Egyptian J. Nutrition and Feeds (2023), 26(3):343-351

AMELIORATIVE EFFECTS OF MANNANOLIGOSACHRIDE ON PERFORMANCE, HEPATIC ACTIVITY, AND GUT HEALTH IN BROILER CHICKENS

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(Received 13/09/2023, accepted 01/11/2023)

SUMMARY

Mannanoligosaccharide (MOS) have more positive effects, such as growth promotion, intestinal flora optimization, and reducing oxidative damage in broiler chickens. This study was designed to investigate the effect of dietary supplementation with MOS to improve productivity and enhance hepatic and intestinal status. Day-old chicks (n= 240) were divided into four treatments (three replicates of each) as follows: control –non supplemented group fed a basal diet recommended to strain (C), three other groups were fed a basal diet supplemented with 0.25, 0.5 and 1g MOS/kg diet, respectively. At 35 days of age, BWG, FC, FCR and EPEF were recorded. Plasma total protein, albumin, lipid profile and hepatic enzymes concentration levels were determined. Total counts of cecum microbiota and histological changes of ileum were determined. Dietary supplementation with MOS significantly improved BWG, FC, or FCR and EPEF values at levels of 0.5 and 1.0g/kg of diet compared with 0.25 level and control. Also, significant differences in plasma triglycerides and ALT levels were observed. Carcass traits were improved in supplemented groups than control. Reduction of coliforms bacteria in chicken intestine was counted with increased in villi height: crypt depth ratio. In general, dietary supplementations with different levels of MOS had a positive effect on performance and improved the gut microbial and histological status.

Keywords*: Broiler chickens, mannanoligosaccharide, performance, carcass traits, microbial population, and histomorphological changes.*

INTRODUCTION

Antibiotic overuse can contribute to the production of resistant pathogenic bacteria to antibiotics, which cause severe damage to human and animal health; therefore, many countries prohibit their use and resort to using effective nutritional supplements such as probiotics and/or prebiotics that positively improve growth rates and the immune systems of birds and animals (Castanon, 2007; Cosby *et al.*, 2015; Johnson *et al.*, 2016; Attia *et al.*, 2017; and Ghazalah *et al.*, 2021). Mannanoligosaccharide is a functional carbohydrate derived from the outer cell wall of Saccharomyces cerevisiae yeast. It has been widely used as an alternative to antibiotics due to its effectiveness in improving immune status, in addition to improving digestion rates and feed utilization (Spring *et al.*, 2015; Salami *et al.*, 2022). Some researchers studied the effect of using MOS in broiler chicks and laying hen diets, such as Yang *et al.* (2009), Mostafa *et al.* (2015), and Leinonen and Kyriazakis (2016), and they reported that using MOS in poultry diets can improve productive performance and feed efficiency and could enhance economic return to meat and egg producers, as well as reduce the environmental impacts of using antibiotics. Mannan oligosaccharides can prevent pathogenic bacteria containing type 1 fimbriae with mannose-seeking lectins [Escherichia coli (E. coli) and Salmonella spp.] from attaching to and colonizing the intestine (Spring *et al.*, 2000; Fowler *et al.*, 2017). Moreover, MOS enhance the growth of beneficial bacteria such as Lactobacillus ssp. in the intestinal gut and, therefore, support the growth rate and performance of the bird, as reported by Cavazzoni *et al.* (1998), Brzoska *et al.* (1999), Joerger (2003), Patterson and Burkholder (2003), and Mostafa *et al.* (2015).

Žikić *et al.* (2011) used MOS at levels 1, 0.75, and 0.5 kg/ton in the starter, grower, and finisher diets, respectively, to study the influence on the growth performance of broiler chickens and reported that using

MOS led to a significant improvement in growth rates from the fourth week until the sixth week. Also, Kim *et al.* (2011) used MOS at levels of 0.25 and 0.05% in broiler diets at 28 days and found that there was an improvement in productive performance with the increase in Mannan oligosaccharide levels in broiler diets. In addition, Sims *et al.* (2004) showed that supplemented turkey diets with mannan oligosaccharides at levels of 0.125 and 0.1% can improve the productive performance at 18 weeks of age. Moreover, Ghazalah *et al.* (2021) found that supplementation with MOS at a 2 g/kg diet improved growth performance, digestibility, and blood parameters without having adverse effects on the intestines of broiler chickens. However, Brümmer *et al.* (2010) found that using MOS at levels 2 and 4 g/kg in broiler diets has no effect on productive performance at 14 days of age. Also, Eseceli *et al.* (2010) supplemented broiler diets with Mannan oligosaccharide (starter 0.15%, grower 0.1%, finisher 0.05%) and found no effect on broiler performance. So, the aim of this study was to investigate the influences of different dietary levels of MOS on performance, carcass traits, blood biochemical parameters, microbial population, and histomorphological changes in broiler chicks.

MARTIALS AND METHODS

One-day-old Ross 308 broiler chicks (n = 240) with an average weight of 42g were divided into four experimental groups (60 chicks in 3 replicates) in three periodical periods (starter from 1–14 days, grower from 15–28 days, and finisher from 29–35 days). The experimental groups were divided as follows: control group, chicks fed a basal diet as recommended by Ross-broiler-pocket-guide-2018 (Table 1). The later three groups, MOS 0.25, MOS 0.50, and MOS 1.0, were fed a basal diet supplemented with MOS at 0.25, 0.50, and 1.0 g/kg, respectively.

Table (1): Ingredients and calculated analysis of basal diets.

Ingredients	Starter %	Grower%	Finisher %
Yellow Corn	61.40	64.60	68.20
Soybean Meal (48%)	27.07	23.00	19.10
Corn Gluten 60%	6.11	6.40	6.50
Vegetable Oil	0.64	1.60	2.00
Ca Carbonate	1.70	1.50	1.45
Mono CaPh	1.86	1.73	1.65
L- Lysine HCL	0.32	0.31	0.27
DL-methionine	0.30	0.26	0.23
NaCl(Salt)	0.30	0.30	0.30
Premix*	0.30	0.30	0.30
Total	100	100	100
Calculated chemical analysis**			
CP	22.00	20.50	19.00
ME	3007	3120	3188
C/P ratio	136.70	152.19	167.78
Ca	1.03	0.93	0.88
A.P	0.50	0.47	0.44
Lys	1.34	1.22	1.08
Meth	0.52	0.48	0.45
Meth + Cys.	1.01	0.94	0.87

**Each 1 kg of premix per 1 ton of feed includes: 12000000 I.U. VIT. A, 2000000I.U. VIT.D3, 10000 mg. VIT.E, 2000 mg. VIT.K3, 1000 mg. VIT.B1, 5000 mg. VIT.B2, 1500 mg. VIT.B6, 10 mg. VIT. B12, 10000 mg Pantothenate acid, 30000 mg. Nicotinic acid, 1000 mg. Folic acid, 50 mg. Biotin 60000mg. Mn, 80 mg. Zn, 50000 mg. Iron, 30000 mg. Cu, 10000 mg. Iodine, 1000 mg. Se 100 mg, cobalt 100 mg, carrier (CaCo3) add to 3 Kg. ** According to NRC (1994).*

Chicks within groups were reared on the floor pens under similar conditions of management until 35 days of age (DOA). At 35 DOA, body weight gain (BWG), feed consumption (FC), feed conversion ratio (FCR), and European production efficiency factor (EPEF) were calculated. Also, five birds of each treatment were taken to collect blood samples by jugular vein puncture, centrifuged at 4000 rpm for 15 minutes, then plasma was separated and kept in the refrigerator for biochemical analysis. Plasma total protein, albumin, ALT, AST, cholesterol, and triglyceride levels were determined by using a commercial kit (Spectrum). Antibody titers against Newcastle disease virus (NDV) were determined by a hemagglutination inhibition test, according to Munir *et al.* (2012). After blood was collected, chicken samples of each treatment were individually weighed, slaughtered, and the percentage of carcass weight, giblets, and lymphoid organs were calculated. For total microbial and coliform counts, five samples from cecum contents (5 g) were collected in a falcon sterile tube and stored at 4ºC for 24 hours. The bacteriological examinations of the cecum digesta samples included total bacterial and coliform counts, according to Dave and Shah (1996). About 2 cm of ileum were taken and fixed in a 10% formalin solution for histological examination.

The statistical analysis of the data obtained was conducted using the general linear model (GLM) procedure of SAS® (SAS, 2004) by applying a one-way ANOVA. Means were compared using Duncan´s range test (Duncan, 1955), where the level of significance was set at minimum ($P \leq 0.05$), and the statistical model was performed as follows:

$$Yi = \mu + Li + Eij$$

Where: Yi= is the effect of the observation, μ = overall mean., Li= the effect of dietary levels of MOS., Eij = experimental random error.

RESULTS AND DISCUSSION

Growth performance:

Table 2 shows the effects of different levels of dietary supplementation with MOS on the productive performance of broilers at 35 days old. There was a significant increase in BWG in the supplemented groups compared with the control group. This result may be related to the development of the microbial population in the digestive tract in supplemented groups (Table 5). Also, it might be related to the increase in villi height in the small intestine (Žikić *et al.,* 2011).

Table (2): Productive performance of broiler chicks supplemented with different levels of dietary MOS at 35 days of age.

Traits	Control	MOS 0.25 g/kg	MOS 0.50 g/kg	MOS 1.0 g/kg	SEM	P-value
BWG (g)	1749[b]	1722.14[a]	1808.49[a]	1782.21[a]	16.82	0.008
FC (g)	2747.93	2715.65	2790.2	2717.73	16.65	0.124
FCR	1.66 [b]	1.68 [b]	1.53 [a]	1.51 [a]	0.07	0.008
EPEF	285.19[b]	281. 93[b]	300.40 [a]	299.06 [a]	9.11	0.005

*[a and b]Means within the same row with different common superscripts differ significantly. SEM: standard error of means BWG, average body weight gain (g); FC, Average feed consumed (g/kg); FCR, feed conversion ratio; EPEF: European production efficiency factor [% livability * BW (kg) * 100/feed conversion ratio *trial duration (d)].*

These results are similar to those found by Benites *et al.* (2008), Žikić *et al.* (2011), Fowler *et al.* (2015), and Habib *et al.* (2020), who reported that prebiotics can increase the LBW of broilers. However, Eseceli *et al.* (2010) found that MOS had no positive effect on the growth rate of broilers.

On the other hand, feed consumption (FC) was not significantly affected by using MOS in broiler diets at 35 days of age. The same results were achieved by Waldroup *et al.* (2003), Brümmer *et al.* (2010), Kim *et al.* (2011), and Habib *et al.* (2020), who found that FC wasn't affected by supplementing broiler diets with MOS. Nevertheless, Alsenosy *et al.* (2012), Bednarczyk *et al.* (2016), and Mostafa *et al.* (2015) reported that supplementing broiler diets with a 0.5 g/kg diet of MOS increased the FC of chicks at 35 days old.

Also, feed conversion ratio (FCR) improved significantly by increasing Mannan oligosaccharide levels in broiler diets. This result agreed with those found by Bai *et al.* (2013), Xiao *et al.* (2012), and Waldroup *et al.* (2003). However, Alsenosy *et al.* (2012) and Bednarczyk *et al.* (2016) found that FCR didn't improve by using Mannan oligosaccharides in the broiler diet at 35 days of age.

Carcass traits:

Data in Table 3 showed that the relative weight of the edible parts (liver, gizzard, and heart) had not significantly affected by supplemented levels of Mos. However, chicks supplemented with MOS dressed higher by 1-2% than control with significant differences; also, the lowest value of pad fat was detected by 1.0 kg/kg Mos with significant differences. These results disagreed with Waldroup *et al.* (2003), Konca *et al.* (2009), and Habib *et al.* (2020), who found that dietary addition of MOS (1 g/kg) has no significant effect on carcass traits or carcass cuts of turkey. However, Abdel-Raheem and Abd-Allah (2011) found that using 2g of MOS/kg of the starter diets and 0.5 g/kg of the grower diets of broiler chicks decreased the carcass yield of broiler chicks. Low pad fat values which contributed by Mannan oligosaccharides supplemented birds compared to control group could be explained by the low cholesterol values detected in treated birds (Table 6).

Table (3): Carcass characteristics of broiler chicks supplemented with different levels of dietary MOS.

Traits	Control	MOS 0.25 g/kg	MOS 0.50 g/kg	MOS 1.0 g/kg	SEM	P-value
Carcass %	69.19 ^{b}	70.93^{a}	71.96^{a}	71.21^{a}	3.25	0.009
Liver %	3.30	3.27	3.30	3.23	0.44	0.124
Gizzard %	2.06	2.24	2.13	2.20	0.31	0.142
Heart %	0.61	0.59	0.59	0.63	0.07	0.111
Pad Fat %	1.86^{a}	1.72^{a}	1.58^{b}	1.54^{b}	0.15	0.001

$^{a\ and\ b}$ Means within the same row with different common superscripts differ significantly. SEM: standard error of means

Immunological parameters:

The relative weights of the lymphoid organs and antibody titers are presented in Table 4. The obtained results showed that there were insignificant increases in spleen, bursa, and thymus% by supplemented broiler diets by MOS. These results might be related to the fact that using MOS in broiler diets decreased harmful bacteria such as E. coli in the small intestine, which can improve the immune status of broiler chicks. These results agree with those found by Ali El-Far *et al.* (2016) and Attia *et al.* (2017), who found an increase in immune organ percentage as a result of using MOS in broiler diets. However, Abo Egla *et al.* (2013) found that MOS in broiler diets has no significant effect on all immune organs.

Table (4): Immune organs relative weight and antibody titers of broiler chicks supplemented with different levels of dietary MOS.

Traits	Control	MOS 0.25 g/kg	MOS 0.50 g/kg	MOS 1.0 g/kg	SEM	P-value
Spleen %	0.157	0.165	0.155	0.166	0.03	0.091
Bursa %	0.196	0.185	0.189	0.202	0.06	0.124
Thymus %	0.219	0.229	0.231	0.224	0.05	0.225
			Antibody titers against NDV			
NDV	4.77^{b}	4.93^{b}	5.43^{a}	5.77^{a}	0.21	0.005

$^{a\ and\ b}$ Means within the same row with different common superscripts differ significantly. SEM: standard error of means.

NDV, Newcastle disease virus.

Furthermore, there was a significant increase in NDV antibody titers associated with dietary Mos levels. These results were in harmony with those obtained by Asif *et al.* (2022), who found that the supplementation of Mos significantly ($P < 0.05$) affected the antibody titer against NDV, where birds fed MOS showed significantly ($P < 0.05$) the highest antibody titer against NDV.

Microbial population:

The observations in Table 5 showed that there was a significant decrease in both total bacteria and total coliforms with MOS supplementation in broiler diets. These results may be related to the fact that MOS binds harmful bacteria, so the coliform count decreased. The results are similar to those obtained by Mostafa *et al.* (2015) and Navidshad *et al.* (2015), who found a significant decrease in harmful bacteria count in experimental groups treated with MOS in broiler diets.

Plasma biochemical constituents:

The effect of different levels of MOS in broiler diets on some blood components is shown in Table 6.

Protein fractions:

The data in Table 6 showed a non-significant effect of different levels of MOS on total protein, albumin, and globulin. However, birds fed diets supplemented with 0.5 kg/ton of MOS recorded the highest albumin and lowest globulin levels (1.64 and 2.45, respectively) compared with other groups. These results agree with Attia *et al.* (2017), El-Sheikh *et al.* (2009), and Muhammad *et al.* (2020), who found a non-significant difference ($P > 0.05$) in all MOS treatment groups for broiler chicks in terms of total protein, albumin, and globulin.

Table (5): Microbial enumeration in broiler chicks supplemented with different levels of dietary MOS.

Traits	Control	MOS 0.25 g/kg	MOS 0.50 g/kg	MOS 1.0 g/kg	SEM	P-value
Total bacteria	8.09 ^{a}	8.08^{b}	9.81 ^{a}	9.92^{a}	0.16	0.003
Total coliforms	2.64 ^{a}	2.14 ^{a}	1.44^{b}	1.89^{b}	0.17	0001

$^{a\ and\ b}$ Means within the same row with different common superscripts differ significantly. SEM: standard error of means

Lipids profile:

Data obtained in Table 6 showed that there was a significant decrease in cholesterol in plasma in experimental groups treated with MOS compared with control (160.40 vs. 154.75, 151.75, and 152.90 mg/dl, respectively). Also, triglycerides decreased in birds fed diets supplemented with MOS. These results were in harmony with those obtained by Youssef *et al.* (2023), who found that MOS had a hypocholesterolemic influence on laying hens. MOS may affect cholesterol levels by affecting bile salt hydrolase action (Ooi and Liong, 2010). Also, these results agree with the results found by Abdel-Raheem and Abd-Allah (2011), who found that cholesterol and triglycerides were significantly affected by using MOS in broiler chickens at 42 days of age.

Liver enzymes:

Data showed an insignificant increase in AST in plasma in experimental Mannan oligosaccharide groups compared with the control group (217.00, 231.00, and 222.41 IU/L, respectively). On the other hand, ALT values were decreased by supplemented Mannan oligosaccharide levels. These results agree with Khairalla (2022), who found that MOS in broiler diets at levels of 0.05, 0.10, and 0.15% significantly reduced serum AST and ALT concentrations; they also agree with the findings of Youssef *et al.* (2023), who showed that MOS reduced AST and ALT concentrations compared to the control group in laying hens.

Table (6): blood biochemical constituents in broiler chicks supplemented with different levels of dietary MOS.

Traits	Control	MOS 0.25 g/kg	MOS 0.50 g/kg	MOS 1.0 g/kg	SEM	P-value
			Protein fractions(g/dl)			
Total protein	4.00	3.80	3.91	3.84	0.16	0.092
Albumin	1.62	1.56	1.64	1.58	0.19	0.84
Globulin	2.38	2.30	2.45	2.26	0.18	0.065
			Lipids profile(mg/dl)			
Cholesterol	160.40 ^{a}	154.75± ^{b}	151.75 ^{b}	152.90 ^{b}	3.31	0.008
Triglycerides	101.25 ^{a}	73.40 ^{b}	83.30 ^{b}	87.09 ^{b}	3.11	0.001
			Liver enzymes(IU/L)			
AST	210.60	217.00	231.00	222.41	3.76	0.055
ALT	78.80 ^{a}	44.20^{b}	50.20 ^{b}	59.71 ^{b}	3.37	0.001

$^{a\ and\ b}$ Means within the same row with different common superscripts differ significantly. SEM: standard error of means
AST, aspartate aminotransferase; ALT, alanine aminotransferase

Histomorphological parameters:

The results presented in Table (7) indicate the effects of MOS addition on the histomorphological parameters of the small intestine. Dietary addition of MOS significantly affected the intestinal histomorphological parameters, as the highest villus was detected in the 1.0 g/kg group, followed by 0.5 and 0.25 g/kg, while the lowest villus was observed in the control group. In contrast, crypt depth decreased significantly with MOS supplementation (Figure 7). These results were in harmony with those obtained by Youssef *et al.* (2023), who found that birds receiving MOS at diet dosages of 0.1 and 0.2 g/kg showed larger villi counts, heights, and crypt depth than birds fed a control diet. This study found that utilizing MOS improved intestine histomorphology features, which could increase feed utilization and performance.

Table (7): Ileum histometric parameters in broiler chickens supplemented with different levels of dietary MOS.

Traits	Control	MOS 0.25 g/kg	MOS 0.50 g/kg	MOS 1.0 g/kg	SEM	P-value
(VH) (µm)	5.98^{d}	7.96^{c}	10.33^{b}	11.68^{a}	0.27	0.001
(CD) (µm)	2.42^{a}	1.61^{b}	1.51^{b}	1.80^{b}	0.13	0.005
VH:CD	2.52^{c}	5.04^{b}	7.04^{a}	6.77^{a}	0.41	0.001

[a,b,c and d] Means within the same row with different common superscripts differ significantly. SEM: standard error of means. VH, villus height; CD, crypt depth; VH/CD, villus height/crypt depth

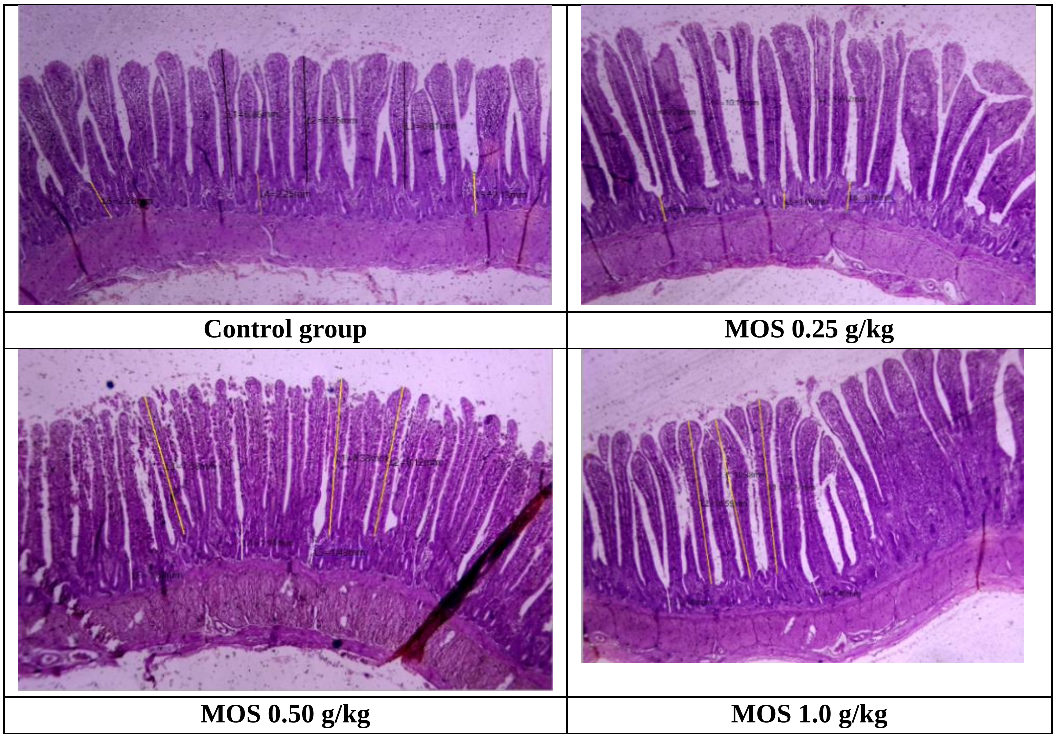

Fig (1): Transverse sections of the ileum in broiler chickens fed a basal diet supplemented with MOS at 0.25, 0.50 and 1.0 g/kg. x40. H&E.

CONCLUSION

Dietary supplementation with different levels of MOS had a positive effect on productive performance and improved the gut microbial and histological status.

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إضافة المانان أوليجوسكاريد لعلائق دجاج إنتاج اللحم لتحسين الأداء الإنتاجي وإنزيمات الكبد وتطور الأمعاء

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أجريت هذه التجربة بهدف دراسة تأثير إضافة المانان أوليجوسكاريد على الأداء الإنتاجي ،إنزيمات الكبد ،صفات الذبيحة، تطور الأمعاء وبعض صفات الدم في دجاج انتاج اللحم. تم تقسيم عدد 240 كتكوت تسمين عمر يوم من سلالة الروس 308 بالتساوي إلى أربع معاملات غذائية كل منها فى ثلاث مكررات على النحو التالي:مجموعة الكنترول : غُذيت على العليقة القاعدية بدون إضافات وثلاث مجموعات أخرى غُذيت على عليقة قاعدية مضافا إليها 0.25 و 0.5 و 1 جم / كجم علف من المانان أوليجوسكاريد على التوالي ،واستمرت التجربة لمدة 35 يومًا. تم تسجيل وزن الجسم الحى ، وزن الجسم المكتسب BWG والإستهلاك الغذائي FC ومعامل التحويل الغذائي FCR ومعامل الكفاءة الأوروبيEPEF. تم عمل مواصفات الذبيحة على عمر 35 يوم ، تم قياس مستويات البروتين الكلي والألبومين والدهون وإنزيمات الكبد في الدم، تم تحديد العدد الكلي للبكتيريا وعدد بكتيريا القولون وقياس التغيرات النسيجية في اللفائفي. أظهرت النتائج تحسن قيم BWG أو FC أو FCR و EPEFبشكل ملحوظ عند مستويات 0.5 و 1.0 جم/كجم مقارنة بمجموعة الكنترول والمستوى الاقل من الإضافة، كما لوحظ انخفاض معنوي في مستويات تركيز الدهون الثلاثية و ALTفي البلازما نتيجة إضافة المانان أوليجوسكاريد. تم تحسين صفات الذبيحة في المجموعات التجريبية مقارنة بمجموعة الكنترول. حدث انخفاض في عدد بكتيريا القولون في الأمعاء مع تحسين في تطور الشكل النسيجي للأمعاء.

بشكل عام، كان لإضافة المانان أوليجوسكاريد بمستويات مختلفة تأثير إيجابي على الأداء الإنتاجي وتحسين الحالة الميكروبية والنسيجية للأمعاء.