica* serovar Typhimurium (4), *S. enterica* serovar Infantis (2) and *S. enterica* serovar Heidelberg (1). The most predominant serotypes were *S. enterica* serovar Enteritidis and *S. enterica* serovar Typhimurium (Table 2).

Antimicrobial susceptibility testing

The Disc diffusion results of the isolated Salmonellae showed total resistance (100%) for neomycin. Higher resistance (91.7%) was recorded for oxytetracycline and colistin sulphate, followed by (83.3%) for amoxicillin, erythromycin, streptomycin, and norfloxacin. Ampicillin/sulbactam, doxycycline, and sulfamethoxazole-trimethoprim exhibited resistance with (75%). Florfenicol showed both resistance and sensitivity with (50%). All the isolated Salmonellae showed multidrug resistance (100%), and 11 different antimicrobial resistance pattern profiles were recorded. The reported multidrug resistance index (MDRI) ranged from 0.73 to 0.91 (Tables 3 and 4).

Table 3 Antimicrobial susceptibility of Salmonella isolates

Antimicrobial agent	AMX	SAM	DO	OT	N	E	S	SXT	CT	FFC	NOR
Susceptible (%)	2 (16.7%)	3 (25%)	3 (25%)	1 (8.3%)	0 (0%)	2 (16.7%)	2 (16.7%)	3 (25%)	1 (8.3%)	6 (50%)	2 (16.7%)
Resistant (%)	10 (83.3%)	9 (75%)	9 (75%)	11 (91.7%)	12 (100%)	10 (83.3%)	10 (83.3%)	9 (75%)	11 (91.7%)	6 (50%)	10 (83.3%)

Amoxicillin (AMX), ampicillin/sulbactam (SAM), doxycycline (DO), oxytetracycline (OT), neomycin (N), erythromycin (E), streptomycin (S), sulfamethoxazole-trimethoprim (SXT), colistin sulphate (CT), florfenicol (FFC), norfloxacin (NOR)

Table 4 Antimicrobial resistance pattern profiles of Salmonella isolates

Antimicrobial agent pattern profiles	Antimicrobial agent	NO. of isolates	No. of resistance markers	MDRI	MDR
1	AMX-DO-OT-N-E-S-CT-FFC-NOR	1	9	0.82	MDR
2	AMX-DO-OT-N-E-S-CT-NOR	1	8	0.73	MDR
3	AMX-SAM-DO-N-E-S-SXT-CT-FFC	1	9	0.82	MDR
4	AMX-SAM-DO-OT-N-E-SXT-CT	1	8	0.73	MDR
5	AMX-SAM-DO-OT-N-E-S-SXT-FFC-NOR	1	10	0.91	MDR
6	AMX-SAM-DO-OT-N-S-SXT-CT-FFC-NOR	1	10	0.91	MDR
7	AMX-SAM-DO-OT-N-E-SXT-CT-NOR	1	9	0.82	MDR
8	AMX-SAM-DO-OT-N-S-SXT-CT-NOR	1	9	0.82	MDR
9	SAM-OT- N-E-S-SXT-CT-FFC-NOR	1	9	0.82	MDR
10	AMX-SAM-OT-N-E-S-SXT-CT-NOR	2	9	0.82	MDR
11	DO-OT-N-E-S-CT-FFC-NOR	1	8	0.73	MDR

Amoxicillin (AMX), ampicillin/sulbactam (SAM), doxycycline (DO), oxytetracycline (OT), neomycin (N), erythromycin (E), streptomycin (S), sulfamethoxazole-trimethoprim (SXT), colistin sulphate (CT), florfenicol (FFC), norfloxacin (NOR). MDRI: multidrug resistance index, MDR: multidrug resistance

Molecular characterization of Salmonella virulence determinants

The examined MDR Salmonella isolates using the PCR technique showed the detection of *inv*A and *fim*H virulence genes in all of the examined isolates.

Efficacy of Mannan and β-glucan on turkey poults-challenged with MDR *S. enterica* serovar Enteritidis

Clinical signs, mortalities, and postmortem lesions

The turkey poults in the negative control group showed no clinical signs or mortalities. Meanwhile, the positive control group showed clinical signs in the form of diarrhea, off food, dropping wings and blindness in one or both eyes and (30%) mortalities. The Postmortem examination showed enteritis and intestines filled with diarrhea, congested internal organs, perihepatitis and pericarditis.

The group supplemented with MOS and β-glucan 1 g/kg showed off food, depression, slight diarrhea and dropping wings, congested internal organs, perihepatitis, pericarditis and 20% mortalities. The group supplemented with MOS and β-glucan 2 g/kg and the antibiotic group revealed the presence of off food, dropping wings, perihepatitis and 10% mortalities (Table 5).

Table 5 Clinical signs, mortalities, postmortem lesions

Group No.	Clinical signs	Postmortem lesions	Mortalities (%)
1	No clinical signs	No lesions	0/10 (0%)
2	Diarrhea, off food, and dropping wings. Some chicks showed blindness in one or both eyes.	Enteritis, the intestine filled with diarrhea, congested internal organs Perihepatitis and pericarditis	3/10 (30%)
3	Off food, depression, slight diarrhea and dropping wings	congested internal organs Perihepatitis and pericarditis	2/10 (20%)
4	Off food and dropping wings	Perihepatitis	1/10 (10%)
5	Off food and dropping wings.	Perihepatitis	1/10 (10%)

1: negative control, 2: positive control, 3: MOS+β-glucan (1g/kg), 4: MOS+β-glucan (2g/kg), 5: Antibiotic

Growth performance

The IBW did not differ significantly among groups ($P>0.05$). At the end of the first week, there was no significant difference in the BW, BWG, FI, and FCR among groups ($P>0.05$). In the second week, the negative control exhibited the highest BW, BWG, FI, and best FCR, whereas MOS and β-glucan supplementation at 2 g/kg showed increased BW and BWG followed by the antibiotic group compared with the positive control group ($P<0.01$). The feed intake in the group received 2 g/kg MOS and β-glucan was not differed significantly from the negative control group, while it reduced in the positive control group, 1 g/kg MOS and β-glucan group, and the antibiotic group. The FCR was improved in the negative control group and 2 g/kg MOS and β-glucan group. The same pattern persisted during the third week, where MOS and β-glucan (2 g/kg) supplementation increased the BW, BWG, and FI compared to the positive control group and the MOS and β-glucan (1 g/kg) ($P<0.01$). The FCR was improved in the negative control group and MOS and β-glucan (2 g/kg) group compared with the positive control group ($P<0.01$). Overall, MOS and

Table 6 Growth performance of turkey poults infected with salmonella fed on Mannan-oligosaccharides and *β*-glucan

Parameters	Control (-)	Control (+)	MOS+*β*-glucan (1 g/kg)	MOS+*β*-glucan (2 g/kg)	Antibiotic	SEM	*P*-value
First week							
IBW (g)	48.2	46.4	47.2	47.9	48.0	0.311	0.575
BW (g)	133	131	130	133	131	0.383	0.055
BWG (g)	84.9	84.7	82.4	84.8	82.9	0.438	0.322
FI (g)	148	144	146	149	147	0.572	0.124
FCR	1.74	1.71	1.78	1.76	1.77	0.010	0.287
Second week							
BW (g)	278.11^{a}	225.67^{e}	237.89^{d}	263.78^{b}	251.22^{c}	5.60	0.0001
BWG (g)	145^{a}	94.6^{e}	108^{d}	131^{b}	120^{c}	4.72	0.0001
FI (g)	235^{a}	192^{c}	205^{c}	225^{ab}	220^{b}	4.77	0.0001
FCR	1.62^{c}	2.03^{a}	1.89^{b}	1.72^{c}	1.83^{b}	0.03	0.0001
Third week							
BW (g)	465^{a}	345^{e}	371^{d}	416^{b}	394^{c}	12.41	0.0001
BWG (g)	187^{a}	119^{d}	133^{cd}	152^{b}	142^{bc}	7.03	0.0001
FI (g)	324^{a}	231^{d}	248^{cd}	277^{b}	264^{bc}	9.71	0.0001
FCR	1.73^{c}	1.94^{a}	1.87^{ab}	1.82^{bc}	1.86^{ab}	0.02	0.002
Overall performance							
BW (g)	465^{a}	345^{e}	371^{d}	416^{b}	394^{c}	12.41	0.0001
BWG (g)	417^{a}	298^{e}	324^{d}	368^{b}	346^{c}	12.24	0.0001
FI (g)	707^{a}	566^{d}	600^{c}	651^{b}	636^{b}	14.67	0.0001
FCR	1.70^{d}	1.90^{a}	1.85^{ab}	1.77^{c}	1.84^{b}	0.02	0.0001

[a,b,c,d]Means within same row with different superscript are statistically different at $P<0.05$ according to Tukey's Honesty significant difference test. *BW,* body weight; *BWG,* body weight gain; *FI,* feed intake; *FCR,* feed conversion ratio; *SEM,* Standard error of the means

β-glucan supplementation at 2 g/kg significantly improved BW, BWG, and FCR relative to the positive control, achieving performance close to the negative control group and superior feed efficiency compared to the antibiotic group, confirming its potential as an effective antibiotic alternative under challenge condition (Table 6).

Blood biochemical parameters

As shown in Table 7, significant differences were detected among treatments in the serum levels AST, creatinine, total protein, globulin, T3, and T4, while ALT and uric acid were not significantly different. The MOS and β-glucan supplementation, particularly at 2 g/kg, increased globulin and T4 levels compared with other groups ($P<0.01$). The total protein level was higher in MOS and β-glucan supplemented-groups and antibiotic-treated group than the control groups ($P<0.01$). The albumin level was increased in the 1 g/kg MOS and β-glucan group compared with the negative control group ($P=0.017$). Thyroid hormones (T3 and T4) were elevated in MOS+β-glucan and antibiotic groups relative to control groups ($P<0.01$). Creatinine levels differed significantly among treatments with MOS and β-glucan–supplemented birds showing higher values than control groups ($P<0.01$). However, all values remained within the normal physiological range.

Table 7 Effects of diets supplemented by mannan-oligosaccharides and β-glucan (g/kg) on blood biochemical parameters of turkey poults

Indices	Control (-)	Control (+)	MOS+*β*-glucan (1 g/kg)	MOS+*β*-glucan (2 g/kg)	Antibiotic	SEM	*P*-value
ALT (U/L)	5.40	5.80	6.20	7.00	6.40	0.200	0.094
AST (U/L)	44.6^{b}	48.8^{ab}	56.4^{a}	48.2^{b}	51.6^{ab}	1.09	0.002
Creatinine (mg/dl)	0.211^{c}	0.220^{bc}	0.252^{a}	0.240^{a}	0.230^{ab}	0.003	0.0001
Uric acid (mg/dl)	3.10	3.58	2.82	3.00	3.58	0.110	0.101
Total protein (g/dl)	3.22^{b}	3.27^{b}	4.60^{a}	5.19^{a}	4.84^{a}	0.170	0.0001
Albumin (g/dl)	1.23^{b}	1.30^{ab}	1.91^{a}	1.57^{ab}	1.83^{ab}	0.080	0.017
Globulin (g/dl)	1.99^{c}	2.07^{c}	2.69^{b}	3.61^{a}	3.01^{b}	0.140	0.0001
T3 (ng/mL)	3.57^{b}	3.58^{b}	4.15^{a}	4.34^{a}	4.28^{a}	0.070	0.0001
T4 (ng/mL)	19.2^{c}	18.8^{c}	22.4^{b}	24.6^{a}	22.8^{ab}	0.490	0.0001

[a,b,c]Means within same row with different superscript are statistically different at $P < 0.05$ according to Tukey's Honesty significant difference test. *AST,* Aspartate transaminase; *ALT,* Alanine aminotransferase; *T3,* Triiodothyronine; *T4,* thyroxine; *SEM,* Standard error of means

Immune status

MOS and β-glucan supplementation, particularly at 2 g/kg, increased IgA, IgG, and IgM levels compared with both control groups ($P<0.01$). Supplementation with MOS and β-glucan at 2 g/kg resulted in the highest levels of IL-1β and IFN-γ ($P<0.01$), exceeding those of the MOS and β-glucan at1 g/kg and antibiotic-treated groups, while both control groups recorded the lowest responses (Table 8).

Histopathological results

Liver sections from the control negative group of turkey (Fig. 1A) revealed normal morphology of hepatic acini, portal triads, central veins, and sinusoids. Sections from the liver of the positive control group (Fig. 1B) showed degenerative changes in large numbers of hepatocytes, congested hepatic blood vessels, thickening of bile duct wall by chronic inflammatory reaction with focal aggregate of lymphocytes beside hyperplasia of bile duct epithelium with cholestasis. While sections from the 1 g/kg MOS and β-glucan group (Fig. 1C) revealed mild degree of hepatic degenerative changes, congested hepatic blood vessels and minute foci of perivascular inflammatory cells aggregations (mainly lymphocytes). Sections from the 2 g/kg MOS and β-glucan group (Fig. 1D) showed apparent normal hepatic parenchyma with focal aggregates of lymphocytes infiltrates. In the other hand, liver sections from the antibiotic group (Fig. 1E) showed normal histology of hepatic acini, sinusoids, Kupffer cells, central veins, and portal triad.

Spleen sections from the control negative group of turkey (Fig. 2A) revealed normal histology of white pulps around eccentric arterioles and normal red pulps. Sections from the control positive group (Fig. 2B) revealed necrotic or apoptotic lymphoid elements at most lymphoid populations of white pulp that represented by depletion of lymphoid follicles around ellipsoid arterioles. Depletion few numbers of white pulp lymphoid populations with re-establishment of most lymphoid elements within white pulp were noticed at the 1 g/kg MOS and β-glucan group (Fig. 2C) and the 2 g/kg MOS and β-glucan group (Fig. 2D). Spleen sections from the antibiotic group (Fig. 2E) showed normal histology of white pulp lymphoid follicles around eccentric arterioles and normal red pulps. The latter had sinusoids filled with erythrocytes, lymphocytes, reticular fibers, and macrophages.

Bacteriological examinations and Salmonella fecal shedding

All the experimental groups were free from Salmonella at −6, −3 and 0 days before challenge. The negative control group was free from Salmonella at all time points. At 3 dpc, Salmonella fecal shedding showed no significant difference between group 2 (positive control) and group 3. Also, no significant difference was recorded in the fecal shedding of group 4 (fed on MOS and β-glucans 2 g/kg) and group 5 (treated with an antibiotic). At 5, 7 and 14 dpc, a significant reduction in shedding was recorded in the treated groups 3, 4 and 5 in comparison with the positive control. Comparing groups 3, 4, and 5, the results showed that shedding at 5 and 7 dpc differed significantly, and a significant reduction was recorded in groups 4 and 5. At 7 and 14 dpc, group 4 and 5 showed a significantly lower Salmonella reduction than group 3. At 14 dpc, no significant reduction in fecal shedding was recorded between group 4 and group 5 (Table 9). The detected S. enterica serovar Enteritidis colonies in the shedding examinations were confirmed serologically by poly O, poly H, and the serotype-specific antisera.

Discussion

According to bacteriological examinations in the current study, Salmonella was recovered from diseased turkey flocks in Dakahlia Governorate with 12%. These results were nearly similar to Abd El-Tawab et al. (2015) who isolated Salmonella from turkey flocks in Dakahlia Governorate,

Table 8 Effects of diets supplemented by different levels of mannan oligosaccharides and β-glucan (g/kg) on serum immune indices of turkey poults

Indices	Control (-)	Control (+)	MOS + *β*-glucan (1 g/kg)	MOS + *β*-glucan (2 g/kg)	Antibiotic	SEM	*P*-value
IgA (mg/100 ml)	2.90^{b}	2.83^{b}	3.33^{b}	4.68^{a}	4.53^{a}	0.170	0.0001
IgG (mg/100 ml)	1.69^{c}	1.67^{c}	2.73^{b}	3.78^{a}	3.50^{a}	0.190	0.0001
IgM (mg/100 ml)	0.670^{c}	0.651^{c}	1.25^{b}	2.04^{a}	1.98^{a}	0.120	0.0001
IL1β (ug/mL)	142^{c}	138^{c}	151^{b}	162^{a}	153^{b}	1.93	0.0001
IFN-γ (pg/ml)	5.47^{bc}	5.33^{c}	6.18^{bc}	7.90^{a}	6.42^{b}	0.210	0.0001

^{a,b,c}Means within same row with different superscript are statistically different at $P < 0.05$ according to Tukey's Honesty significant difference test. *AST,* Aspartate transaminase; *ALT,* Alanine aminotransferase; *SEM,* Standard error of the means

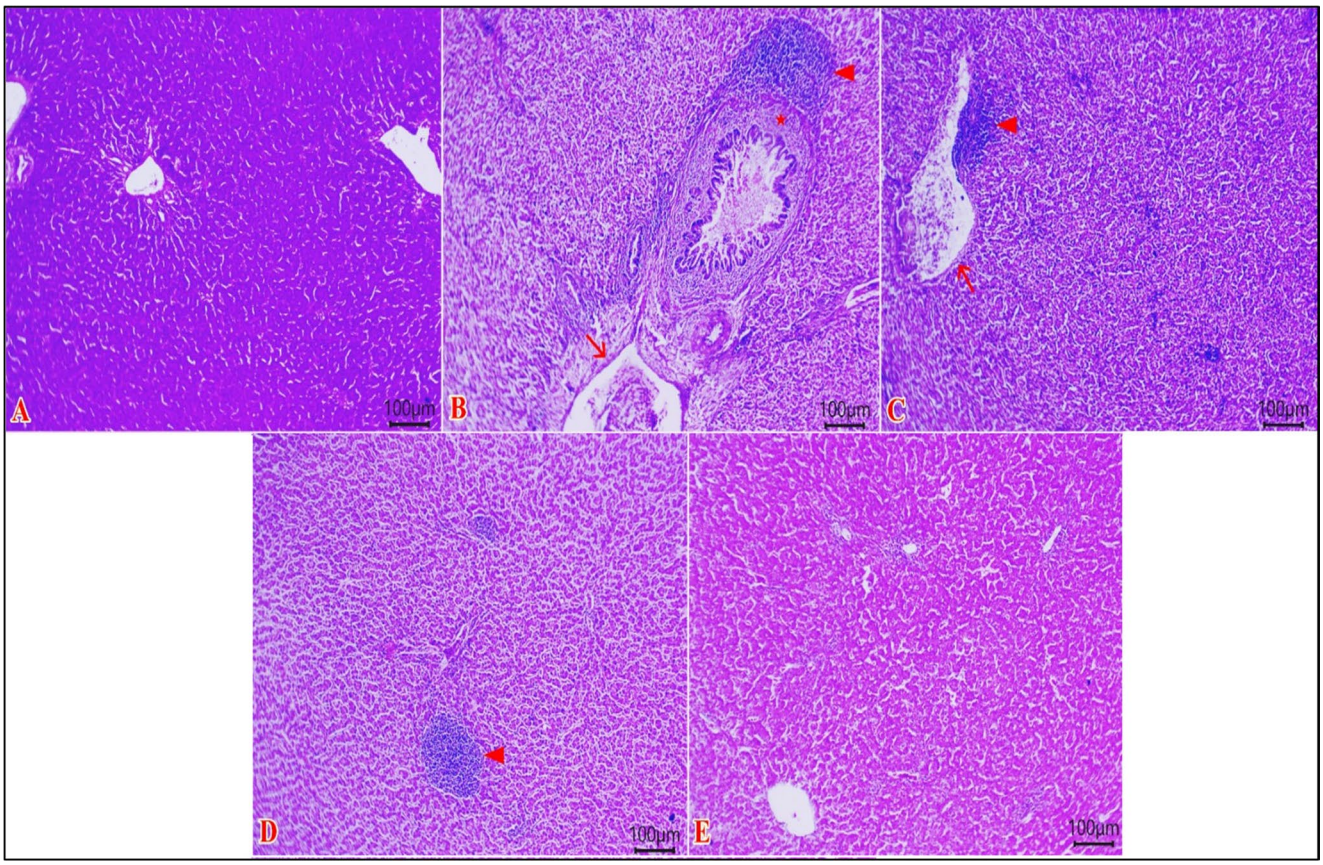

Fig. 1 Photomicrograph of H&E-stained sections of the liver (scale bar 100 µm) of the experimental groups showing: **A** normal morphology of hepatic acini, portal triads, central veins, and sinusoids in the control negative group. **B** degenerative changes in large numbers of hepatocytes, congested hepatic blood vessel (arrow), thickening of bile duct wall by chronic inflammatory reaction (star) with focal aggregate of lymphocytes (arrowhead) beside hyperplasia of bile duct epithelium with cholestasis in the control positive group. **C** mild degree of hepatic degenerative changes, congested hepatic vasculature (arrow) and minute perivascular focal area of inflammatory cells aggregations (arrowhead) in the 1 g/kg MOS and β-glucan group. **D** apparent normal hepatic parenchyma with focal aggregates of lymphocytes infiltrates (arrowhead) in the 2 g/kg MOS and β-glucan group. E: normal histology of hepatic acini, sinusoids, Kupffer cells, central veins, and portal triads in the antibiotic group

Egypt with 10%, and Jahantigh et al. (2015) who found that Salmonella was prevalent in turkeys 14.8%. Lower isolation rates from turkey farms were recorded in Algeria with 5% (Djemaioune et al. 2025) and in Egypt with 4% (Abd El-Tawab et al. 2020). In contrast, higher Salmonella isolation was recorded with 64% from Bangladesh turkey farms (Tawyabur et al. 2020).

In the current study, *S. enterica* serovar Enteritidis, *S. enterica* serovar Typhimurium, *S. enterica* serovar Infantis and *S. enterica* serovar Heidelberg were reported as the most predominant serovars. The obtained results in this study were similar to Papadopoulou et al. (2009), who isolated *S. enterica* serovar Typhimurium from British turkey flocks. However, our finding contrasts with several previous international and local studies that identified different predominant serovars, such as *S. enterica* serovar Kentucky in North Carolina (Santos et al. 2007) and *S. enterica* serovar Kedougou and *S. enterica* serovar Kottbus in Great Britain (Danguy des Desert et al. 2011), and *S. enterica* serovar Albany and *S. enterica* serovar Hadar in Taiwan (Yeh et al. 2018). These discrepancies in serovar distribution and predominance could be fundamentally due to geographical variations and differing climatic conditions, in addition to variation in management practices, including flock density, age, and breed, in addition the level of implementation of farm biosecurity measures. These localized variations within Egypt, compared to Abd El-Tawab et al. (2020) and Sanad et al. (2016) could be due to different farming systems. Additionally, they may reflect the continuous changes in the epidemiological map of Salmonella in Egypt over recent years.

The disc diffusion results in this study demonstrated complete resistance (100%) of Salmonella isolates to neomycin and higher resistance (91.7%) to oxytetracycline and colistin sulphate, followed by 83.3% resistance to amoxicillin, erythromycin, streptomycin, and norfloxacin.

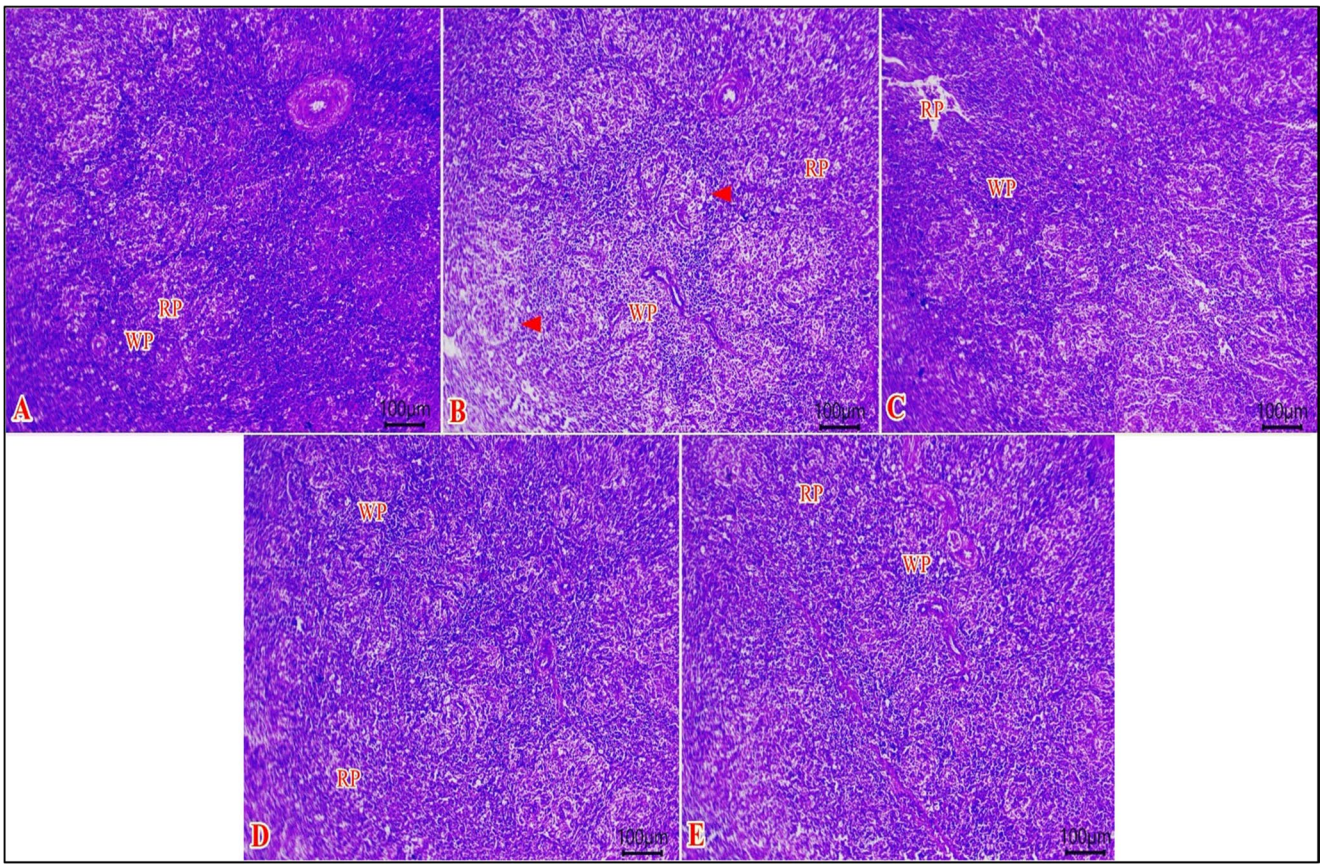

Fig. 2 Photomicrograph of H&E-stained sections of the spleen (scale bar 100 µm) of the experimental groups showing: **A** normal histology of white pulps around eccentric arterioles and normal red pulps in the control negative group. **B** depletion of lymphoid follicles around ellipsoid arterioles (arrowheads) in the control positive group. **C**, **D** depleted few numbers of white pulp lymphoid populations with re-establishment of most lymphoid elements within white pulp at both 1 and 2 g/kg MOS and β-glucan, respectively. E: normal histology of white pulp lymphoid follicles around eccentric arterioles and normal red pulps at in the antibiotic group. White pulp (WP), red pulp (RP)

Table 9 The mean fecal shedding (log_{10} CFU/ml) of *S. enterica* serovar Enteritidis during the experimental period

Days	Control (-)	Control (+)	MOS + *β*-glucan (1 g/kg)	MOS + *β*-glucan (2 g/kg)	Antibiotic	SEM	*P*-value
3 dpc	0^{c}	4.55^{a}	4.26^{a}	3.38^{b}	3.23^{b}	0.17	0.0001
5 dpc	0^{e}	5.785^{a}	3.77^{b}	3.29^{c}	1.09^{d}	0.13	0.0001
7 dpc	0^{e}	5.81^{a}	2.71^{b}	1.57^{c}	$<1^{d}$	0.05	0.0001
14 dpc	0^{d}	4.87^{a}	1.3^{b}	$<1^{c}$	$<1^{c}$	0.09	0.0001

^{a,b,c,d,e}Means within the same row with different superscripts are statistically different at $P<0.05$ according to Tukey's Honesty significant difference test. *dpc,* days post challenge; *SEM,* Standard error of means. Control (-): group 1, control (+): group 2, MOS + β-glucan (1 g/kg): group 3, MOS + β-glucan (2 g/kg): group 4, Antibiotic: group 5

Ampicillin/sulbactam, doxycycline, and sulfamethoxazole-trimethoprim exhibited resistance with 75%. Florfenicol showed both resistance and sensitivity (50%). These findings were in near similarity with Jahantigh et al. (2015), who observed high resistance of Salmonella recovered from turkeys against tetracycline and colistin with 86.5% and 83.8%, respectively. A different resistance pattern from this study was investigated by Santos et al. (2007), who found that Salmonella isolated from turkey farms exhibited resistance to tetracycline, streptomycin, ampicillin, and trimethoprim-sulfamethoxazole with 85.7%, 64.3%, 35.7%, and 7.1%, respectively. Compared to the obtained results in this study, some previous studies identified different frequencies of Salmonella resistance in turkeys against amoxicillin, and tetracycline with 100% (Rumi et al. 2019), and against florfenicol, oxytetracycline, doxycycline, colistin, ampicillin, amoxicillin, trimethoprim-sulfamethoxazole with 97.5%, 89.3%, 78.6%, 77.8%, 75.7%, 75.3%, and 73.7%, respectively (Yeh et al. 2018). Lower resistance of Salmonella isolated from turkey flocks compared to the present study showed resistance to streptomycin and tetracycline with 47.2% and 43.3%, respectively (Sodagari et al. 2023).

Additionally, Palmeira et al. (2016) recorded lower resistance to tetracycline and neomycin with 34.9% and 30.2%, respectively. In this study, multidrug resistance of Salmonella was recorded with 100%, and this agreed with the findings reported by Abd El-Tawab et al. (2020). The emergence of Salmonella antimicrobial resistance and the variations in the resistance pattern between farms may be attributed to the misuse of antimicrobials, stock density, biofilm formation, and biosecurity levels.

Dietary supplementation with MOS and β-glucan in this study showed low clinical signs and mortalities with no PM lesions in comparison with the positive control group. The mortality in MOS and β-glucan 2 g/kg and antibiotic groups was 10%, while in the MOS and β-glucan 1 g/kg group it was 20%. These results may be attributed to the ability of MOS and *β*-glucans in preventing pathogenic bacteria from adhesions to the intestinal tract (Wasilewska et al. 2010; Rahimi et al. 2020; Teng et al. 2021). Our findings were supported by De Oliveira and Hosotani (2023), who mentioned that feeding turkeys with glucomannan lowers mortality rates.

The findings of the present study clearly demonstrated that Salmonella challenge markedly impaired the growth performance of turkey poults compared with the negative control group. In contrast, dietary supplementation with MOS + β-glucan significantly improved growth-related parameters, particularly at the level of 2 g/kg, which resulted in significant increases in body weight and body weight gain, along with an improved feed conversion ratio compared with the positive control group. Notably, the growth performance of poults receiving 2 g/kg MOS + β-glucan approached that of the negative control group and was comparable to that observed in the antibiotic-treated birds. This parity underscores the potential of MOS + β-glucan as an effective non-antibiotic growth promoter in turkey production, capable of maintaining productivity under pathogenic challenges. The positive effects observed in the present study may be attributed to the well-documented role of MOS in modulating intestinal microbial ecology. Mannan-oligosaccharides are known to inhibit the colonization and adhesion of pathogenic bacteria while simultaneously promoting the proliferation of beneficial gut microbiota, thereby contributing to the establishment of a more stable and balanced intestinal environment (Osman et al. 2024). Such modulation of the gut microbiome improves intestinal functionality by enhancing nutrient digestion and absorption, which ultimately translates into improved growth performance (Ghazalah et al. 2021). Improvements in body weight and weight gain have been reported with inclusion levels of 1 g/kg (Kamran et al. 2013; Osman et al. 2024); and 1.5 g/kg (Özpinar et al. 2010). Moreover, higher inclusion levels, such as 2 g/kg MOS, have been reported to further maximize growth performance and feed efficiency (Ghazalah et al. 2021). In addition, the modulation of gut microbiota by MOS has been associated with improvements in intestinal morphology, including increased villus height and expanded absorptive surface area, which further enhances nutrient uptake efficiency (Chand et al. 2016). Consequently, the improvement in gut integrity and digestive efficiency may explain the enhanced growth performance observed in the present study. These findings are in agreement with previous reports indicating that dietary supplementation with mannan-oligosaccharides can significantly improve growth performance in poultry species, including broiler chickens (Nikpiran et al. 2013).

According to Rathgeber et al. (2008), yeast β-glucan demonstrated growth-promoting effects comparable to those of the antibiotic virginiamycin, which is commonly incorporated into broiler diets at subtherapeutic levels. Likewise, Zhang et al. (2008) reported that dietary supplementation with β-glucans improved both average daily gain and feed conversion ratio in broiler chickens. In addition, several studies have confirmed the beneficial influence of β-glucan on broiler performance (Tokunaka et al. 2000; Thanardkit et al. 2002). Salmonella challenge tended to reduce the growth performance of birds fed the control diet (Sacakli et al. 2025). Similar observations have been reported in previous studies (Abudabos et al. 2019; Teng et al. 2020). The impaired growth performance may be associated with intestinal mucosal damage induced by Salmonella infection, which negatively affects nutrient absorption (Sacakli et al. 2025). However, Mountzouris et al. (2009) observed no significant differences in growth performance between Salmonella-infected and healthy broilers. These inconsistent findings may be related to differences in Salmonella type, strain, or infection dose, which could influence the stability of the intestinal environment (Malago et al. 2003).

Serum biochemical profiles provide essential insights into the metabolic efficiency and health status of turkey poults under salmonella challenge. In the present study, the increase in total protein and globulin levels in the 2 g/kg MOS and β-glucan and antibiotic groups compared to both controls, signifies an enhanced capacity for protein synthesis and a robust humoral immune response. Amer et al. (2022) reported an increase in serum total protein levels in β-glucan supplemented groups, while found no significant changes in serum globulin levels. Fadl et al. (2020) revealed improved total protein and globulin compared to the challenged-untreated groups. Gabr (2021) suggested that yeast-derived components have an insignificant effect on total protein and globulin levels in the serum of rabbits. El-Sawy et al. (2015) noticed no effect of yeast glucan supplementation on blood levels of total proteins or albumin of broiler chickens. This protein profile aligns with the histopathological observation

of re-established splenic lymphoid populations, suggesting that the treatment effectively countered the immunosuppressive effects of *Salmonella*.

Regarding thyroid function, the significant increase in T3 and T4 levels in the treated groups suggests an improvement in the birds' metabolic rate. The higher thyroid hormone levels in the high level of MOS and β-glucans (2 g/kg) likely contributed to the superior growth performance and body weight gain observed, as these hormones are pivotal for regulating basal metabolism and protein deposition in poultry. These results are in agreement with Amer et al. (2022) who reported an increase in the thyroid hormones in the glucan supplemented group. T3 and T4 hormones affect growth rate via various metabolic processes, including the stimulation of RNA and protein synthesis (Oster et al. 2018). Furthermore, elevated serum levels of thyroid hormones can be directly translated into better BWG (Nari and Ghasemi 2020).

Furthermore, the stability of AST, ALT, creatinine, and uric acid within physiological ranges indicate that the level 2 g/kg MOS and β-glucans is safe and does not exert adverse effects on hepatic or renal functions. These findings are consistent with Osman et al. (2024), who reported that dietary supplementation with MOS and β-glucan maintains liver enzyme activities. El-Sawy et al. (2015) observed a significant decrease in AST and ALT activities in birds fed 2 g/kg MOS. Furthermore, the stability of these enzymes contradicts the findings of Rokade et al. (2018) and Biswas et al. (2019), who observed a significant increase in AST in broiler chickens fed MOS. Helal et al. (2015) reported a substantial reduction in AST and ALT levels in birds fed prebiotic-enriched diets. Fadl et al. (2020) revealed that Agrimos® (MOS+ beta-glucan) supplementation did not negatively affect serum ALT and AST, this may be related to the antioxidant properties of MOS, which have been reported to neutralize free radicals and mitigate oxidative stress during pathogenic challenges (Dawood et al. 2020). Regarding renal function, Helal et al. (2015) and Muhammad et al. (2020) reported a decrease in serum creatinine in chicks fed prebiotic diets compared to those on a basal diet. Ghazalah et al. (2021) reported that creatinine levels in all MOS-treated groups were significantly lower than those in the control group.

In the present study, the marked elevation of immunoglobulins (IgA, IgG, and IgM) in the high level of MOS and β-glucans (2 g/kg) comparable to the antibiotic treated birds strongly reinforces the immunomodulatory potential of the tested prebiotic. These findings are consistent with previous reports indicating that MOS and β-glucans supplementation can induce systemic immune activation and enhance resistance to enteric pathogens (Osman et al. 2024). The observed increase in IgA levels reflects improved mucosal immunity, which is attributed to the ability of yeast- derived polysaccharides to enhance the proliferation and activity of intraepithelial lymphocytes within the intestinal lamina propria (Tsukada et al. 2003). In parallel, the elevated IgG and IgM levels suggest a strengthened systemic humoral immune response capable of mitigating the stress induced by pathogenic challenge. Mechanistically, mannans and β−1, 3–1, 6-glucans act as microbial associated molecular patterns that interact with host recognition receptors, thereby modulating immune signaling pathways (Ferket et al. 2002). Additionally, MOS may function as a competitive exclusion agent by binding to mannose-specific lectins on pathogenic bacteria, preventing their adhesion to intestinal epithelium and facilitating their clearance (Vandeplas et al. 2010). This is further supported by reports demonstrating that dietary MOS supplementation (1 g/kg) in laying hens significantly elevates serum IgA, IgG, and IgM levels, alongside improved hematological parameters like lymphocyte counts while improving gut health parameters (Youssef et al. 2023). Moreover, yeast cell wall components contribute to the reinforcement of the intestinal barrier integrity through up regulation of mucin-2 and tight junction proteins, such as occludin and claudin, thereby limiting pathogen translocation (Schwartz and Vetvicka 2021); (Bi et al. 2022).

Collectively, These effects are often associated with improved development of primary lymphoid organs, including the bursa of Fabricius, ensuring sustained immune competence (Gheisari and Kholeghipour 2006; Zhang et al. 2008). Furthermore, modulation of gut microbiota, particularly the enrichment of beneficial genera such as Lactobacillus and Bifidobacterium, may indirectly enhance IgA secretion through short-chain fatty acid production and maintenance of epithelial integrity (Dunislawska et al. 2017; Teng and Kim 2018; Slawinska et al. 2019). In addition to humoral immunity, the significant increase in IL-1β and IFN-γ levels in the MOS and β-glucans (2 g/kg) group reflects a robust activation of the cellular immune responses. The observation aligns with previous findings demonstrating that yeast-derived prebiotics effectively modulate cytokine profiles to enhance host defense mechanisms (Fadl et al. 2020). The elevated IL-1β levels may be attributed to the capacity of β-glucans to activate macrophages and modulate the release of pro-inflammatory cytokines (Cao et al. 2018; Amer et al. 2022). At the molecular level, β-glucans bind to specific receptors such as Dectin-1 and complement receptor 3 (CR3) on the surfaces of macrophages, dendritic cells, and monocytes (Bohn and Bemiller, 1995; Tsukada et al. 2003). Thereby triggering intracellular signaling cascades that bridge innate and adaptive immunity (Chae et al. 2006).

Furthermore, the peak response of IFN-γ in the high-dose group can be attributed to enhanced T-cell activation, which play a crucial role in orchestrating cellular immunity (Bazan et al. 2014).

These immunological responses are particularly important in the context of enteric infections, as cytokines-mediated regulation of inflammation contributes to limiting pathogen invasion, including Salmonella species (Xiong et al. 2015). This potent immune response is in agreement with the recent findings of Sacakli et al. (2025) who demonstrated that dietary supplementation of MOS and β-glucans significantly mitigates the negative impact of *S. enterica* serovar Typhimurium challenge. Their study underscores the importance of these prebiotics in maintaining intestinal integrity and enhancing the host's defensive posture during enteric infections. In contrast, the lower immune responses observed in the positive control groups may be associated with the detrimental effects of pathogenic challenges on gut integrity and immune function.

The histopathological architecture of the liver and spleen serves as a critical indicator of the systemic health and immune status of the birds. In the present study, the positive control group exhibited severe tissue disorganization, including widespread hepatocellular degeneration and significant splenic lymphoid depletion. These findings are consistent with previous reports demonstrating that co-infection with *Salmonella* and *Eimeria* in turkeys induces severe systemic inflammation and compromises host immune defenses leading to significant tissue damage and metabolic exhaustion (Pascual et al. 2020; Rafieian-Naeini et al. 2025). In contrast, supplementation with the higher level (2 g/kg) MOS and β-glucan demonstrated a superior restorative capacity compared to the lower dose (1 g/kg), as evidenced by reduced hepatic congestion and substantial recovery of splenic lymphoid populations. This indicates that the treatment, particularly at higher inclusion levels, exerts significant hepatoprotective and immunomodulatory effects in turkeys. These observations are in agreement Amer et al. (2022), who reported that yeast-derived beta-glucans act as potent immunomodulators by enhancing the expression of CD3 and CD20 (T and B lymphocyte markers) in the spleen of the broiler, thereby reversing lymphoid depletion and restoring immune organ integrity following systemic challenges.

At the molecular level, The hepatoprotective effect observed in the higher level MOS + β-glucan and antibiotic groups may be attributed to the down regulation of key pro-inflammatory mediators, including TNF-alpha and NF-kappaB as demonstrated by Fadl et al. (2020). This molecular suppression likely explains the marked reduction in vascular congestion and inflammatory cell infiltration observed in the treated groups. Furthermore, the comparable efficiency between the high- dose prebiotic group and the antibiotic group aligns with the previous findings of Ghazalah et al. (2021) and Osman et al. (2024), supporting the hypothesis that optimized prebiotic supplementation can effectively preserve histological integrity and maintain immune homeostasis. Furthermore, the stability of serum biochemical parameters, including AST and ALT within physiological ranges supports the histopathological findings and indicates that dietary supplementation with MOS and β-glucans at 2 g/kg is safe and does not adversely affect hepatic functions.

Regarding MDR *S. enterica* serovar Enteritidis shedding in the experimental turkey poults, the current study demonstrated lower shedding in the MOS and *β*-glucans (2 g/kg) and antibiotic groups when compared to the positive control and the MOS and *β*-glucans (1 g/kg) groups. By comparing the supplementation of MOS and *β*-glucans (2 g/kg) and MOS and *β*-glucans (1 g/kg), it was found that the experimental poults showed very low shedding at 7 and 14 dpc after supplementation with MOS and *β*-glucans (2 g/kg). The findings in this study indicated the effectiveness of MOS and *β*-glucans (2 g/kg) in lowering *S. enterica* serovar Enteritidis colonization in the intestinal tract of turkeys without producing side effects in comparison with antibiotic treatments. These results are supported by the ability of MOS and *β*-glucans supplementation in preventing pathogenic bacteria adhesions to intestinal epithelial cells (Teng et al. 2021), and the MOS supplementation to turkey poults controls pathogenic bacteria colonization (Wasilewska et al. 2010; Rahimi et al. 2020). The findings of this study agreed with Meijerink et al. (2022), who found lower *S. enterica* serovar Enteritidis counts in the intestinal tracts of chickens at 7 dpc and 21 dpc after feeding with a diet supplemented with glucomannan. Despite the efficacy of the antibiotic treatment in this study in preventing *S. enterica* serovar Enteritidis colonization at 14 dpc, its application may promote antibiotic resistance in case of misuse. For this reason, the MOS and *β*-glucan, particularly at 2 g/kg is preferable to be supplemented.

One limitation of this study is the use of a combination of MOS and β-glucans without investigating each individually to determine their specific effects. Therefore, further studies using MOS and β-glucans separately in turkey diets are needed to investigate their effects on controlling Salmonella infection compared with antibiotic application. Another limitation is the limited tissue and blood analysis. So, further studies are recommended to examine the molecular analysis of the immune genes in the small intestine and intestinal histopathology.

Conclusion

The present study demonstrated that Salmonella infection remains a significant challenge in turkey production, as evidenced by its prevalence, multidrug resistance patterns, and associated negative impacts on growth performance, health status, and immune function. Dietary supplementation with MOS and β-glucans, particularly at 2 g/kg, represent a promising and effective alternative to antibiotics in turkey production systems indicated by improved growth performance, enhanced immunity, and controlling Salmonella infection. Their supplementation also maintained liver and kidney function within normal physiological ranges. Histopathological findings further supported these results, showing substantial restoration of hepatic and splenic architecture in MOS and β-glucans-treated groups. Further studies are recommended to examine the molecular analysis of the immune genes in the small intestine and intestinal histopathology.

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Data availability The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Ethical approval The experimental procedures were approved by the Ethical Committee in the Faculty of Veterinary Medicine, Zagazig University, Egypt, performed in accordance with the Guide for the Care and Use of Laboratory Animals, and were approved under approval number ZU-IACUC/2/F/152/2025.

Competing interests The authors declare no competing interests.

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