

Original Article

Effects of mannan-oligosaccharide supplementation on gut health, immunity, and production performance of broilers

Efeitos da suplementação de mananoligossacarídeo na saúde do intestino, imunidade e desempenho da produção de frangos

M. Asif^a , Z. Hayat^{a,b} , A. Rahman^{a*} , M. F. Qamar^a , S. Nawaz^c , M. Ijaz^a , I. H. Badar^d , A. R. Asif^{a,e}  and M. K. Yar^a 

^aUniversity of Veterinary and Animal Sciences, Department of Animal Sciences, Jhang Campus, Jhang, Pakistan

^bUniversity of Sargodha, College of Agriculture, Department of Animal Sciences, Sargodha, Pakistan

^cUniversity of Agriculture, Faculty of Veterinary Science, Department of Anatomy, Faisalabad, Pakistan

^dUniversity of Veterinary and Animal Sciences, Faculty of Animal Production and Technology, Department of Meat Science and Technology, Lahore, Pakistan

^eHuazhong Agricultural University, Key Laboratory of Animal Genetics, Breeding and Reproduction, Wuhan, China

Abstract

The study was designed to investigate the effect of mannan-oligosaccharide (MOS) supplementation on intestinal histomorphology, immunity against Newcastle disease virus (NDV) and productive parameters of broilers. A total of 1800, day old broiler chicks of Cobb-500 strain were selected and randomly assorted into 6 treatment groups: T1 (basal diet without antibiotics as negative control); T2 (basal diet plus antibiotics as positive control group); T3 (basal diet plus 200g/ton MOS); T4 (basal diet plus 400g/ton MOS); T5 (basal diet plus 600g/ton MOS) and T6 (basal diet plus 800g/ton MOS). Each treatment was having 6 replicates and the feed intake, body weight gain and feed conversion ratio (FCR) were recorded on weekly basis. Results showed that, MOS supplemented birds have significantly higher feed intake, weight gain and FCR ($P < 0.05$). Similarly, supplementation of MOS showed positive effect on villus height and crypt depth both in jejunum and ileum. Goblet cell density was unaffected by MOS addition ($P < 0.05$). Furthermore, birds fed with diets containing MOS, exhibited better productive performance in comparison to positive and negative control groups. In conclusion, MOS can replace antibiotic growth promoters (AGPs) as non-microbial performance-enhancing feed advocates.

Keywords: antibiotic growth promoters, Newcastle disease virus, poultry, villus height, crypt depth.

Resumo

O estudo foi desenhado para investigar o efeito da suplementação de mananoligossacarídeo (MOS) na histomorfologia intestinal, imunidade contra o vírus da doença de Newcastle (NDV) e parâmetros produtivos de frangos de corte. Um total de 1.800 pintos de corte de um dia da linhagem Cobb-500 foram selecionados e distribuídos aleatoriamente em 6 grupos de tratamento: T1 (dieta basal sem antibióticos como controle negativo); T2 (dieta basal mais antibióticos como grupo controle positivo); T3 (dieta basal mais 200g/ton MOS); T4 (dieta basal mais 400g/ton MOS); T5 (dieta basal mais 600g/ton MOS) e T6 (dieta basal mais 800g/ton MOS). Cada tratamento tinha 6 repetições e o consumo de ração, ganho de peso corporal e conversão alimentar foram registrados semanalmente. Os resultados mostraram que as aves suplementadas com MOS apresentam consumo de ração, ganho de peso e CA significativamente maiores ($P < 0,05$). Da mesma forma, a suplementação de MOS mostrou efeito positivo na altura das vilosidades e na profundidade das criptas tanto no jejuno quanto no íleo. A densidade de células caliciformes não foi afetada pela adição de MOS ($P < 0,05$). Além disso, as aves alimentadas com dietas contendo MOS apresentaram melhor desempenho produtivo em comparação aos grupos controle positivo e negativo. Em conclusão, o MOS pode substituir os promotores de crescimento de antibióticos (AGPs) como defensores de alimentos não microbianos que melhoram o desempenho.

Palavras-chave: antibióticos promotores de crescimento, vírus da doença de Newcastle, aves, altura das vilosidades, profundidade da cripta.

*e-mail: abdurrehman@uvas.edu.pk

Received: March 26, 2021 – Accepted: March 15, 2022



This is an Open Access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

1. Introduction

Over past several years, there has been an enormous increase in consumption of poultry products due to enriched nutrients present in it. Poultry sector is playing a pivotal role in minimizing the gap between the requirement and availability of proteins for human. In Pakistan, poultry is one of the well-organized sectors producing 1.39 million tons of meat and contributes 32.7% of total meat production. The profitability of poultry sector depends on efficient manufacturing of feed, proper utilization of nutrients, growth rate, improved feed conversion ratio (FCR) and better gastrointestinal tract (GIT) health of birds. Poultry production is facing several problems, including climatic changes, microbial load and stress during rearing which leads to disturbance of gastrointestinal tract (GIT) that lead to poor performance of birds (Grashorn, 2010; Granstad et al., 2020). Gut microflora which is a key to the proper utilization of nutrients, can affect the immune status of birds as it influences the intestinal wall (Klasing (2007; Huang, 2008). It is well documented that for good performance and healthy GIT showed good effect on overall poultry production (Chen et al., 2009; Gul et al., 2021). Moore et al. (1946) was first who claimed that there is an improvement in performance, when birds fed with streptomycin.

The use of antibiotics in poultry feed is banned due to problem of antimicrobial resistance and appearance of antibiotic residuals in poultry products (eggs and meat). Consequently, it has encouraged the researchers to find out the antibiotics-alternatives to be used in poultry feed. Therefore, use of probiotics, prebiotics, synbiotics, phytobiotics, enzymes, organic acids antimicrobial peptides, hyperimmune egg yolk antibodies, bacteriophages, clay and metals have been extensively studied as AGPs replacer in poultry feed (Gadde et al., 2017; Kamran et al., 2013a, b). Probiotics as stated by Reid (2016; Hutkins et al., 2016) are live strains of strictly selected microorganisms which, when fed to animals in adequate amounts, causes an improvement in health and performance of the host. Phytobiotics are plant derived compounds which are being added to animals feed and improves the productivity and quality of meat (Windisch and Kroismayr, 2006). A prebiotic is a non-nutritive ingredient that may be digestible by intestinal microflora and brings beneficial changes in health by changing the proportion of beneficial bacteria to pathogenic bacteria (De Vrese and Schrezenmeir, 2008; Yasmeen et al., 2021). Many prebiotics including fructo-oligosaccharides (FOS), lactulose, inulin, galactooligosaccharides (GOS), and polydextrose are already used as source of prebiotics in poultry feed.

Mannan-oligosaccharide (MOS) is one of the main prebiotics used in poultry feed that can improve the average daily feed intake, feed conversion ratio and overall performance of broiler chicks when fed in feed as they increase (Kocher et al., 2005). Many studies have revealed that MOS can improve the gut health of the birds by inhibiting the adhesion of harmful bacteria such as *Escherichia coli* and *Salmonella pullorum* to coco-2 cells and by promoting the *Bifidobacterium* in gut (Kocher et al., 2005; Xu et al., 2017). Therefore, this study was designed

to check the effect of MOS supplementation on gut health, immunity against Newcastle disease virus (NDV), and production performance of broilers.

2. Materials and Methods

The current study was approved by the Institutional Ethical Review Committee of University of Veterinary and Animal Sciences (UVAS), Lahore, Pakistan. Experiment has been conducted at research and development farm of Sultan Feed Mills, Sargodha, Pakistan. Before the arrival of chicks, floor brooding area and equipments were cleaned and disinfected. Five days prior to arrival of chicks, the whole shed was fumigated with formaldehyde. Two days before arrival of chicks, brooder have been switched on to maintain inside shed feeling temperature at 32°C and humidity was set to 65±5%. Feed and water were supplied adlib, while light duration was set at 22-24 hours for entire duration.

The research trial was conducted using 1800 Cobb-500 day old chicks. All the birds were randomly divided into 6 treatment groups (T1, T2, T3, T4, T5 and T6) having 300 birds in each group. Each group was consisted of 6 replicates containing 50 birds per replicate. T1 served as negative control, T2 as a positive control supplemented with meduramycine and flavomycine, T3 was supplied with Actigen at level of 0.2gm/kg of feed, T4 was supplied with Actigen at level of 0.4gm/kg of feed, T5 was supplied with Actigen at level of 0.6gm/kg of feed and T6 was supplied with Actigen at level of 0.8gm/kg of feed. The ingredient and chemical composition of experimental diets are given in Table 1 and Table 2.

2.1. Weekly body weight gain

Live weight (g) of each bird was recorded at the beginning of trial. Birds were wing banded and live body weight (g) of each bird was recorded at the start and end of experimental period, 35 days of age in the morning before accesses to feed.

2.2. Feed intake and feed conversion ratio

Feed was weighed at the start and after end of each week. Feed residues were collected and weighed every week to calculate the amount of feed consumed per each bird per day for each treatment (g/bird/day). The FCR was calculated as (FCR = kg feed consumed/kg weight gain of birds).

2.3. Gut histomorphology

At the end of trial, six birds per treatment group were slaughtered by Halal method (Farouk et al., 2014). Their small intestines were removed and washed with normal saline and its segments; duodenum (pancreatic loop), jejunum and ilium were measured in centimeter, and then 2 cm segments were fixed in 10% formalin solution for further processing. Villus height and crypt depth were recorded in jejunum and ilium and goblet cells per villus were counted using microscope. To measure villus height and crypt depth, 2 cm segments of jejunum and

Table 1. Ingredients and their inclusion levels in experimental diets.

Ingredient	T1	T2	T3	T4	T5	T6
Maize	632	632	632	632	632	632
Soybean meal	209	209	209	209	209	209
Canola meal	38	38	38	38	38	38
Rapeseed meal	50	50	50	50	50	50
PBM	50	50	50	50	50	50
Rice polish	0.94	0.34	0.94	0.94	0.94	0.94
Limestone	8.8	8.8	8.8	8.8	8.8	8.8
MCP	0.9	0.9	0.9	0.9	0.9	0.9
Lysine HCl	2.9	2.9	2.9	2.9	2.9	2.9
DL-Methionine	1.85	1.85	1.85	1.85	1.85	1.85
L-Threonine	0.66	0.66	0.66	0.66	0.66	0.66
L-Isoleucine	0.15	0.15	0.15	0.15	0.15	0.15
Salt	2.0	2.0	2.0	2.0	2.0	2.0
Soda	1.0	1.0	1.0	1.0	1.0	1.0
Choline	0.5	0.5	0.5	0.5	0.5	0.5
Phytase	0.1	0.1	0.1	0.1	0.1	0.1
Meduramycine	0	0.5	0	0	0	0
Flavomycine	0	0.1	0	0	0	0
Mannan-oligosaccharide	0	0	0.2	0.4	0.6	0.8
Vit. Premix	0.6	0.6	0.6	0.6	0.6	0.6
Min. Premix	0.6	0.6	0.6	0.6	0.6	0.6

ilium were cut down and washed with physiological saline solution, and then fixed in 10% buffered formalin. Histological sections were examined microscopically. Villi were photographed with Nikon spot camera and PixelPro software was used for all measurements (Brümmer et al., 2010a).

2.4. Antibody titer against Newcastle disease virus

Antibody titer was tested against the Newcastle disease virus (NDV) using hemagglutination inhibition test (HI). After 7 days of vaccination, 2 ml fresh blood was collected from the wing vein of the birds in a sterile way and transferred to the vacutainer. The (Newcastle Virus) suspension was prepared with a known HA titer. 0.025 ml of phosphate saline (PBS) solution was distributed in each well of the microtiter plate. 0.025 ml of serum was placed in the first well. Then the dual serial dilution was made through this suspension across the plate. After that, 0.025 ml of 4HAU of virus/antigen was added to each well and the plate is left for 30 minutes at room temperature. Prepare 1% (v/v) of the chicken RBCs by adding 100ml of PBS into 1 ml of suspended RBCs. Then add 0.025 ml of 1% (v/v) of the chicken RBCs, to each well and mix gently. Red blood cells (RBCs) were allowed to settle for 40 minutes at room temperature. HI titer was the highest serum dilution causing complete inhibition of 4HAU (Shahir et al., 2014).

Table 2. Chemical composition of experimental diets.

Nutrient	Starter diet	Grower diet
Metabolizable energy kcal/kg	2900	2950
Crude protein %	22.19	20.30
Crude fiber %	3.62	2.87
Ether extract %	4.26	3.77
Total ash %	3.92	3.43
Calcium %	0.8	0.78
Avail. Phosphorus %	0.4	0.38
Sodium %	0.15	0.14
Potassium %	0.63	0.68
Chlorine %	0.3	0.28
Avail. Choline mg/kg	1078.16	800.00
D lysine %	1.15	1.1
D Methionine + Cystine %	0.84	0.82
D Threonine %	0.74	0.73
D Tryptophan %	0.20	0.21
D Arginine %	1.18	1.17
D Isoleucine %	0.78	0.75
D Valine %	0.92	0.85
D Leucine %	1.89	1.58

2.5. Statistical analysis

Data were analyzed using SPSS 21.0 software (SPSS Inc., Chicago, IL, USA). Effects of mannan-oligosaccharide supplementation on gut health, immunity, and production performance of broilers were analyzed using one-way ANOVA. The significance level was set at 5% and calculated using Duncan's multiple range test. The data were presented as the means \pm standard deviations.

3. Results

3.1. Average weekly feed intake

Results shown in Table 3 reveals the effect of MOS supplementation on average weekly feed intake in broilers at 5 consecutive weeks. Results showed that supplementation of MOS significantly ($P < 0.05$) affected the feed intake. At 1st week, highest average feed intake (FI) was observed in T5 followed by T3, T2 and T1 groups. At 2nd week of age, higher FI was seen in T6 group followed by T5 and T1. However, at 3rd week, elevated FI was observed in T6 and T3 followed by T2 and T1. At 4th week, highest FI was seen in T6 followed by T2. At the end of trial, highest feed intake was calculated in T4 followed by T6 and lowest FI was observed in T1.

3.2. Average weekly weight gain

Results presented in Table 4 shows the outcome of MOS on average weekly body weight gain (BWG) in broilers at

5 consecutive week intervals. Supplementation of MOS significantly ($P < 0.05$) affected the BWG in broilers. During 1st week, higher BWG was observed in T6 followed by T3 group. During 2nd week, higher BWG was seen in T6 followed by T3. During 3rd week of age, higher BWG was found in T3 and T6 followed by T1, T4 and T5 groups. At 4th week of trial, birds reared on T6 showed significantly ($P < 0.05$) highest body weight gain followed by T5, T4, T3 and T2. At last week, higher BWG was recorded in T6 and T4 group and no significant difference was observed between T1, T2, T3 and T5 groups ($P > 0.05$).

3.3. Feed conversion ratio

The Table 5 demonstrates the impact of MOS supplementation on weekly feed conversion ratio. Supplementation of MOS significantly affected FCR in broilers ($P < 0.05$). 1st week data showed that birds of T6 group had the best FCR followed by T3, T2 and T1. Similarly, at the end of 2nd week best ($P < 0.05$) value of FCR was found in T6 followed by T4 and T3. At the end of 3rd week, best value for FCR was calculated in T6 and T3 followed by T5 and T4. At 4th week, birds of T6 group showed best FCR. However, at the end of trial, best FCR was calculated in T6 group followed by T4 and T2 ($P < 0.05$).

3.4. Length of different intestinal sections

Results presented in Table 6 reveals the impact of MOS addition on the length of duodenum, jejunum and ileum.

Table 3. MOS supplementation and average weekly feed intake of broilers.

Treatment	Week				
	W1	W2	W3	W4	W5
T1	171.43 \pm 0.49 ^a	602.41 \pm 1.39 ^{ab}	1275.23 \pm 4.91 ^{ab}	2059.07 \pm 6.84 ^a	2931.08 \pm 18.76 ^a
T2	173.85 \pm 1.25 ^{abc}	599.82 \pm 4.15 ^{ab}	1284.34 \pm 7.86 ^{ab}	2098.19 \pm 11.80 ^{cd}	2964.32 \pm 16.25 ^{ab}
T3	176.33 \pm 1.13 ^{cd}	613.62 \pm 4.03 ^c	1290.25 \pm 7.92 ^b	2085.78 \pm 9.94 ^{bc}	2972.01 \pm 18.65 ^{ab}
T4	174.94 \pm 1.19 ^{bc}	592.60 \pm 3.28 ^a	1269.23 \pm 7.37 ^a	2088.51 \pm 4.45 ^{bc}	3026.09 \pm 11.02 ^c
T5	178.93 \pm 1.22 ^d	600.30 \pm 2.64 ^{ab}	1268.10 \pm 3.44 ^a	2070.41 \pm 8.12 ^{ab}	2940.92 \pm 11.28 ^a
T6	172.80 \pm 0.98 ^{ab}	606.61 \pm 4.14 ^{bc}	1290.68 \pm 7.71 ^b	2117.24 \pm 8.25 ^c	2996.04 \pm 10.05 ^{bc}

Means \pm standard deviation within a column not sharing same superscripts are significantly different $P < 0.05$.

Table 4. MOS supplementation and average weekly weight gain of broilers.

Treatment	Week				
	W1	W2	W3	W4	W5
T1	178.33 \pm 0.86 ^a	472.32 \pm 1.84 ^a	892.75 \pm 3.46 ^b	1296.32 \pm 17.29 ^a	1808.87 \pm 23.59 ^a
T2	178.74 \pm 1.30 ^a	472.84 \pm 3.40 ^a	873.69 \pm 6.55 ^a	1349.42 \pm 20.58 ^b	1834.35 \pm 9.67 ^a
T3	182.71 \pm 0.96 ^b	489.95 \pm 1.57 ^{bc}	928.95 \pm 5.78 ^c	1337.96 \pm 10.51 ^b	1827.72 \pm 18.62 ^a
T4	175.82 \pm 1.02 ^a	479.77 \pm 9.24 ^{ab}	899.74 \pm 8.64 ^b	1337.56 \pm 10.44 ^b	1881.61 \pm 11.10 ^b
T5	178.36 \pm 1.00 ^a	470.15 \pm 2.79 ^a	902.76 \pm 6.07 ^b	1334.02 \pm 11.36 ^b	1817.85 \pm 15.77 ^a
T6	190.42 \pm 0.56 ^c	494.93 \pm 4.43 ^c	927.10 \pm 7.11 ^c	1404.14 \pm 8.38 ^c	1897.28 \pm 15.46 ^b

Means \pm standard deviation within a column not sharing same superscripts are significantly different $P < 0.05$.

Table 5. MOS supplementation and Feed conversion ratio.

Treatment	Week				
	W1	W2	W3	W4	W5
T1	0.96±0.01 ^b	1.28±0.00 ^b	1.43±0.00 ^b	1.60±0.02 ^c	1.63±0.01 ^b
T2	0.97±0.01 ^b	1.27±0.01 ^b	1.47±0.01 ^c	1.55±0.01 ^b	1.61±0.01 ^{ab}
T3	0.97±0.00 ^b	1.25±0.01 ^{ab}	1.39±0.01 ^a	1.56±0.01 ^b	1.63±0.02 ^b
T4	1.00±0.01 ^c	1.25±0.03 ^{ab}	1.42±0.02 ^{ab}	1.56±0.01 ^b	1.61±0.01 ^{ab}
T5	1.00±0.00 ^c	1.28±0.01 ^b	1.41±0.01 ^{ab}	1.55±0.01 ^b	1.62±0.01 ^b
T6	0.91±0.01 ^a	1.23±0.00 ^a	1.39±0.00 ^a	1.51±0.01 ^a	1.58±0.01 ^a

Means ± standard deviation within a column not sharing same superscripts are significantly different $P < 0.05$.

Results showed that MOS significantly ($P < 0.05$) affected the length of intestinal sections. At the end of trial, birds supplemented with T2 group have significantly ($P < 0.05$) highest length of duodenum followed by T4. Smallest length of duodenum was found in negative control group T1. Highest jejunum length was found positive control group T2 followed by T5 and lowest values for jejunum length was found in T6. Similarly, significantly ($P < 0.05$) highest ileum length was found positive control group and T5 followed by T4 and T3. Lowest values for ileum length were found in negative control group.

3.5. Histomorphological parameters

Results presented in Table 7 indicating the effects of MOS addition on histomorphological parameters of small intestine. Addition of MOS significantly ($P < 0.05$) affected the villus height in jejunum. At the end of trial, birds of groups T3 and T6 showed highest ($P < 0.05$) villus height in jejunum. Lowest values for villus height in jejunum were observed in negative control group (T1). Data for villus height in ileum revealed that supplementation of MOS had non-significant effect ($P > 0.05$). As for as crypt depth is concerned, in jejunum portion crypt depth was significantly highest ($P < 0.05$) in T5 and T4. In ileum, supplementation of MOS showed non-significant effect on crypt depth. Similarly, MOS had non-significant effect on number of goblet cells/10000 μm^2 as highest values were found in positive control group.

3.6. Antibody titer against NDV

Data presented in Table 8 shows the impact of MOS supplementation on antibody titer against NDV. Supplementation of MOS significantly ($P < 0.05$) affected the antibody titer against NDV. Birds fed on T6 and T5 showed significantly ($P < 0.05$) highest antibody titer against NDV. Lowest values of the titer were found in negative control group.

4. Discussion

Current research illustrates that the addition of MOS to birds at different week interval have a positive effect on feed intake. Results showed that birds fed with feed T6 (800g/ton of MOS) consumed significantly highest

Table 6. MOS supplementation and length of different intestinal sections.

Treatment	Duodenum	Jejunum	Ilium
T1	27.33±0.30 ^a	74.00±1.15 ^b	65.33±1.45 ^{ab}
T2	31.17±0.43 ^c	80.83±1.66 ^d	71.17±1.88 ^c
T3	28.82±0.31 ^b	75.17±1.43 ^{bd}	67.67±1.68 ^{bc}
T4	29.00±0.60 ^b	73.17±0.79 ^b	68.33±0.79 ^{bc}
T5	28.33±0.67 ^{ab}	78.33±1.08 ^{cd}	71.33±1.03 ^c
T6	27.83±0.47 ^{ab}	67.33±1.00 ^a	61.50±1.61 ^a

Means ± standard deviation within a column not sharing same superscripts are significantly different $P < 0.05$.

average FI than negative and positive control group. Similar outcomes were observed by Zakeri and Kashefi (2011) and Fernandes et al. (2014) as supplementation of MOS during 1st week of age has greater feed intake than the control group. In the same way, Iji et al. (2001) revealed that addition of MOS to diet enhanced the FI of birds as compared to the control group. In contrast, Abdelwahid et al. (2017), Koc et al. (2010) and Al-Sultan et al. (2016) observed that MOS supplemented had no difference on FI.

It was observed that addition of MOS to birds feed at different week interval had a positive effect on average weekly weight gain. Same outcomes were found by Abdelwahid et al. (2017), as he observed that supplementation of MOS at 0.2% significantly increased the BWG in broiler chicks during 0-21 days of age. Similarly, Shahir et al. (2014) found that MOS supplemented group showed better WG than control group and probiotic group for 1-21 days. In contrast, according to the study of Abdelwahid et al. (2017) and Koc et al. (2010) no significant difference was observed in WG during 1-21 days of age between MOS supplemented and positive control group Attia et al. (2014a, b; Gibson et al., 2004).

The present study showed that the supplementation of MOS to birds at different week interval had a positive effect on weekly FCR. The results of Bozkurt et al. (2008) were similar to this study, they observed better FCR in MOS added group than all other groups (negative control, positive control with AGP and dextran oligosaccharide) during 0-21 days of age. Similarly, results of Koc et al. (2010) were

Table 7. MOS supplementation and histomorphology of jejunum and ileum.

Treatment	Villus height (µm) in Jejunum	Villus height (µm) in Ilium	Crypt depth (µm) in Jejunum	Crypt depth (µm) in Ilium	No. of Goblet cells/10000µm ² area
T1	526.11±156.1 ^a	446.92±88.59	81.56±17.60 ^a	72.22±10.57	14.67±3.56 ^a
T2	604.14±93.60 ^{ab}	374.07±51.78	91.74±28.86 ^a	79.90±43.76	22.00±6.10 ^b
T3	787.69±209.1 ^c	450.76±264.5	103.57±13.14 ^a	81.74±34.68	16.50±2.59 ^{ab}
T4	744.96±46.70 ^{bc}	467.31±86.60	146.03±33.77 ^b	62.13±9.53	19.00±6.20 ^{ab}
T5	629.22±144.4 ^{ab}	438.03±97.57	148.71±50.74 ^b	64.57±21.66	18.17±2.32 ^{ab}
T6	784.74±94.87 ^c	446.97±78.00	103.60±18.03 ^a	74.59±24.49	20.17±5.31 ^a

Means ± standard deviation within a column not sharing same superscripts are significantly different P < 0.05.

also in line to our study, as they recorded the better FCR in MOS supplemented group. However, results presented in the study of Abdelwahid et al. (2017) contrasted with our study as they reported that there was no significant improvement in FCR due to supplementation of MOS.

Supplementation of MOS significantly affected the length of intestinal sections. At the end of trial, birds supplemented with T2 group have highest length of duodenum followed by T4. Similar results were found by Padihari et al. (2014) and Castillo et al. (2008) as they observed that addition of MOS at a level of 500g/ton significantly increased the duodenum length as compared to negative and positive control groups. Supplementation of MOS didn't show any effect on jejunum length (Dimitroglou et al. 2010; Padihari et al., 2014; Chand et al., 2019).

Addition of MOS to broiler chick feed significantly affected the histomorphology of jejunum and ileum. At the end of trial, birds of group T3 and T6 showed significantly highest villus height in jejunum. In the same way, Mostafa et al. (2015) found that supplementation of MOS had positive effect on villus height in jejunum and ileum. In contrast to our results, Abudabos et al. (2015; Ao and Choct, 2013; Dev et al., 2020) observed that supplementation of MOS had no significant effect on villus height in jejunum. Supplementation of MOS has no effect on villus height and crypt depth in ileum. In contrast to our results, Biswas et al. (2018) found that addition of MOS to basal diet had significant effect on crypt depth in ileum. Supplementation of MOS at different inclusion rates had no effect on number of goblet cell per villus but these results were significantly superior and inferior to negative and positive control groups, respectively. In contrast to our result Baurhoo et al. (2007, 2009) found that MOS had significantly affected the goblet cell number.

Supplementation of MOS significantly affected the antibody titer against NDV. Birds fed on T6 and T5 showed highest antibody titer against NDV. Lowest values for titer were found in negative control group. Similar results were also published by Shahir et al. (2014) as they observed that MOS supplemented group gained higher antibody titer against NDV as compared to control group but lower than probiotic group. Results of Muhammad et al. (2020) and Bonato et al. (2020) were also similar to our study they recorded that MOS supplemented group has significantly higher antibody titer against NDV as than control group

Table 8. MOS supplementation and antibody titer against NDV.

Treatment	Antibody titer against NDV
T1	4.67±0.23 ^a
T2	4.83±0.17 ^{ab}
T3	5.33±0.21 ^{bc}
T4	5.67±0.23 ^{cd}
T5	6.17±0.25 ^d
T6	6.00±0.21 ^d

Means ± standard deviation within a column not sharing same superscripts are significantly different P < 0.05.

but lower than probiotic group. Similar results were also found by Waqas et al. (2019) and Borsatti et al. (2020) as MOS supplemented group showed higher antibody titer against NDV as compared to control group.

5. Conclusion

The results extracted from the following research are indicative that birds fed with diets containing MOS, exhibited improvement in weight gain, enhanced immunity against NDV and commutatively better productive performance in comparison to positive and negative control group. In conclusion, MOS can be used in place of AGPs as non-microbial performance-enhancing feed advocates and can play a part in minimizing the irrational use of antibiotics in poultry feed.

References

- ABDELWAHID, H.H., IBRAHIM, G.A., NAFISA, M., and BASHEER, E.O., 2017. Impact of different dietary supplementation with (Y-MOS) yeast on performance and carcass characteristics of broiler chickens. *International Journal of Multidisciplinary and Current Research*, vol. 5, pp. 1337-1341.
- ABUDABOS, A., AL-BATSHAN, H. and MURSHED, M., 2015. Effects of prebiotics and probiotics on the performance and bacterial colonization of broiler chickens. *South African Journal of Animal Science*, vol. 45, no. 4, pp. 419-428. <http://dx.doi.org/10.4314/sajas.v45i4.8>.

- AL-SULTAN, S.I., ABDEL-RAHEEM, S.M., EL-GHAREEB, W.R. and MOHAMED, M.H., 2016. Comparative effects of using prebiotic, probiotic, synbiotic and acidifier on growth performance, intestinal microbiology and histomorphology of broiler chicks. *The Japanese Journal of Veterinary Research*, vol. 64, suppl. 2, pp. S187-S195.
- AO, Z. and CHOCT, M., 2013. Oligosaccharides affect performance and gut development of broiler chickens. *Asian-Australasian Journal of Animal Sciences*, vol. 26, no. 1, pp. 116-121. <http://dx.doi.org/10.5713/ajas.2012.12414>. PMID:25049713.
- ATTIA, Y.A., AL-HAMID, A.E.A., IBRAHIM, M.S., AL-HARTHI, M.A., BOVERA, F. and ELNAGGAR, A.S., 2014a. Productive performance, biochemical and hematological traits of broiler chickens supplemented with propolis, bee pollen, and mannan oligosaccharides continuously or intermittently. *Livestock Science*, vol. 164, pp. 87-95. <http://dx.doi.org/10.1016/j.livsci.2014.03.005>.
- ATTIA, Y.A., AL-HAMID, A.E.A., IBRAHIM, M.S., AL-HARTHI, M.A., BOVERA, F. and ELNAGGAR, A.S., 2014b. Productive performance, biochemical and hematological traits of broiler chickens supplemented with propolis, bee pollen, and mannan oligosaccharides continuously or intermittently. *Livestock Science*, vol. 164, pp. 87-95. <http://dx.doi.org/10.1016/j.livsci.2014.03.005>.
- BAURHOOD, B., GOLDFLUS, F. and ZHAO, X., 2009. Purified cell wall of *Saccharomyces cerevisiae* increases protection against intestinal pathogens in broiler chickens. *International Journal of Poultry Science*, vol. 8, no. 2, pp. 133-137. <http://dx.doi.org/10.3923/ijps.2009.133.137>.
- BAURHOOD, B., LETELLIER, A., ZHAO, X. and RUIZ-FERIA, C., 2007. Cecal populations of lactobacilli and bifidobacteria and *Escherichia coli* populations after in vivo *Escherichia coli* challenge in birds fed diets with purified lignin or mannanoligosaccharides. *Poultry Science*, vol. 86, no. 12, pp. 2509-2516. <http://dx.doi.org/10.3382/ps.2007-00136>. PMID:18029796.
- BISWAS, A., MESSAM, R., KUMAWAT, M., NAMIT, M., MANDAL, A. and MIR, N., 2018. Effects of prebiotics on intestinal histomorphometry and gut microflora status of broiler chickens. *Indian Journal of Animal Research*, vol. 8, no. 6, pp. 1-5. <http://dx.doi.org/10.18805/ijar.B-3579>.
- BONATO, M., BORGES, L.L., INGBERMAN, M., FÁVARO JUNIOR, C., MESA, D., CARON, L.F. and BEIRÃO, B.C.B., 2020. Effects of yeast cell wall on immunity, microbiota and intestinal integrity of salmonella-infected broilers. *Journal of Applied Poultry Research*, vol. 29, no. 3, pp. 545-558. <http://dx.doi.org/10.1016/j.japr.2020.03.002>.
- BORSATTI, L., BROCH, J., AVILA, A.S., SCHNEIDERS, J.L., ROCHA, C.S., OXFORD, J.H. and NUNES, R.V., 2020. Essential oils and prebiotic on broiler diets as feed additives. *Semina: Ciências Agrárias*, vol. 41, no. 4, pp. 1307-1316. <http://dx.doi.org/10.5433/1679-0359.2020v41n4p1327>.
- BOZKURT, M., KUCUKYILMA, K., CATLI, A.U. and CINAR, M., 2008. Growth performance and slaughter characteristics of broiler chickens fed with antibiotic, mannan oligosaccharide and dextran oligosaccharide supplemented diets. *International Journal of Poultry Science*, vol. 7, no. 10, pp. 969-977. <http://dx.doi.org/10.3923/ijps.2008.969.977>.
- BRÜMMER, M., JANSEN VAN RENSBURG, C. and MORAN, C.A., 2010a. *Saccharomyces cerevisiae* cell wall products: the effects on gut morphology and performance of broiler chickens. *South African Journal of Animal Science*, vol. 40, no. 1, pp. 14-21.
- CASTILLO, M., MARTÍN-ORUE, S., TAYLOR-PICKARD, J., PEREZ, J. and GASA, J., 2008. Use of mannanoligosaccharides and zinc chelate as growth promoters and diarrhea preventative in weaning pigs: effects on microbiota and gut function. *Journal of Animal Science*, vol. 86, no. 1, pp. 94-101. <http://dx.doi.org/10.2527/jas.2005-686>. PMID:17911238.
- CHAND, N., SHAMSULLAH, S., RAFIULLAH, R., KHAN, R.U., MOBASHAR, M., NAZ, S., ROWGHANI, E. and KHAN, M.A., 2019. Mannanoligosaccharide (MOS) in Broiler Ration during the Starter Phase: 1. Growth performance and intestinal histomorphology. *Pakistan Journal of Zoology*, vol. 51, no. 1, pp. 173-176. <http://dx.doi.org/10.17582/journal.pjz/2019.51.1.173.176>.
- CHEN, K.-L., KHO, W.-L., YOU, S.-H., YEH, R.-H., TANG, S.-W. and HSIEH, C.-W., 2009. Effects of *Bacillus subtilis* var. natto and *Saccharomyces cerevisiae* mixed fermented feed on the enhanced growth performance of broilers. *Poultry Science*, vol. 88, no. 2, pp. 309-315. <http://dx.doi.org/10.3382/ps.2008-00224>. PMID:19151345.
- DE VRESE, M. and SCHREZENMEIR, J., 2008. Probiotics, prebiotics, and synbiotics. *Advances in Biochemical Engineering/Biotechnology*, vol. 111, pp. 1-66. PMID:18461293.
- DEV, K., MIR, N.A., BISWAS, A., KANNOUJIA, J., BEGUM, J., KANT, R., & MANDAL, A. 2020. Dietary synbiotic supplementation improves the growth performance, body antioxidant pool, serum biochemistry, meat quality, and lipid oxidative stability in broiler chickens. *Animal Nutrition*, vol. 6, no. 3, pp. 315-332. <https://doi.org/10.1016/j.aninu.2020.03.002>.
- DIMITROGLOU, A., MERRIFIELD, D.L., SPRING, P., SWEETMAN, J., MOATE, R. and DAVIES, S.J., 2010. Effects of mannan oligosaccharide (MOS) supplementation on growth performance, feed utilisation, intestinal histology and gut microbiota of gilthead sea bream (*Sparus aurata*). *Aquaculture (Amsterdam, Netherlands)*, vol. 300, no. 1-4, pp. 182-188. <http://dx.doi.org/10.1016/j.aquaculture.2010.01.015>.
- FAROUK, M.M., AL-MAZEEDI, H.M., SABOW, A.B., BEKHIT, A.E., ADEYEMI, K.D., SAZILI, A.Q. and GHANI, A., 2014. Halal and Kosher slaughter methods and meat quality: a review. *Meat Science*, vol. 98, no. 3, pp. 505-519. <http://dx.doi.org/10.1016/j.meatsci.2014.05.021>. PMID:24973207.
- FERNANDES, B., MARTINS, M., MENDES, A., MILBRADT, E., SANFELICE, C., MARTINS, B., AGUIAR, E. and BRESNE, C., 2014. Intestinal integrity and performance of broiler chickens fed a probiotic, a prebiotic, or an organic acid. *Brazilian Journal of Poultry Science*, vol. 16, no. 4, pp. 417-424. <http://dx.doi.org/10.1590/1516-635X1604417-424>.
- GADDE, U., KIM, W., OH, S. and LILLEHOJ, H.S., 2017. Alternatives to antibiotics for maximizing growth performance and feed efficiency in poultry: a review. *Animal Health Research Reviews*, vol. 18, no. 1, pp. 26-45. <http://dx.doi.org/10.1017/S1466252316000207>. PMID:28485263.
- GIBSON, G.R., PROBERT, H.M., LOO, J.V., RASTALL, R.A. and ROBERFROID, M.B., 2004. Dietary modulation of the human colonic microbiota: updating the concept of prebiotics. *Nutrition Research Reviews*, vol. 17, no. 2, pp. 259-275. <http://dx.doi.org/10.1079/NRR200479>. PMID:19079930.
- GRANSTAD, S., KRISTOFFERSEN, A.B., BENESTAD, S.L., SJURSETH, S.K., DAVID, B., SØRENSEN, L., FJERMEDAL, A., EDVARDSEN, D.H., SANSON, G., LØVLAND, A. and KALDHUSDAL, M., 2020. Effect of feed additives as alternatives to in-feed antimicrobials on production performance and intestinal clostridium perfringens counts in broiler chickens. *Animals (Basel)*, vol. 10, no. 2, pp. 240. <http://dx.doi.org/10.3390/ani10020240>. PMID:32028636.
- GRASHORN, M., 2010. Use of phytobiotics in broiler nutrition—an alternative to in-feed antibiotics. *Journal of Animal and Feed Sciences*, vol. 19, no. 3, pp. 338-347. <http://dx.doi.org/10.22358/jafs/66297/2010>.

- GUL, F., AHMAD, B., AFZAL, S., ULLAH, A., KHAN, S., AMAN, K., KHAN, M.T., HADI, F., KIRAN, K., ZAHRA, M., MAQBOOL, T., MOHSIN, U., NADEEM, T., JAVED, M.A., ALI, Q. and AHMAD, L., 2021. Comparative analysis of various sources of selenium on the growth performance and antioxidant status in broilers under heat stress. *Brazilian Journal of Biology = Revista Brasileira de Biologia*, vol. 83, pp. e251004. PMID:34614126.
- HUANG, G.L., 2008. Extraction of two active polysaccharides from the yeast cell wall. *Zeitschrift für Naturforschung C*, vol. 63, no. 11-12, pp. 919-921. <http://dx.doi.org/10.1515/znc-2008-11-1224>. PMID:19227846.
- HUTKINS, R.W., KRUMBECK, J.A., BINDELS, L.B., CANI, P.D., FAHEY JUNIOR, G., GOH, Y.J., HAMAKER, B., MARTENS, E.C., MILLS, D.A., RASTAL, R.A., VAUGHAN, E. and SANDERS, M.E., 2016. Probiotics: why definitions matter. *Current Opinion in Biotechnology*, vol. 37, pp. 1-7. <http://dx.doi.org/10.1016/j.copbio.2015.09.001>. PMID:26431716.
- JJI, P.A., SAKI, A.A. and TIVEY, D.R., 2001. Intestinal structure and function of broiler chickens on diets supplemented with a mannan oligosaccharide. *Journal of the Science of Food and Agriculture*, vol. 81, no. 12, pp. 1186-1192. <http://dx.doi.org/10.1002/jsfa.925>.
- KAMRAN, Z., MIRZAA, M., AHMAD, S., SAMAD, H., SOHAIL, M. and SAADULLAHB, M., 2013a. Performance of broiler chickens fed mannan oligosaccharides as alternatives to antibiotics from one to twenty-two days of age. *Journal of Animal and Plant Sciences*, vol. 23, pp. 1482-1485.
- KAMRAN, Z., MIRZAA, M., AHMAD, S., SAMAD, H., SOHAIL, M. and SAADULLAHB, M., 2013b. Performance of broiler chickens fed mannan oligosaccharides as alternatives to antibiotics from one to twenty-two days of age. *Journal of Animal and Plant Sciences*, vol. 23, no. 5, pp. 1482-1485.
- KLASING, K.C., 2007. Nutrition and the immune system. *British Poultry Science*, vol. 48, no. 5, pp. 525-537. <http://dx.doi.org/10.1080/00071660701671336>. PMID:17952723.
- KOC, F., SAMLI, H., OKUR, A., OZDUVEN, M., AKYUREK, H. and SENKOYLU, N., 2010. Effects of Saccharomyces cerevisiae and/or mannanoligosaccharide on performance, blood parameters and intestinal microbiota of broiler chicks. *Bulgarian Journal of Agricultural Science*, vol. 16, no. 5, pp. 643-650.
- KOCHER, A., DENEV, S., NIKIFOROV, I., DINEV, I. and SCHEIDEMANN, C., 2005. The use of mannan oligosaccharides (Bio-Mos) in sow and piglet diets. In: *Proceedings of Fourth BOKU-Symposium Tierernährung Tierernährung ohne antibiotische Leistungsförderer*, 27 October 2005, Vienna, Austria. Vienna: University of Natural Resources and Life Sciences (BOKU).
- MOORE, P., EVENSON, A., LUCKEY, T., MCCOY, E., ELVEHJEM, C. and HART, E., 1946. Use of sulfasuxidine, streptothricin, and streptomycin in nutritional studies with the chick. *The Journal of Biological Chemistry*, vol. 165, no. 2, pp. 437-441. [http://dx.doi.org/10.1016/S0021-9258\(17\)41154-9](http://dx.doi.org/10.1016/S0021-9258(17)41154-9). PMID:20276107.
- MOSTAFA, M., THABET, H. and ABDELAZIZ, M., 2015. Effect of bio-mos utilization in broiler chick diets on performance, microbial and histological alteration of small intestine and economic efficiency. *Asian Journal of Animal and Veterinary Advances*, vol. 10, no. 7, pp. 323-334. <http://dx.doi.org/10.3923/ajava.2015.323.334>.
- MUHAMMAD, S.L., SHEIKH, I.S., BAJWA, M.A., MEHMOOD, K., RASHID, N., AKHTER, M.A., ULLAH JAN, S., RAFEEQ, M., HAMEED, S. and FAYYAZ, A., 2020. Effect of Mannan Oligosaccharide (MOS) on growth, physiological and immune performance parameters of broiler chickens. *Pak-Euro Journal of Medical and Life Sciences*, vol. 3, no. 2, pp. 76-85.
- PADIHARI, V.P., TIWARI, S.P., SAHU, T., GENDLEY, M.K. and NAIK, S.K., 2014. Effects of mannan oligosaccharide and Saccharomyces cerevisiae on gut morphology of broiler chickens. *Journal of World's Poultry Research*, vol. 4, no. 3, pp. 56-59.
- REID, G., 2016. Probiotics: definition, scope and mechanisms of action. *Best Practice & Research. Clinical Gastroenterology*, vol. 30, no. 1, pp. 17-25. <http://dx.doi.org/10.1016/j.bpg.2015.12.001>. PMID:27048893.
- SHAHIR, M.H., AFSARIAN, O., GHASEMI, S. and TELLEZ, G., 2014. Effects of dietary inclusion of probiotic or prebiotic on growth performance, organ weight, blood parameters and antibody titers against influenza and newcastle in broiler chickens. *International Journal of Poultry Science*, vol. 13, no. 2, pp. 70-75. <http://dx.doi.org/10.3923/ijps.2014.70.75>.
- WAQAS, M., MEHMOOD, S., MAHMUD, A., HUSSAIN, J., AHMAD, S., KHAN, M.T., REHMAN, A., ZIA, M.W. and SHAHEEN, M.S., 2019. Effect of yeast based mannan oligosaccharide (Actigen™) supplementation on growth, carcass characteristics and physiological response in broiler chickens. *Indian Journal of Animal Research*, vol. 53, no. 11, pp. 1475-1479.
- WINDISCH, W. and KROISMAYR, A., eds., 2006. The effect of phytobiotics on performance and gut function in monogastrics. In: BIOMIN, eds. *World nutrition forum: the future of animal nutrition*. Argentina: Engromix, pp. 85-90.
- XU, X., QIAO, Y., PENG, Q., GAO, L. and SHI, B., 2017. Inhibitory effects of YCW and MOS from Saccharomyces cerevisiae on Escherichia coli and Salmonella pullorum adhesion to Caco-2 cells. *Frontiers in biology*, vol. 12, no. 5, pp. 370-375. <http://dx.doi.org/10.1007/s11515-017-1464-0>.
- YASMEEN, R., ZAHID, B., ALYAS, S., AKHTAR, R., ZAHRA, N., KOUSER, S., HASHMI, A.S., ATHAR, M., TAYYAB, M. and ANJUM, A.A., 2021. Ameliorative effects of Lactobacillus against Aflatoxin B1. *Brazilian Journal of Biology = Revista Brasileira de Biologia*, vol. 84, pp. e250517. PMID:34932626.
- ZAKERI, A. and KASHEFI, P., 2011. The comparative effects of five growth promoters on broiler chickens humoral immunity and performance. *Journal of Animal and Veterinary Advances*, vol. 10, no. 9, pp. 1097-1101. <http://dx.doi.org/10.3923/javaa.2011.1097.1101>.