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Research Article

Effects of Chitosan Oligosaccharides Addition to Japanese Quail's Diets on Growth, Carcass Traits, Liver and Intestinal Histology, and Intestinal Microflora

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Abstract

This research was conducted to determine effects of chitosan oligosaccharides (COS) addition to quail diets on growth, carcass traits, liver and intestinal histology, and intestinal microflora. Two hundred forty Japanese quail chicks were distributed among three treatments groups, with four replicates. A group was fed with a basal starter diet for 1-21th and a grower diet for 22-42th days (Control). The experimental groups were fed the same diets, in addition to 75 mg/kg (Trial I) or 150 mg/kg (Trial II) of COS. The final live weights of the quails in the Control and Trial I groups were higher than in the Trial II group. There were no differences among the groups in gain, feed intake, feed conversion, and carcass traits. Steatosis in the Trial II group was less than in the Control and Trial I groups. Crypt depth and villus length were higher in the Trial II group than in the other groups. The number of bacteria and yeast in the intestine were lower in the Trial I and II groups than in the Control group. In conclusion, the addition of 75 mg/kg of COS had no adverse effect on the tested parameters, and it increased the crypt depth, villus length, and beneficially on intestinal microflora.

Keywords: *Quail, Chitosan oligosaccharides, Growth performance, Villus, Steatosis, Intestinal microflora*

Bıldırcın Rasyonlarına Kitosan Oligosakkarit İlavesinin Besi Performansı, Karkas Özellikleri, Karaciğer ve Barsak Histolojisi ile Barsak Mikroflorası Üzerine Etkisi

Özet

Bu çalışma, bıldırcın rasyonlarına farklı oranlarda kitosan oligosakkarit (KOS) ilavesinin besi performansı, karkas verim özellikleri, karaciğer ve barsak histolojisi ile barsak mikroflorası üzerine etkilerini belirlemek amacıyla yapıldı. Araştırmada 240 adet Japon bıldırcını kullanıldı. Civcivler herbiri dört alt gruptan oluşan üç ana gruba ayrıldı, Kontrol grubu temel başlangıç (1-21. gün) ve büyütme (22-42. gün) yemleriyle beslendi. Deneme grupları araştırma süresince bu yemlere 75 mg/kg (Deneme I) veya 150 mg/kg (Deneme II) KOS ilave edilerek beslendi. Araştırma sonu itibariyle Kontrol ve Deneme I grubunun canlı ağırlığı Deneme II'den önemli derecede yüksek bulundu. Canlı ağırlık artışı, yem tüketimi, yemden yararlanma oranı ve karkas verim özellikleri bakımından gruplar arasında farklılık görülmedi. Deneme II grubundaki bıldırcınların karaciğerindeki yağlanmanın Kontrol ve Deneme I'den daha az olduğu gözlendi. Kript derinliği ve villus uzunluğu Deneme II grubunda diğer gruplardan önemli derecede yüksek bulundu. İnce barsaklardaki bakteri ve maya sayıları Deneme I ve II gruplarında Kontrol grubundan daha düşük bulundu. Sonuç olarak, bıldırcın rasyonlarına 75 mg/kg kitosan oligosakkarit ilavesinin incelenen parametreler üzerinde olumsuz bir etki oluşturmadığı, kript derinliğini ve villus yüksekliğini artırdığı, ince barsak mikroflorası üzerinde faydalı olduğu tespit edilmiştir.

Anahtar sözcükler: *Bıldırcın, Kitosan oligosakkarit, Besi performansı, Villus, Steatosis barsak mikroflorası*

INTRODUCTION

The use of antibiotics as feed additives, caused residues in animal tissues, and by this resulted in a decrease in the effectiveness of antibiotics in therapy. The using of antibiotics as feed additive was banned in the European Union in 2006 [1]. Since the ban, various oligosaccharides, which are natural feed additives, also called prebiotics,

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have been used in poultry feed to support growth, improve gut microbial flora, and strengthen the immune system [2-4]. Prebiotics are defined as non-digestible food ingredients that have a beneficial effect on the host animal by selectively stimulating the growth or activity, or both, one or more types of bacteria in the colon [5].

Chitosan and chitosan oligosaccharides (COS), which are recognized as prebiotics, are obtained from chitin via chemical and enzymatic hydrolysis. Chitin, a cellulose-like polymer, is found in the exoskeletons of arthropods, such as crabs, shrimps, lobsters, and insects [6]. Due to the high molecular weight, insolubility, allergic, and high viscosity of chitin, its usefulness as a natural feed additive is limited [7]. Chitosan, especially COS, has a lower molecular weight, higher solubility, and lower viscosity than chitin [8].

Studies conducted in different animals have shown that COS improves growth performance and that it has hypocholesterolemic, antidiabetic, antitumoral, antifungal, antioxidant, and immune strengthening functions [9], as well as free radical scavenging properties [10]. The addition of COS to broiler chicken diets improved their live weight, feed intake [11], and feed conversion [3]. However, Keser et al.[12] reported that the addition of COS to broiler chicken diets had no effects on their growth. Zhou et al.[11] found that the addition of this additive to broiler chicken diets had no impact on breast meat and gizzard weight but increased the liver weight.

Other studies reported that undigested oligosaccharides in the intestinal tract promoted beneficial microorganisms and increased both the villus length and crypt width in the digestive system of broiler chickens [13,14].

Chitosan and COS have antimicrobial effects on bacteria, yeast, and fungi. Chitosan and its oligomers have a stronger bactericidal effect on gram (+) bacteria. The effect of chitosan depends on its origin, molecular weight, and pH [14]. Previous studies of the effects of oligosaccharides on gut microbial flora of poultry demonstrated that they reduced colonization by *Salmonella* and *Escherichia coli* [13,15].

The present study was conducted to determine the effects of the addition of different levels of COS to quail diets on growth performance, carcass traits, liver and intestinal histology, and intestinal microflora.

MATERIAL and METHODS

Experimental Animals, Experimental Design, Diets, Management

The ethical committee approval of Kafkas University (KAÜ-HADYEK: 2014-028) was taken in order to conduct this study.

Two hundred forty 1-d-old unsexed Japanese quail chicks *(Coturnix coturnix japonica)* were used in this study. They were randomly divided into three main groups with four replicates of 20 chicks each. The study lasted for 6 weeks, with the first 3 weeks as the starter period and the last 3 weeks as the grower period. In the starter and grower periods, the birds were fed a basal diet *(Table 1)* as recommended by the NRC [16]. One of the main groups was fed these basic diets (Control), and the other groups were fed the same diets, but 75 mg/kg of COS (Trial I) or 150 mg/kg of COS (Trial II) added their diets. The COS (GlycoBio Company, Dalian, China) used in this study contained 40% COS and 60% cyclodextrine as a carrier. The birds were housed in wire cages, and feed and water were available at all times during the experimental period.

Data Collection

Live weights of quails and also feed intake of each group were recorded weekly. The feed conversion was also calculated on a group basis. At the end of the experiment, 16 quails from each group (two males and two females

Table 1. *Composition and nutrient content of the diets used in the study, %*
Tablo 1. *Araştırmada kullanılan karma yemlerin bileşimi ve besin madde içerikleri, %*

Ingredients	Starter Diet 1 to 21th days	Grower Diet 22 to 42th days
Corn	30.75	42.50
Soy bean meal	21.45	24.00
Sunflower meal	10.00	10.00
Wheat	20.00	18.25
Full fat soybean	11.00	-
Vegetable oil	3.50	2.00
DCP	1.60	1.50
Lime stone	0.66	1.00
Vit. Min. prem.*	0.35	0.35
Colin chloride	0.10	-
Salt	0.25	0.30
Methionine	0.20	0.10
Lysine	0.06	-
Threonine	0.06	-
Sodium bicarbonate	0.02	-
Nutritional content, DM basis		
Dry matter	92.20	91.08
Metabolic energy, kcal/kg**	3019	2910
Crude protein	22.25	20.22
Crude fat	7.91	4.65
Crude fibre	5.34	4.99
Crude ash	8.28	7.08

** **Per 2,5 kg containing following nutrients:** Vit A: 6.000.000 IU, Vit D_3: 32.000.000 IU, Vit E: 40.000 mg, Vit K_3: 1.600 mg, Vit B_1: 1.200 mg, Vit B_2: 3.200 mg, Niacin: 24.000 mg, Cal.D-Pantothenate: 7.200 mg, Vit B_6: 2.000 mg, Vit B_{12}: 6,4 mg, D-Biotin: 80 mg, Folic acid: 800 mg, Vit C: 40.000 mg, Manganese: 42.000 mg, Iron: 33.600 mg, Zinc: 33.600 mg, Copper: 3.600 mg, Cobalt: 80 mg, Iodine: 400 mg, Selenium: 72 mg, Molybdenum: 416 mg; ** Provided by calculation [17]*

from each subgroup) were slaughtered for determination of carcass traits. The carcasses were cut into parts using a method described previously [17].

Analyses of Feed Contents

The composition of dry matter, crude protein, crude fiber, crude fat, and crude ash of the feeds used in the experiment were determined according to the procedure in AOAC [18].

Histological Analyses

Liver and ileum tissue samples were also taken from the slaughtered quails. Cryostat (Leica CM 1510 S, USA) sections (5 µm thick) were taken to determine the amount of fat in the hepatocytes. The tissue sections were then placed onto glass slides and stained with Oil Red O. Additionally, liver and ileum samples were fixed in 10% neutral formalin solution. After routine tissue examinations, sections 5 µm thick were stained with Crossman's triple stain and hematoxylin & eosin (H & E) [19]. The stained sections were then examined and photographed under light microscopy (Olympus BX-051, Japan). The crypt depth, villus length, and villus width in the ileum were measured.

Microbiological Analysis

The contents of the small intestine of each bird were placed in a sterile flask. They were transferred to the laboratory and stored at +4°C thermal conditions. Cultures were prepared immediately. The intestinal contents were serially diluted to tenfold diagram using anaerobic dilution solution and phosphate buffer solution for enumeration of anaerobic and aerobic bacterial populations, respectively [20]. From the prepared intestinal content, 0.1 ml was inoculated in suitable medium and then incubated in aerobic and anaerobic conditions. The bacteria and fungi were identified according to the morphology of the colonies and microscopic analysis, in addition to the characteristics of the gram stain, spotting, motility, and biochemical activities.

Statistical analysis

A one-way ANOVA was used for the data analysis. Statistical significance among the groups was determined with Duncan's Multiple Range test [21]. The results are given as the average ± standard error (X ± Sx).

RESULTS

The live weight of the quails in the Trial II group was lower than in the Control and Trial I groups at the end of the study (P<0.01). Average daily gain, feed intake, and food conversion did not differ among the groups during the starter (day 1 to day 21), grower (day 22 to day 42), and overall experiment periods (day 1 to day 42) *(Table 2)*.

Table 2. *Effects of dietary chitosan oligosaccharides addition to the diet on the performance of quails*
Tablo 2. *Bıldırcın rasyonlarına kitosan oligosakkarit ilavesinin performans üzerine etkileri*

Weeks/Periods/Item	Control	Trial I	Trial II	Significance
	Live weight, g			
Hatching	8.30±0.04	8.34±0.02	8.33±0.02	NS
1	26.13±0.25a	24.85±0.23b	26.16±0.28a	**
2	54.05±0.58a	48.35±0.75b	53.06±1.08a	**
3	99.55±0.74a	94.41±0.73b	97.00±1.36ab	*
4	140.39±0.64a	133.66±0.82b	135.81±1.14b	**
5	167.80±0.81b	174.39±1.67a	171.05±0.74ab	**
6	206.23±1.21a	203.42±1.19a	195.12±1.05b	**
Starter period (from 1st to 21st day)				
Average daily gain, g	4.35±1.15	4.10±1.27	4.22±1.09	NS
Average feed intake, g	9.74±3.63	10.26±3.99	10.34±3.81	NS
Feed conversion, g feed/g gain	2.10±0.30	2.37±0.34	2.30±0.32	NS
Grower period (from 22nd to 42nd day)				
Average daily gain, g	5.08±0.59	5.19±0.53	4.67 0.63	NS
Average feed intake, g	23.11±2.93	24.28±2.01	23.75±2.87	NS
Feed conversion, g/g	4.74±0.92	4.86±0.93	5.59±1.60	NS
Overall experiment (from 1st to 42nd day)				
Average daily gain, g	4.71±0.60	4.65±0.66	4.45±0.57	NS
Average feed intake, g	16.42±3.64	17.27±3.71	17.04±3.68	NS
Feed conversion, g/g	3.42±0.73	3.62±0.71	3.94±1.03	NS

NS: *No significant;* ***a, b:*** *Values in the same row with a different letter are significantly different (* P<0.05, ** P<0.01)*

Table 3. *Effects of chitosan oligosaccharides on slaughter and carcass traits of quails*
Tablo 3. *Kitosan oligosakkarit ilavesinin bıldırcınların kesim ve karkas özelliklerine etkisi*

Item	Control	Trial I	Trial II	Significance
Slaughter weight, g	178.10±4.39	175.22±6.97	181.27±6.12	NS
Cold carcass weight, g	122.12±2.36	117.84±4.38	119.91±3.42	NS
Cold carcass percentage, %	68.73±0.65	67.44±0.85	66.42±0.91	NS
Leg percentage, %	23.69±0.32	23.61±0.33	23.99±0.35	NS
Breast percentage, %	38.28±0.76	38.58±0.68	38.80±0.43	NS
Wing percentage, %	8.91±0.18	9.34±0.20	8.69±0.20	NS
Heart percentage, %	1.43±0.04	1.36±0.05	1.39±0.04	NS
Liver percentage, %	3.68±0.28	3.70±0.32	3.67±0.27	NS
Gizzard percentage, %	3.15±0.20	3.28±0.17	3.39±0.15	NS

NS: *No significant*

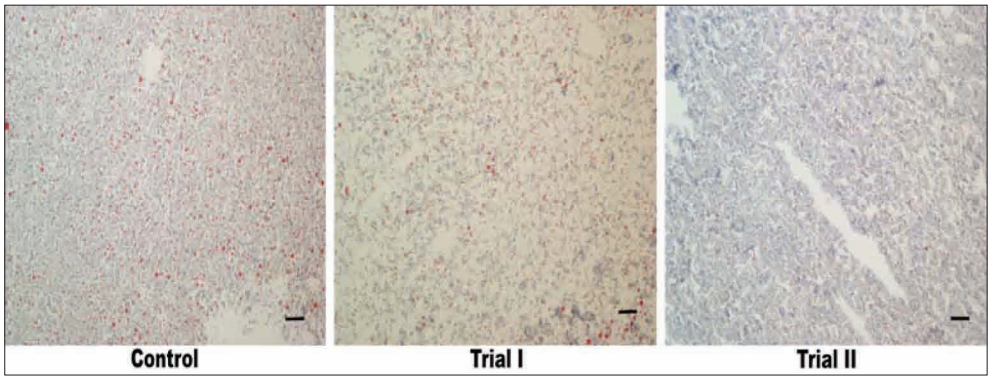

Fig 1. Microscopic aspect of liver in experimental groups. Oil Red O. Scale bar: 100 µm

Şekil 1. Araştırma gruplarındaki karaciğerin mikroskopik görünümü Oil Red O. Bar: 100 µm

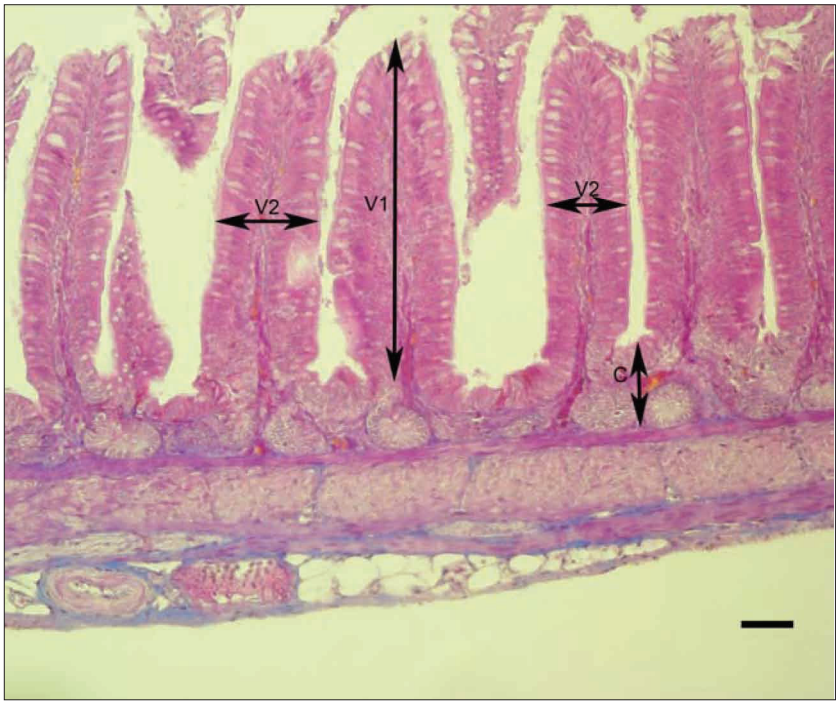

Fig 2. Ileal villus length, villus width and crypt depth in the Control group. V1: Villus length, V2: Villus width, C: Crypt depth, Triple stain, Bar: 100 µm (Only measurement of the control is given, due to the other groups' measurement similar to the control)

Şekil 2. Kontrol grubunun ileum villus uzunluğu, villus genişliği ve kript derinliği. V1: Villus uzunluğu, V2: Villus genişliği, C: Kript derinliği, Üçlü boyama, Bar: 100 µm (Diğer grup ölçümleri Kontrole benzediği için sadece Kontrol grubu ölçümleri verilmiştir)

Effects of COS addition to the diets on the slaughter and carcass traits is shown in *Table 3*. As seen in *Table 3*, the slaughter and carcass traits did not differ among the groups.

In the histological examination of the liver, steatosis was observed in all the groups. Steatosis was more common in the Control and Trial I groups, and lipid deposition was higher than in the Trial II group *(Fig. 1)*.

Table 4. *Ileal crypt depth, villus length and villus width in the experimental groups, µm*
Tablo 4. *Gruplara ait ileumdaki kript derinliği, villus uzunluğu ve villus genişlikleri, µm*

Item	Control	Trial I	Trial II	Significance
Crypt depth	55.70±0.60^{b}	57.22±0.58^{b}	69.40±0.65^{a}	***
Villus length	317±3.88^{b}	312±3.67^{b}	340±5.62^{a}	***
Villus width	81.80±1.04	79.90±1.04	78.16±1.20	NS

NS: *No significant;* ***a, b:*** *Values in the same row with a different letter are significantly different (*** P<0.001)*

Table 5. *Distribution of intestinal microbial flora agents in the experimental groups, CFU/ml*
Tablo 5. *Gruplardan elde edilen ince bağırsak mikroflora etkenlerinin dağılımı*

Bacteria		Control	Trial I	Trial II	Significance
Gram (-)	*Escherichia coli*	4x10^{6}±3.28a	3.6x10^{6}±4.50a	2.3x10^{6}±2.48b	***
	Pseudomonas aeruginosa	5.4x10^{5}±1.15	5.4x10^{5}±1.09	3.6x10^{5}±0.36	NS
	Fusobacterium spp.	3.6x10^{5}±0.16a	3.6x10^{5}±0.26a	1.8x10^{5}±0.00b	***
Gram (+)	*Lactobacillus* spp.	3.6x10^{5}±0.65a	1.8x10^{5}±0.38b	0.9x10^{5}±0.00b	***
	Staphylococcus spp.	2.2x10^{6}±1.21a	2.0x10^{6}±1.55a	7.6x10^{5}±0.40b	***
	Bacillus spp.	2.0x10^{6}±1.56a	1.2x10^{6}±1.08b	8.1x10^{5}±0.85c	***
	Clostridium spp.	9.0x10^{5}±0.76a	6.3x10^{5}±0.67b	6.3x10^{5}±0.62b	**
	Streptococcus spp.	1.7x10^{6}±2.62a	8.1x10^{5}±0.83b	5.6x10^{5}±1.05b	***
	Enterococcus faecalis	1.0x10^{6}±1.41a	8.1x10^{5}±0.54a	5.4x10^{5}±0.5b	***
Yeast	*Candida* spp.	9.0x10^{5}±1.34a	2.7x10^{5}±0.33b	1.8x10^{5}±0.35b	***

NS: *Non significant;* ***a, b:*** *Values in the same row with a different letter are significantly different (** P<0.01, *** P<0.001)*

The histological structure of the ileum was similar among the groups. The crypt depth and villus length were significantly lower in the Control and Trial I groups (P<0.01) than in the Trial II group *(Table 4)*. There was no statistical difference in the villus width among the groups *(Table 4, Fig. 2)*.

The intestinal microorganisms isolated from the intestinal contents and the average amounts are given in *Table 5*. Ten different microorganisms were isolated from the intestinal contents: six gram (+) bacteria, three gram (-) bacteria, and one *Candida* yeast. The concentrations of *Enterococcus faecalis*, *Fusarium* spp., *E. coli,* and *Staphylococcus* spp. were significantly lower in the Trial II group than in the Control and Trial I groups (*P*<0.001). The concentrations of *Clostridium spp.*, *Streptococcus spp.*, *Lactobacillus* spp., *Bacillus* ssp., and *Candida* spp. were significantly lower in the Trial I and Trial II groups than in the Control group. There were no statistical differences in the concentration of *Pseudomonas aeruginosa* among the groups.

DISCUSSION

At the end of the starter period (21st day), the live weight of the quails was significantly higher in the Control group than in the Trial I group *(Table 2)*. This result is in accordance with the results of Razdan and Petterson [22]. The live weight of the quails in the Control and Trial I groups was higher than in the Trial II group at the end of the grower period (42th day). Previous studies reported that different amounts of COS added to the diet did not affect the live weight of broiler chickens [12,23]. Contrary to these results, other researchers found that the addition of COS to the diet increased the live weight of broiler chickens [4,11]. The average daily gain, feed intake, and feed conversion did not differ among the groups either in the starter and grower periods or in the entire experimental period *(Table 2)*. Similarly, other studies reported that the addition of COS to the diet did not change the daily gain of broiler chickens [2,12,23]. However, Li et al.[3] found that the addition of COS to the diet positively affected the daily gain of broiler chickens. The lack of change in the daily gain, feed intake, and feed conversion in this study might be related to the low viscosity and low molecular weight of the COS that was used. Discrepancies in the growth performance parameters between this research and other studies could be associated with the different molecular weights, deacetylation degrees, or doses of the chitosan or COS used in these experiments.

As seen in *Table 3*, slaughter and carcass traits did not differ among the groups. Our results indicated that the addition of 75 or 150 mg/kg of COS to the quail diets had neither a positive nor a negative effect on carcass traits. However, Tufan and Arslan [23] reported that 50 or 100 mg/kg of COS added to the diet of broiler chickens increased

the carcass ratio, leg and wing ratio, but not the breast, heart and gizzard ratio, and decreased the liver ratio. Zhou et al.[11] found that the addition of 14 or 28 g/kg of COS to broiler chicken diets enhanced the liver weight but did not change the breast meat ratio.

Liver steatosis was lower in the Trial II group than in the Control and Trial I groups *(Fig. 1)*. The lower fat accumulation in the Trial II group might be related to the higher level of COS added to the diet and the low fat digestibility and fat micelle binding properties of chitosan/COS. In support of this view, Razdan and Petterson [22] found that raw fat digestibility was decreased (26%) in broiler chickens fed a diet containing chitosan. Razdan et al.[24] reported that feeding chitosan reduced the concentration of bile acid in the small intestine and the total plasma cholesterol concentration. They also reported that chitosan had hypolipidemic potencies. Additionally, Genc et al.[25] established that the addition of mannan-oligosaccharide to the diets of fish reduced the accumulation of liver fat.

In this study, the crypt depth and villus length were greater in the Trial II group than in the Control and Trial I groups, which is in accordance with the results of Liu et al.[26]. The exact mode of action of COS supplementation in the diet on intestinal microflora is unknown. However, COS can selectively stimulate the growth of beneficial microorganisms, such as Lactobacillus and Bifidobacterium, thereby potentially inhibiting the growth of putrefactive and pathogenic bacteria [5]. A likely explanation for the increased villus height and crypt width could be the reduced number of pathogenic microorganisms in the small intestine following COS supplementation. In support of this idea, Mourao et al.[27] reported that COS supplementation to pigs reduced numbers of pathogen microorganisms and consequently enhanced the villus length. However, Baurhoo et al.[28] reported that the addition of mannan-oligosaccharide to broiler chicken diets had no effect on crypt depth.

In the present study, the addition of COS to the diet seemed to reduce the concentration of bacteria and *Candida* yeast *(Table 5)*. No et al.[29] and Simunek et al.[30] reported similar results. The dietary addition of 75 or 150 mg/kg of COS decreased the concentrations of intestinal pathogen microorganisms *(E. coli*, *Clostridium* spp., and *Staphylococcus* spp.), and this effect was more apparent in the group supplemented with 150 mg/kg of COS. Li et al.[3] established that the addition of 100 mg/kg of COS to broiler chicken diets reduced the cecal *E. coli* concentration. Other researchers [4,13,15] found similar results.

In conclusion, 75 mg/kg addition of COS did not change the final live weight of quails, but the addition of 150 mg/kg of COS decreased the final live weight. Overall, the results of the experiment suggest that dietary COS addition at both doses does not affect the average live weight gain, feed intake, feed conversion, or composition of the carcass traits. Given the increased ileal crypt depth and villus length and the positive effects on intestinal microflora, it conclude that 75 mg/kg of COS can be added to the diets of quails.

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