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Growth Performance, Nutrient Digestibility, Biochemical Properties, Hematological Traits, and Intestinal Histopathology of Broiler Chicks Fed Mannan Oligosaccharides

Abdallah Ali Ghazalah[1], Mohamed Ahmed Fouad El-Manylawi[1], Hady Fathy Abbas Motawe[2], Marwa Salah Khattab[3], and Yara Ibrahem Youssef[2*]

[1]*Animal Production Department, Faculty of Agriculture, Cairo University, Giza, Egypt*
[2]*Regional Centre for Food and Feed, Agricultural Research Centre, Giza, Egypt*
[3]*Pathology Department, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt*

*Corresponding's Email: yara.ibrahem30@gmail.com; ORCID: 0000-0002-5653-3936

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ABSTRACT

Antibiotics as growth promoters in poultry diets are currently restricted, so other feed additives, such as prebiotics and probiotics, have been suggested as an antibiotics alternative to improve the performance and gut health of poultry. The current experiment was conducted to study the effects of adding Mannan oligosaccharides (MOS) as a potential replacement for an antibiotic on productive performance, nutrient digestibility, some blood parameters, and caecal microbiota of broiler chickens. For conducting the current research experiment, a total of 180 one–day old Ross broiler chicks were randomly divided into one control group fed a basal diet and four dietary treatments with six replicates for each treatment. The treatment groups were fed a basal diet supplemented with different levels of MOS 0.5, 1, and 2 g/Kg diet and Lincomycin 4.4 mg/Kg diet during 35 days of the feeding trial. With exception of the growing period, the group fed a basal diet supplemented with 2 g MOS/Kg feed had considerably higher body weight and weight gain, while having the lowest feed consumption and best feed conversion ratio compared to the other treatment groups, during all experimental periods. Moreover, dietary supplementation of MOS resulted in a significant decrease in the counts of caecal E. coli and Enterococcus, while Lactobacillus and Yeast bacteria counts were significantly higher, compared to non-supplemented groups. Broiler chicks having a 2 g MOS/kg diet recorded lower values of cholesterol, low-density lipoprotein (LDL), urea, and liver enzymes, including Aspartate transaminase (AST) and Alanine aminotransferase (ALT), while they recorded significantly higher high-density lipoprotein (HDL), compared to the other experimental groups. Group treated with MOS 2 g/Kg feed improved digestibility of crude protein, ether extract, crude fiber, nitrogen-free extract, and organic matter, compared to the control group, Additionally, MOS supplementation also increased the intestinal villi length, width, and crypt depth and decreased intestinal inflammation, compared to the control group. In conclusion, supplementation of MOS at 2 g/kg diet improved growth performance, digestibility, and blood parameters without having adverse effects on the intestine of broiler chickens, comparable to the Lincomycin.

Keywords: Broilers, Digestibility, Growth Performance, Lincomycin, Mannan oligosaccharides

INTRODUCTION

Optimizing the performance of broilers chicks is the main target of poultry production. To ensure the highest production, broiler chicks must be healthy and offered diets that contain all nutrient requirements needed for optimal production. Using antibiotics as growth promoters in poultry diets is now restricted due to the negative effects on broiler chicks, animal products, and human health (Kovitvadhi et al., 2019). In the past decades, antibiotics have been used to build the immune response of broiler chicks as well as growth promoters (AGP). However, applying antibiotics in poultry feed for long periods may lead to the development of bacteria resistant to drugs, which can be transmitted to the consumer (Sweeney et al., 2018). As a result, the European Union and several advanced countries have completely outlawed the use of antibiotics as growth promoters (Rafeeq and Murad et al., 2017). Consequently, other feed additives such as prebiotics and probiotics have been suggested as an alternative to antibiotics to improve performance and gut health in poultry (Higgins et al., 2008). Nowadays, mannan oligosaccharides (MOS) is a promising prebiotic, obtained from the outer cell wall of the yeast, and can decrease pathogenic bacteria of the gut (Pourabedin et al., 2015; Mahfuz et al., 2019) and enhance intestinal mucosal health leading to a balance between useful and harmful microorganisms (Kim et al., 2016). In addition, MOS can serve as an energy source for useful microorganisms like *Lactobacillus* and *Bifidobacterium* species (Leblebicier and Aydogan, 2018). The mode of action of MOS provides nutrients to the intestinal microflora and prevents the attachment of pathogens, such as *Escherichia Coli* (*E. coli*) to the intestinal mucosa (Attia et al., 2014). Moreover, the decrease in harmful bacteria and increase in beneficial bacteria may be due to receptor site competition and the production of volatile fatty acids by bacteriocins in combination with IgA antibodies, resulting in a strong immunological response (Kim et al., 2009). Therefore, the current study aimed to evaluate the effect

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of prebiotics (MOS) and antibiotics (Lincomycin) as growth promoters on growth performance, nutrient digestibility, blood biochemical, hematological parameters, and gut histology in broiler chicks.

MATERIALS AND METHODS

Ethical approval

The experimental protocol used in the present study was approved by the Institutional Animal Care and Use Committee of Cairo University, Egypt (CU-IACUC) with approval number CU/II/F/33/20.

Experimental design and management

The current experiment was carried out at the Faculty of Agriculture, Cairo University, Egypt using a total of 180, one-day-old, unsexed Ross broiler chicks with average initial body weight (BW) of 40 ±1 g. The broiler chicks were randomly distributed into five treatment groups (6 replicates with 6 broiler chicks per replicate) using a completely randomized design. The treatment groups were fed basal diets supplemented with MOS at 0, 0.5, 1, and 2 g/kg diet, and the last one was supplemented with Lincomycin antibiotic as an antibiotic growth promoter (AGP) at 4.4 mg/kg diet, respectively. Commercial Mannan oligosaccharides (Bio-MOS®) were purchased from the Multi Vita for Animal Nutrition Company, Egypt. Lincomycin was obtained as Lincomycin Hydrochloride (Lincomix 44 premix®) from the International Free Trade Corporation (IFT) company, The basal diets were mycotoxins-free and contained 23, 21. and 19% Crude Protein (CP) along with 3000, 3150, and 3200 kcal ME/kg, and offered to broiler chickens during 1-14, 15-28, and 29-35 days as starter, grower, and finisher diets, respectively. Aflatoxin and other mycotoxins levels in diets were below the detection limit (1 ppb) (Ross et al., 1997). There were no medications or antibiotics in the basal diets. At the poultry research unit, faculty of agriculture, Cairo University, Egypt, according to the National Research Council (NRC, 1994), the diets were formulated to meet the nutrient requirements of broiler chicks during the starting, growing, and finishing periods.

Table 1. Ingredients and calculated analysis of the experimental basal diets for Ross broiler chicks introduced during experimental periods (35 days) at the poultry research unit, Cairo University, Egypt

Ingredients	Starter (kg)	Grower (kg)	Finisher (kg)
Corn, grains (7.5% CP)	54.32	58.74	65.04
Soybean meal (46.0% CP)	34.46	28.98	23.72
Corn gluten meal (60.0% CP)	4.98	5.01	4
Vegetable oil	2.01	3.25	3.17
Dicalcium phosphate	1.7	1.75	1.75
Limestone	1.54	1.15	1.3
Vit.Mixture[1]	0.05	0.03	0.07
Min. Mixture[2]	0.1	0.1	0.1
Salt	0.42	0.4	0.4
L-lysine-HCl	0.15	0.22	0.15
DL-Methionine	0.15	0.25	0.18
Choline chloride	0.12	0.12	0.12
Total	100	100	100
Calculated values			
ME (KCal/Kg)	3000	3150	3200
CP	23	21	19
Ether Extract	4.6	5.97	6.09
Crude Fiber	2.45	3.09	2.34
Lysine	1.36	1.3	1.13
Methionine	0.61	0.58	0.51
Methionine + Cystine	0.98	0.94	0.85
Calcium	0.96	0.9	0.85
Available P	0.45	0.48	0.44

[(1)]Vitamins mixture supplied per kg of diet: Vit A=12000 I.U., Vit D3=2000 I.U, Vit E =10 g, Vit K3=2 g, Vit B1= 1 g, Vit B2 = 5 g, Vit B6 = 1.5 g, Vit B12 = 10 µg, Biotin = 50 µg, Pantothenicacid = 10 g, Niacin = 30 g, Folicacid = 1 g. [(2)]Minerals mixture supplied per kg of diet: Manganese = 60 g, Zinc = 50 g, Iron = 30 g, Copper = 10 g, Iodine = 1 g, Selenium = 0.1 g and Cobalt = 0.1 g.

Ross broiler chicks were fed the starter diet from the first day to fourteen days of age, followed by the grower diet from 15 to 28 days of age, and then switched to the finisher diet during 29 to 35 days of age Table 1. Feed and water were available *ad-libitum* during the experimental period (35 days). The temperature was adjusted at 30 ± 0.5 ºC in the first week then lowered by 2ºC each successive week, and then maintained at 24 ± 0.5 ºC. Relative humidity was about 60% to 70% during the first week of age then, dropped to 50-60% from the second week of age until the end of the experiment. During the first week of the experiment, the broiler chicks were exposed to 23 hours of light and 1 hour of darkness during the day. The daily lighting program was adjusted to 20 hours light and 4 hours dark from the second

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week up to the end of the experiment. Chicks were vaccinated against Infection Bronchitis (IB) at 6 days of age, Avian Influenza a (H5N1) Virus at day 9, infection bursal disease (IBD) at 13 and 24 days of age. During all periods of growth, body weight (BW) and feed intake (FI) were recorded weekly for each replicate; accordingly, body weight gain and feed conversion ratio were easily calculated.

Caecal contents

At 35 days of age, five chickens from each treatment group were slaughtered and caecal contents were taken for bacterial count including *Lactobacillus*, *Enterococcus*, *E. coli*, Yeast, and *Salmonella* as well as determining pH (Collin et al., 1995).

Blood parameters

Blood samples were collected at the end of the experiment from the wing vein of three individuals per treatment into 2mL sterile vials and allowed to clot for 4 hours. The serum was collected and kept at -20°C until further analysis after centrifugation (10 minutes, 2000 rpm). Liver function indicators including aspartate aminotransferase (AST) and alanine aminotransferase (ALT); kidney function indices including urea and creatinine were determined in the collected serum using Bahman et al. (2011) methods. Lipid profiles including triglycerides, low-density lipoprotein (LDL), high-density lipoprotein (HDL), and cholesterol were measured in serum using commercially available kits (Biosystem S.A., Costa Brava, 30, Barcelona, Spain) according to manufacturer's instructions. Moreover, at 35 days of age, another blood samples were collected into 2mL sterile vials containing anticoagulant (EDTA K3) for measuring red blood cell count (RBC), hemoglobin (Hb) content, total leukocyte counts (WBCs), lymphocytes (L) and heterophils (H) according to Ewuola and Egbunike (2008). At the same time,`H/L ratio was calculated. The hematology parameters were determined by an automated hematology analyzer for animals (Sysmex XT-2000iV, KOBE, JAPAN) using Sysmex software (version, 00-11) of an automated hematology analyzer for the animal, (XT-2000iV, KOBE, JAPAN) and (Sysmex) software (version, 00-11) according to manufacturer's instructions.

Digestibility trial

A digestibility trial was conducted after the end of the experiment using four broiler chicks from each treated group over a four-day collecting period. To determine the apparent digestibility (AD) of nutrients, the total collection method cited by Abou-Raya and Galal (1971) was executed. The number (N), ether extract (EE), crude fiber (CF), and ash content of dried excreta, as well as those of feed, were determined using N. 928.08, 2003.06, 2011.25, and 920.153 procedures, respectively, according to AOAC (2016). The amount of nitrogen-free extract (NFE) on a dry matter basis was obtained by the difference that is by deducting the sum of the percentage of ash, crude protein, EE, and CF from hundred for food materials. For dung material, urinary organic matter was calculated as urinary-N X 2.62 (Sturkie, 1954). Fecal NFE on a dry matter basis was then calculated as follows:

Fecal NFE% = 100 – (ash% + FCP% + EE% + urinary OM% + CF %).

Fecal nitrogen of dried excreta collected from the experimental digestibility trial was determined according to the method by (Jakobsen et al., 1960).

Histopathology

A tissue specimen was collected from the intestine of broiler chickens of all groups (two chickens of each replicate) and fixed in 10% neutral buffered formalin for 72 hours. Fixed tissue was processed by xylene and ethyl alcohol, embedded in paraffin, sectioned into 4µm thick sections using a microtome (Leica 2135, Germany) into 4µm thick sections and stained by hematoxylin and eosin stain (Suvarna et al., 2012). Stained tissue was examined using a light microscope and photographed using a digital camera (Olympus XC30, Tokyo, Japan). Morphometric analysis of the length, width, and crypt depth of intestinal villi was performed using TS view Software. Three intestinal villi were measured in three captured images at magnification 40× to calculate the mean for each chicken (Mohamed et al., 2020).

Economic efficiency of feeding

To determine the economic efficiency (EE) of dietary treatments, broiler meat production administration factors in all treatments were considered to be constants but weight gains and feed consumption per treatment were calculated. The price of feed additives (MOS and Lincomycin) and basal diets were calculated according to the cost of the native market at the timing of the experiment. The EE of feeding was represented as (Net revenue / total cost) (Saad et al., 2014).

Statistical analysis

The data were statistically analyzed by the least-squares procedure of the general linear model (GLM) of SAS program (SAS, 2010). The separation of means was done using Duncan's New Multiple Range Test (Duncan, 1955) for

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comparisons among the significant means at 0.05 significant levels. The fixed model used in the analysis was $Y_{ij} = \mu + T_i + \varepsilon_{ij}$, where, Y_{ij} is the value of the respective variable, μ signifies the overall mean of the respective variable, Ti denotes i^{th} treatments effect, i refers to 1, 2, …, and 5 (1 = Control, 2 = 0.5g Mannan, 3 = 1g Mannan, 4 = 2g Mannan, and 5 = 4.4mg Lincomycin), and ε_{ij} is the experimental random error.

RESULTS

Productive performance

The performance traits were affected by the supplementation of MOS and lincomycin as an AGP into the diets of broiler chickens (Table 2). There were highly significant differences between treatment groups in terms of BW, BWG, FI, and FCR during all periods from 1 to 35 days ($p < 0.05$). The results revealed that the group fed a basal diet supplemented with 2 g MOS/Kg had the highest significant growth performance in BW and BWG, lower in FI, and expressed the best FCR when compared to the other treatment groups at the starter, grower, and finisher experimental periods.

Table 2. Broiler production performance at 35 days of age as affected by diets supplemented with different levels of Mannan oligosaccharides or Lincomycin at the poultry research unit, Cairo University, Egypt

Traits	Control	MOS (0.5 g/kg)	MOS (1 g/kg)	MOS (2 g/kg)	Lincomycin (4.4 mg/kg)	SEM
1-14 days						
BW, (g)	492.71^{d}	511.5^{ab}	500.33^{c}	516^{a}	506.5^{b}	2.02
BWG, (g)	452.71^{d}	471.5^{ab}	460.33^{c}	476^{a}	466.5^{b}	2.02
FI, (g)	531.17^{a}	499.67^{b}	501.67^{b}	495.67^{b}	501.67^{b}	2.2
FCR	1.17^{a}	1.06^{d}	1.09^{b}	1.04^{e}	1.08^{c}	0.005
BW, (g)	1502.67^{b}	1565^{a}	1525.33^{b}	1571.5^{a}	1525^{b}	7.78
BWG, (g)	1009.96^{b}	1053.5^{a}	1025^{b}	1055.5^{a}	1018.5^{b}	7.28
FI, (g)	1613.17^{a}	1587.83^{ab}	1591.33^{ab}	1577.33^{b}	1597.33^{ab}	10.5
FCR	1.6^{a}	1.51^{c}	1.55^{b}	1.50^{c}	1.57^{ab}	0.01
BW, (g)	2225.67^{c}	2322.5^{ab}	2282^{b}	2354.17^{a}	2288.33^{b}	13.88
BWG, (g)	723^{b}	757.5^{ab}	756.67^{ab}	782.67^{a}	763.33^{ab}	14.75
FI, (g)	1403.83^{a}	1327.83^{cd}	1345.33^{b}	1318.83^{d}	1335.33^{bc}	5.6
FCR	2.02^{a}	1.76^{bc}	1.80^{b}	1.69^{c}	1.76^{bc}	0.03
BW, (g)	2225.67^{c}	2322.5^{ab}	2282^{b}	2354.17^{a}	2288.33^{b}	13.88
BWG, (g)	2185.67^{c}	2282.5^{ab}	2242^{b}	2314.17^{a}	2248.33^{b}	13.88
FI, (g)	3548.17^{a}	3415.33^{bc}	3438.33^{b}	3391.83^{c}	3434.33^{b}	12.02
FCR	1.63^{a}	1.5^{c}	1.54^{b}	1.47^{d}	1.53^{b}	0.008

$^{a,b,c, \text{ and } d}$ Means different letters within each row are significantly different ($p < 0.05$), SEM: Standard error mean from the least square mean.
BW: Body weight, BWG: Body weight gain, FI: Feed intake, FCR: Feed conversion ratio, and MOS: Mannan oligosaccharides.

Microbial content of caecum

There were highly significant differences in *E. coil*, *Lactobacillus*, *Enterococcus* bacteria, and yeast counts in the caecum of experimental broiler chicks at 35 days of age (Table 3). A significant decrease in the caecal *E. coli* and *Enterococcus* counts, and a significant increase in the *Lactobacillus* and Yeast counts were found due to the inclusion of Bio-MOS in supplementing the diets of broilers. Compared to the Lincomycin and control groups, the group that fed a basal diet supplemented with 2 g MOS/Kg diet had the significantly highest *Lactobacillus* count and yeast, while it had the lowest *E. coli* and *Enterococcus* counts, compared to other treatments. On the other hand, there was a significant difference ($p < 0.05$) in the pH level among groups, obtaining the highest level in the control group (7.48) and the lowest level in the group fed supplemented with a 2 g MOS/Kg/diet (6.94). However, the pH was generally neutralized in all experimental groups.

Table 3. Broiler microbiota as affected by diets supplemented with different levels of Mannan oligosaccharides diet or Lincomycin at 35 days of age

Treatment	*Lact.* count (cfu/g)	*E. coli* (cfu/g)	pH	*En*. count (cfu/g)	Yeast (cfu/g)	*Salmonella*
Control	4.66^{c}	4.4^{a}	7.48^{a}	6.16^{a}	2.8^{b}	ND
MOS (0.5 g/kg)	5.13^{ab}	2.96^{b}	7.1^{c}	5.13^{b}	3.36^{ab}	ND
MOS (1 g/kg)	4.8^{b}	3.2^{b}	7.26^{b}	5.03^{bc}	3.53^{a}	ND
MOS (2 g/kg)	5.46^{a}	1.96^{c}	6.94^{d}	4.8^{d}	3.56^{a}	ND
Lincomycin(4.4 mg/kg)	4.7^{bc}	3.86^{ab}	7.31^{b}	4.9^{c}	2.3^{c}	ND
SEM	0.16	0.29	0.10	0.24	0.20	-

$^{a,b,c, \text{ and } d}$ Means, different letters within each column, are significantly different ($p < 0.05$), SEM: Standard error mean from least square mean, Cfu/g: Colony forming unite/gram, *Lact*: *Lactobacillus*, *E. coli*: *Escherichia coli*, En: *Enterococcus*, ND: Not detected, MOS: Mannan oligosaccharides.

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Blood parameters

As presented in Table 4, there was a significant decrease in the cholesterol, LDL, urea, AST, and ALT, of the group that fed 2 g MOS/Kg diet while the HDL was significantly ($p < 0.05$) increased compared to the control and the other treatment groups. In contrast, the creatinines of all treated groups with MOS or Lincomycin were significantly ($p < 0.05$) lowers than those in the control group. The results of blood count (CBC) as affected by MOS or Lincomycin treatment in broilers chickens were presented in Table 5. The highest present level of Hb was obtained in the group that fed a 2 g MOS/kg diet, compared to the other groups ($p < 0.05$). In contrast, the lowest Heterophile level was found in the group treated with 2 g MOS in comparison with the control and other treatment groups. In addition, the H/L ratio was significantly decreased ($p < 0.05$) in MOS (2 g/kg diet) and Lincomycin treated groups in comparison to the control group. The percentage of lymphocytes, as well as the percentage of RBCs and WBCs, did not show any significant between the experimental groups.

Table 4. Broiler's blood serum analysis as affected by dietary supplementation with Mannan oligosaccharides or Lincomycin at 35 days of age

Treatment	Cholesterol (mg/dl)	LDL (mg/dl)	HDL (mg/dl)	Urea (mg/dl)	Creatinine (mg/dl)	AST (U/L)	ALT (U/L)
Control	129.23^{a}	59^{a}	38.7^{c}	4.23^{c}	0.23^{a}	114^{a}	17^{a}
MOS (0.5 g/kg)	126.1^{b}	47.6^{b}	65.96^{a}	4.5^{a}	0.2^{b}	90.66^{b}	13.66^{b}
MOS (1 g/kg)	113.43^{c}	42^{c}	61^{b}	4.06^{d}	0.2^{b}	78.66^{c}	14^{b}
MOS (2 g/kg)	106.3^{d}	39.13^{d}	66.56^{a}	4^{e}	0.2^{b}	66.33^{d}	11^{d}
Lincomycin (4.4 mg/kg)	128.83^{a}	47.66^{b}	60.33^{b}	4.43^{b}	0.2^{b}	76.66^{c}	12^{c}
SEM	0.43	2.66	2.38	0.1	0.009	2.54	1.06

$^{a,b,c,d, \text{ and } e}$ Means different letters within a column, are significantly different ($p < 0.05$), SEM: Standard error mean from least square mean, LDL: Low-density lipoprotein, HDL: High-density lipoprotein, AST: Aspartate transaminase, ALT: Alanine aminotransferase, MOS: Mannan oligosaccharides.

Table 5. Broiler's blood hematology as affected by diets supplemented with different levels of Mannan oligosaccharides (g/ kg diet) or Lincomycin (mg/ kg diet) at 35 days of age

Variable	Control	MOS (0.5 g/kg)	MOS (1 g/kg)	MOS (2 g/kg)	Lincomycin (4.4 mg/kg)	SEM
Hb (%)	9.94^{e}	10.23^{d}	10.35^{b}	10.62^{a}	10.32^{c}	0.11
RBCs (Cells/µL)	2.16	2.81	2.8	2.95	2.80	0.11
WBCs (19-30×10^{3}/mm^{3})	25.66	21	23.33	20.66	23.66	1.58
Lymphocytes (%)	69	67.33	64.66	69	70	1.75
Heterophile (%)	36.5^{a}	35.67^{b}	33.75^{c}	30.33^{e}	32.5^{d}	0.06
H/L ratio	0.52^{a}	0.52^{b}	0.52^{b}	0.43^{c}	0.46^{c}	0.03

$^{a,b,c,d, \text{ and } e}$ Means different letters within each row are significantly different ($p < 0.05$), SEM: Standard error mean from least square mean, Hb: Hemoglobin, RBCs: Reed blood cells, WBCs: White blood cells, MOS: Mannan oligosaccharides, H: Heterophile, L: Lymphocytes.

Nutrient digestibility

The results of nutrient digestibility in broiler chickens treated with MOS or Lincomycin are shown in Table 6. It was observed that supplementing broiler chickens' diet with 1 g and 2 g MOS improved the digestibility of CP, NFE, and OM, compared to the control and Lincomycin groups. In contrast, the treatment with 2 g MOS or Lincomycin significantly increased the digestibility of EE and CF compared to the control.

Table 6. Digestibility of nutrients in broilers chickens' fed diet supplemented with different levels of Mannan oligosaccharides or Lincomycin at the poultry research unit, faculty of agriculture, Cairo University, Egypt

Treatment	Crude protein (%)	Ether extract (%)	Crude fiber (%)	Nitrogen free extract (%)	Organic matter (%)
Control	89.01^{b}	75.08^{b}	20^{bc}	73.24^{c}	72.21^{c}
MOS (0.5 g/Kg diet)	89.98^{ab}	69.63^{c}	18.58^{c}	75.23^{bc}	73.94^{b}
MOS (1 g/Kg diet)	91.21^{a}	76.06^{b}	19.75^{bc}	76.79^{b}	75.51^{ab}
MOS (2 g/Kg diet)	89.85^{ab}	80.54^{a}	20.85^{b}	77.66^{a}	76.8^{a}
Lincomycin (4.4 mg/Kg diet)	85.44^{c}	79.08^{a}	24.33^{a}	74.67^{c}	71.52^{d}
SEM	0.63	0.61	0.58	0.63	0.63

$^{a,b,c, \text{ and } d}$ Means different letters within each row are significantly different ($p < 0.05$), SEM: Standard error mean from least square mean, MOS: Mannan oligosaccharides.

Intestinal histomorphometry and histopathology

The intestinal villi length in chickens supplemented with MOS recorded a significant increase, compared to the control group. The highest increase was observed in the group supplemented with a 2 g MOS/kg diet, compared to all other groups. The intestinal width was also increased significantly in a dose-dependent manner in the groups supplemented with MOS, compared to control. The crypt depth also was increased in all groups supplemented with MOS with the highest increase in MOS (0.5 g/kg) and the least increase in MOS (2 g/kg) as compared with the control group (Table 7).

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Microscopy of the intestine in the control group revealed mucous exudates in the lumen, slight shortening of intestinal villi, few goblet cell hyperplasias', and few inflammatory cells infiltration in the lamina propria and submucosa (Figure 1a). Similar lesions were detected in the group provided with a 0.5 g MOS/kg diet. However, the intestinal villi were longer and wider, with moderate mononuclear cells infiltrating the lamina propria and submucosa. In addition severe epithelial and goblet cell hyperplasia were observed (Figure 1b). Microscopy of the chicken intestine in the group fed MOS at 1 g/kg diet revealed severe goblet cell hyperplasia and widening of intestinal villi with mononuclear cell infiltration (Figure 1c). For group fed MOS at 2 g/kg basal diet, the intestinal villi were tall in some sections with few mononuclear leukocyte infiltrations and moderate goblet cell hyperplasia (Figure 1d). In the lincomycin group, the tips of intestinal villi were necrosed in some intestinal sections. Goblet cell hyperplasia was moderate and the lamina propria and submucosa were infiltrated by numerous mononuclear cells (Figure 1e).

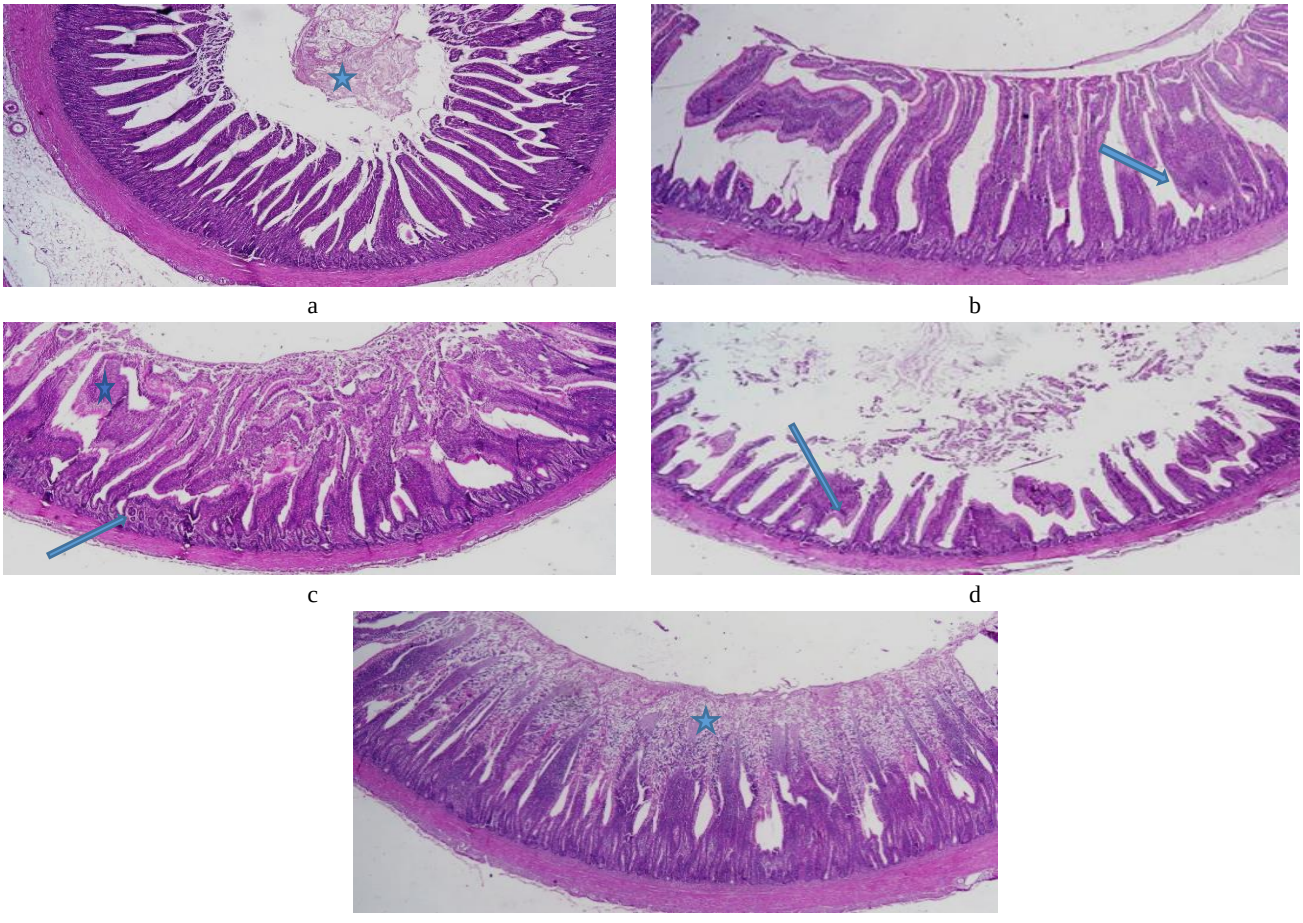
The economic efficiency

As shown in Table 8, the dietary treatment with MOS (2 g/kg diet) exhibited the best values of net return and EE. According to Table 8, the lowest cost/kg body weight (28.19 EGP) was observed when chicks were fed diets supplemented with lincomycin, followed by those supplemented with MOS (at 0.5, 1, and 2 g/kg), respectively, while the highest cost per kg BW (28.85 EGP) was recorded in the control group.

Table 7. The morphology of broiler chickens' intestine at 35 days of age affected by diets supplemented with different levels of Mannan oligosaccharides or Lincomycin

Treatment	Length (µm)	Width (µm)	Depth (µm)
Control	818.43^{d}	153.45^{c}	120.71^{e}
MOS (0.5 g/kg)	1089.53^{b}	159.06^{b}	279.44^{a}
MOS (1 g/kg)	1029.08^{c}	174.26^{a}	240.18^{b}
MOS (2 g/kg)	1389.57^{a}	187.37^{a}	221.36^{c}
Lincomycin (mg/kg)	1038.44^{c}	158.49^{b}	190.86^{d}
SEM	58.27	12.47	14.84

$^{a,b,c,d, \text{ and } e}$ Means different letters within each column are significantly different ($p < 0.05$), SEM: Standard error mean from the least square mean, MOS: Manna oligosaccharides.



a b c d e

Figure 1. Intestine of Ross broiler chickens fed Manna oligosaccharides at 35 days of age. **a:** showing mucous exudates (star) in the lumen and slight shortening of intestinal villi (control group), **b:** Manna oligosaccharides (MOS, 0.5 g/kg) showing long and wide intestinal villi with epithelial hyperplasia (arrow), **c:** MOS (1 g/kg) showing severe goblet cell hyperplasia (star) and widening of intestinal villi with mononuclear leukocytes (arrow), **d:** MOS (2 g/kg) showing few mononuclear leukocytes infiltration and moderate goblet cell hyperplasia (arrow) and **e:** Lincomycin (4.4 mg/kg) showing necrosis of intestinal villi tips (star) with severe diffuse mononuclear leukocytes infiltration.

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Table 8. Effect of dietary Mannan oligosaccharides and lincomycin on the economic efficiency of broiler chickens' production from 1 to 35 days of age

Treatment	FI (g/bird)	Feed cost (L.E/bird)	*Total cost (L.E/bird)	BW (g/bird)	**Total Revenue (L.E/bird)	Net Revenue (L.E/bird)	E.E	Relative E.E (%)
Control	3548.17	20.85	28.85	2225.67	66.77	37.92	1.31	100
MOS (0.5 g/kg)	3415.33	20.22	28.22	2322.50	69.68	41.46	1.47	112
MOS (1 g/kg)	3438.33	20.49	28.49	2282.00	68.46	39.97	1.40	107
MOS (2 g/kg)	3391.83	20.50	28.50	2354.17	70.62	42.12	1.48	113
Lincomycin (mg/kg)	3434.33	20.19	28.19	2288.33	68.65	40.46	1.44	110

*including chick price which was 8 L.E. **assuming the price of 1 kg live weight was 30 L.E ***assuming the economic efficiency of the control was 100, BW: Body weight, E.E: Economic efficiency, MOS: Mannan oligosaccharides, FI: Feed intake.

DISCUSSION

Productive performance

In the current study, BW and BWG were the highest, and FCR was the best in the group fed 2 g MOS/Kg diet at trial days of the experiment as compared with the other experimental groups. This could be related to the mechanism of MOS, which results in a decrease in pathogenic bacteria load and an increase in beneficial bacteria production, leading to the establishment of a healthy intestinal environment. Consequently, absorption of nutrients in the intestine is enhanced and the performance is improved. In addition, MOS promotes the growth of caecal beneficial bacteria such as *Bifidobacterium* and *Lactobacillus* spp. in the intestine (Sadeghi et al., 2013). Therefore, the intestinal digestion and absorption of the nutrients were enhanced with the increased surface area of the villi (Chand et al., 2016) In addition, Mannan-oligosaccharide has been shown to enhance the growth parameters of the broiler chicks (Nikpiran et al., 2013).

Current results were in the same line with previous studies that found significant improvement of BW, BWG and FCR, when broiler chickens fed on a basal diet supplemented with 2 g MOS/Kg diet (Markovic et al., 2009; Ozduven et al., 2009; Esecel et al., 2012). Regarding the dose-response of MOS, the ideal dosage of MOS for optimal growth is approximately 2g/kg diet according to Tucker et al. (2003). Generally, Ozpinar et al. (2010) and Tufail et al. (2019) found that adding MOS to a broiler's diet increased chicks' BW and BWG compared to chicks that fed a basal diet without any supplementation.

Lincomycin is commonly used to prevent the infection caused by gram-positive pathogenic organisms in poultry. Antibiotic residues could be found in animal products such as eggs, meat, and milk, and they can cause a wide variety of symptoms in consumers, such as bacterial resistance or allergic reactions (Apata, 2009). Lincomycin, at low doses of 2.2 to 4.4 mg/kg, has been demonstrated in numerous trials to improve weight gain and efficiency of food utilization (Stutz and Lawton, 1984; Dafwang et al., 1987). In contrast, Proudfoot et al. (2007) reported that there was not be more effective in both economically and biologically performance' improving by using lincomycin at 2.2mg/kg. As a result, antibiotics used as a growth promoter in animal feed can improve growth rates while still causing the development of resistance.

In the present study, broilers fed on a basal diet supplemented with lincomycin at a 4.4 mg/kg diet improved productive performance, compared to the control group, but was lower than the performance of MOS groups. In this regard, Jamal et al. (2017) reported that the FCR of the lincomycin group was better compared to the control group.

Microbial content of caecum

In the present study, the groups that fed on a basal diet supplemented with different levels of MOS showed an increase in *Lactobacillus*, *Bifidobacterium* and yeast count at the same time showed a decrease in *E. coli* and *Enterococcus* count. These findings might be attributed to MOS which increases the population of lactic acid bacteria in the intestinal tract of birds, resulting in the improved immune response against harmful bacteria such as *E. coli* and *Enterococcus* (Joerger, 2003; Patterson and Burkholder, 2003). Furthermore, the MOS supplements have specific receptors for *E. coli fimbriae* (sensitive to mannose) and *Salmonella* spp., resulting in the removal of these microorganisms with the digesta flow rather than binding to the intestinal receptor (Huyghebaert et al., 2011).

In this respect, (Castillo et al., 2008) stated that MOS can minimize the count of hindgut pathogenic bacteria during high exposure to the pathogen. This improvement in the intestinal microbial population is positively reflected in growth performance as indicated in the present study. The results of the current study were concurrent with those of Baurhoo et al. (2009a) indicating a decline in caecum *E. coli* count and an increase in *Lactobacillus* and *Bifidobacteria* spp. in broilers fed a diet supplemented with MOS, compared with broilers fed on a basal diet. A decline of caecum *E. coli* of broilers fed a basal diet supplemented with MOS, compared to broilers fed a basal diet without any supplements in the current study agrees with various studies (Baurhoo et al., 2009a; Baurhoo et al., 2009b; Koc et al., 2010).

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Concerning the comparison between lincomycin and MOS groups, the antibiotic additive is considered to be most effective against Gram-positive bacteria (Butaye et al., 2003), in comparison to the MOS treatments, the colonization of several common bacteria, such as *lactobacilli*, was inhibited. In agreement with current results, Jamroz et al. (2004) reported a greater decline of caecum *E. coli* for broilers fed a basal diet supplemented with 2 g MOS/Kg diet as compared with the group fed a basal diet supplemented with antibiotic (Avilamycin), Baurhoo et al. (2009a) reported that broilers fed a diet supplemented with 2 g MOS/Kg had a significant increase in *Lactobacilli* spp. and reduction of *E. coli* count in the caecum.

Results obtained herein showed that the pH levels were significantly decreased in the groups supplemented with MOS; this may be due to that MOS may increase ingesta fermentation which improves gut health in the distal intestine, resulting in the production of short-chain fatty acids (SCFA). The SCFAs play an important role in cell proliferation in the mucosa of the intestine. This lowers the pH of the brush boundary micro environment and prevents pathogen attachment (Lan et al., 2004; Oliveira et al., 2008). So, increased SCFAs in the intestinal digesta resulted in acidic gut ph. This mechanism may explain the better effects of MOS and microbial phytase concomitant use (Karimian and Rezaeipour, 2020).

Blood parameters

There was a gradually significant decrease in cholesterol and LDL as MOS levels increased in the diet while the group fed a basal diet supplemented with lincomycin showed a similar cholesterol level to the control group. Therefore, the group fed a basal diet supplemented with a 2 g MOS/kg diet showed the lowest cholesterol and LDL among all experimental groups. Concerning HDL, the groups fed a basal diet supplemented with 0.5g or with 2 g MOS/ kg diet had significantly higher values. The most important mechanism upon which prebiotics reduce blood cholesterol levels is certainly through decreased intestinal lipid absorption by binding bile acids, leading to enhanced cholesterol excretion and hepatic synthesis of new bile acids (Ooi and Liong, 2010). Synthesis of bile acids from cholesterol in the liver is the most important way of cholesterol excretion (Wilson et al., 1998). In this connection, Biswas et al. (2019) reported that cholesterol level was decreased ($p < 0.05$) in the 0.2% MOS group compared with other treated groups. Similarly, Kannan et al. (2005) and Juskiewicz et al. (2003) stated that serum total cholesterol concentration was significantly lower in broilers fed a 0.05% MOS diet when compared to a control diet. On the other hand, Muhammad et al. (2020) found a non-significant difference between levels of Mannan oligosaccharides (0.5, 1, 1.5 g/kg diet) among serum biochemical parameters and cholesterol levels.

Additionally, among all experimental groups, the group fed a basal diet supplemented with a 2g MOS/ kg diet had the lowest ALT and AST levels. When compared to the control group, they were significantly lower in all treatment groups supplemented with MOS and Lincomycin. The current result was in agreement with Helal et al. (2015) who found a significant reduction in the AST and ALT in the chicks that received prebiotic in their diet as compared with chicks fed on antibiotics and basal diet. The result was in the same line with explanations of Jameel et al. (2014) who indicated a significant decrease in ALT and AST and declared that the differences might be due to the inclusion of different pre/probiotics, experimental plan executed, and management differences during rearing and environmental conditions. However, Muhammad et al. (2020) reported that the different levels of MOS (0.5, 1, 1.5 g/kg diet) showed non-significant differences among serum biochemical parameters (ALT, AST) of treatment groups. Also, Biswas et al. (2019) reported that at 42 days, a significant increase ($p < 0.05$) was recorded in AST at 0.2% MOS supplemented groups; whereas, no significant difference was observed in ALT concentration among the treated groups. The creatinine levels were significantly low for all experimental treated groups in comparison to those fed basal diet without supplementation. This was in line with the results of Helal et al. (2015) and Salim et al. (2011), who found a significant reduction in creatinine levels in the chicks that received prebiotics compared to the control group. However, Muhammad et al. (2020) reported no significant difference in creatinine levels among treatment groups.

The blood parameters showed that the group fed 2 g MOS/Kg diet had the significantly lowest blood urea among all experimental groups. The result agrees with Biswas et al. (2019) and disagrees with (Muhammad et al., 2020) who reported a non-significant difference among the urea levels between treatment groups.

The current result is in agreement with Iqbal et al. (2018) who reported that the different levels of MOS (0.25, 0.50, and 1%) for 15 weeks in Japanese quail breeders who found significant changes in HDL levels in treated groups compare the control group.

According to hematological results obtained in this study, a non-significant difference was recorded in RBCs, WBCs, and lymphocytes between all treatments group. The results are in agreement with Muhammad et al. (2020). Also, partly agree with the finding of Al-Saad et al. (2014) since no differences in RBCs were found between broilers fed a control diet supplemented with antibiotics (Lincomycin 100g/ton) and those fed diet supplemented with MOS (1000g/ton).

Whereas, the current result showed that the Hb was significantly the highest in the group fed dietary 2 g MOS/Kg diet compared with the control group. Moreover, Hb levels were significantly higher in all experimental treated groups

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as compared to the control group. The current result was not in the same line with studies of Muhammad et al. (2020) reported a non-significant difference among levels of MOS (0.5, 1, 1.5 g/kg diet) regarding Hb of treatment groups. However, Abdel-Fattah and Fararh (2009) reported that there was a significant increase in Hb when they were used 1 kg of prebiotic (Bio-MOS®)/ton of feed compared with the basal diet fed, but a significant increase in RBCs count was detected in broilers fed prebiotic.

Nutrient digestibility

The current results showed a significant increase in CP digestibility in the diet supplemented with1 g MOS/Kg feed compared to other groups, and a significant decrease in group fed diet supplemented with lincomycin. However, the groups fed diets supplemented with 0.5 and 2 g MOS/Kg feed were similar in CP digestibility with the control group. The current study was in the same line with Afrouziyeh et al. (2014) who did not find a significant difference in CP digestibility between birds consuming treatments (0.1% MOS, 0.2% MOS, and 0.3 % MOS) and the control one. Also agree with Navidshad et al. (2015) in which the digestibility coefficients of CP were similar for the control and 2 g/kg Bio-Mos® supplemented diets.

Overall, groups fed a diet supplemented with MOS at 2 g/kg feed had the best OM digestibility among all experimental groups, this is probably due to the increase in the digestion and absorption of nutrients and reduced the number of pathogenic bacteria (*E. coli*, *Enterococcus*) as well as increased the beneficial bacteria *(Lactobacilli*) in the intestine that is important to improve growth performance. This is in agreement with Afrouziyeh et al. (2014) who recorded that the organic matter (OM) was significantly higher in broilers fed a diet supplemented with 0.2 g MOS/Kg diet compared to the other treatment groups. Similarly, Huang et al. (2005) showed that oligosaccharide supplementation enhanced nutrient digestibility and feed efficiency in broiler chicks. The improvement of nutrient digestibility in broilers fed diets supplemented with oligosaccharides might be attributed to gut health improvement (Tuohy et al., 2003).

Intestinal histopathology

In this study, the length of intestinal villi, villus width, and crypt depth in all treated groups of broiler chickens were significantly increased compared to the control group. The improvement observed in the intestinal villi length can be attributed to the increase in *Lactobacillus* count in the treated groups. Accordingly, increased levels of beneficial bacteria such as *lactobacillus* can lead to promoting the development of a healthy intestinal environment, resulting in an increase in villi length due to competitive exclusion (Baurhoo et al., 2009b).

Dietary inclusion of antibiotics also reduces the weight and length of the intestines in poultry (Postma et al., 1999). Results obtained in the present study were in accordance with those of Baurhoo et al. (2009a) who reported that on day 34 of age, dietary MOS of 2 g and 5 g MOS/kg significantly increased villus height in all intestinal segments, compared to virginiamycin (0.002%) and bacitracin (0.006%). Similarly, Sinovec et al. (2005) reported that MOS (0.2%) significantly improved broiler villus height in all areas of the small intestine when compared to birds supplemented with (Flavomycin 0.002%) and control diets. Furthermore, the study of Karimian and Rezaeipour (2020) reported that the dietary MOS 2 g/kg diet significantly increased villus height compared to the basal diet. Although, previous studies indicated no variation in villus height between broilers fed MOS and those fed other dietary treatments (Oliveira et al., 2008; Baurhoo et al., 2009b).

A greater surface area for nutrient absorption due to long villi and shallow crypts, and a low renewal rate allow efficient enzyme synthesis and intestinal cell maturation (Yang et al., 2009). Previous studies however reported that the depths of crypts were increased in broiler chicks fed MOS (0.1 and 0.5% of diet), compared to the control group which is similar to the current study (Iji et al., 2001; Oliveira et al., 2008). Present results however are not in the same line with those of Yang et al. (2009) who indicated that MOS reduced crypt depth in the broiler's small intestine. Similarly, Sinovec et al. (2005) showed that the crypt depth was reduced in all parts of the small intestine of broilers in response to 0.2% MOS in the diet, compared to both Flavomycin at 0.002% and control diets.

Increased stress and pathogen load can alter immune responses facilitating infection, host tissue damage, and inflammation (Berghman, 2016). Inflammation, therefore, is considered the most predominant reaction of innate defense to alterations in tissue homeostasis (Medzhitov, 2008). In the current study, the intestine of chicken in the control group and group supplemented with lincomycin showed mild to moderate inflammation of the intestine whereas the groups supplemented with MOS especially the group supplemented with the high dose of MOS showed a decrease in intestinal inflammation and few leukocytes infiltration. Likewise, a previous study showed that MOS and live yeast supplementation was able to control intestinal inflammation by attenuating *E. coli*-induced intestinal disruption in broilers (Tarradas et al., 2020).

Economic efficiency

The current investigation indicated that the group fed diet supplemented with lincomycin had the least feed cost among all groups and the group fed diet supplemented with 0.5 g MOS/Kg diet recorded lower feed cost compared to

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other MOS doses. Besides, both Lincomycin and MOS treatment groups exhibited higher values of net revenue and EE compared to the control. However, chicks which were fed diets supplemented with MOS at 2 g/kg were the best. Hooge et al. (2003) confirmed our results on broiler chicks that fed during their first 49 days of age, with 0 (negative control), 0.5, and 1 g Bio-Mos/kg in different periodical diets. Also, Nazir et al. (2017) reported that the broiler chicks fed on a diet supplemented with lincomycin (6.5 mg/kg diet) were more economical, compared to the control group. However, Eseceli et al. (2010) did not show any significant differences in feed cost kg-1 BWG between three broiler chick groups fed on diets supplemented with 0.5, 1, or 1.5 g Bio-Mos/kg diet.

There are a very limited number of researchers interested in the economic aspect of Bio-Mos inclusion in poultry diets. Therefore, a higher dose rate of MOS may be evaluated for better results provided that economic parameters are not compromised. These improvements could be attributed to the improved body weight gain, feed conversion, and low prices of Mannan oligosaccharides and lincomycin, compared to the control group.

CONCLUSION

In the current study, three Mannan oligosaccharides concentrations were used (0.5, 1, and 2 g/kg), in addition to Lincomycin (4.4) mg/kg as feed additives. Mannan concentration of 2 g/kg supplementation had the best results for growth performance, histopathological examination, and biochemical properties. Further investigations are needed to optimize the recommended concentrations.

DECLARATIONS

Authors' contribution

All authors contributed equally in conceiving the idea and writing the first draft and the revised version.

Competing interests

The authors declare no conflicts of interest in the present study.

Ethical consideration

Ethical issues (including plagiarism, consent to publish, misconduct, double publication and/ or submission, and redundancy) have been checked by the authors.

Consent to publish

Hereby, the authors agreed to publish the article.

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