Effects of Dietary Chitosan Oligosaccharide Supplementation on Meat Quality, Antioxidant Capacity, Small Intestinal Mucosal Structure, and Intestinal Microbiota in Broiler Chickens: Postprint

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Abstract

This study aimed to investigate the effects of dietary chitosan oligosaccharide (COS) supplementation on meat quality, antioxidant capacity, small intestinal mucosal architecture, and intestinal microbiota in broiler chickens. Three hundred healthy 1-day-old Arbor Acres (AA) male broilers were randomly assigned to 5 groups with 6 replicates per group and 10 birds per replicate. The blank control group received a basal diet without antibiotics, the positive control group received a basal diet supplemented with 100 mg/kg chlortetracycline, and the experimental groups received basal diets supplemented with 50, 100, or 150 mg/kg COS, respectively. The trial lasted 42 days. The results demonstrated that compared with the blank control group: 1) dietary supplementation with 100 mg/kg COS significantly increased the redness (a*) values of breast and thigh muscles ($P<0.05$). 2) dietary supplementation with 50 and 100 mg/kg COS significantly enhanced serum total superoxide dismutase (T-SOD) activity at 1-21 days of age ($P<0.05$); dietary supplementation with 100 mg/kg COS significantly elevated serum total antioxidant capacity (T-AOC) and activities of T-SOD and glutathione peroxidase (GSH-Px) at 22-42 days of age ($P<0.05$); and dietary supplementation with 50 mg/kg COS significantly increased serum T-SOD and GSH-Px activities at 22-42 days of age ($P<0.05$). 3) dietary supplementation with 100 mg/kg COS significantly increased jejunal villus height and villus height/crypt depth ratio at both 1-21 and 22-42 days of age ($P<0.05$). 4) dietary supplementation with 50 and 100 mg/kg COS significantly reduced ileal Escherichia coli populations ($P<0.05$). 5) dietary supplementation with 100 mg/kg COS significantly improved average daily gain of broiler chickens ($P<0.05$). In conclusion, dietary COS supplementation can enhance antioxidant capacity, improve meat quality and intestinal mucosal architecture, and

modulate intestinal microbiota structure in broiler chickens. The optimal supplementation level of COS in broiler diets is 100 mg/kg.

Full Text

Effects of Dietary Chitosan Oligosaccharide Supplementation on Meat Quality, Antioxidant Activity, Intestinal Mucosal Structure and Intestinal Flora of Broilers

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Abstract: This experiment was conducted to investigate the effects of dietary chitosan oligosaccharide (COS) supplementation on meat quality, antioxidant activity, intestinal mucosal structure, and intestinal flora of broilers. A total of 300 one-day-old healthy Arbor Acres (AA) male broilers were randomly allocated to 5 groups with 6 replicates per group and 10 birds per replicate. The blank control group was fed a basal diet without antibiotics, the positive control group was fed the basal diet supplemented with 100 mg/kg chlortetracycline, and the experimental groups were fed the basal diet supplemented with 50, 100, and 150 mg/kg COS, respectively. The experiment lasted for 42 days. The results showed that, compared with the blank control group: (1) dietary supplementation with 100 mg/kg COS significantly increased the redness (a*) value of both breast and thigh muscle ($P < 0.05$); (2) dietary supplementation with 50 and 100 mg/kg COS significantly increased serum total superoxide dismutase (T-SOD) activity during days 1–21 ($P < 0.05$), dietary supplementation with 100 mg/kg COS significantly increased serum total antioxidant capacity (T-AOC) and T-SOD and glutathione peroxidase (GSH-Px) activities during days 22–42 ($P < 0.05$), and dietary supplementation with 50 mg/kg COS significantly increased serum T-SOD and GSH-Px activities during days 22–42 ($P < 0.05$); (3) dietary supplementation with 100 mg/kg COS significantly increased jejunal villus height and villus height/crypt depth during both days 1–21 and days 22–42 ($P < 0.05$); (4) dietary supplementation with 50 and 100 mg/kg COS significantly decreased ileal* Escherichia coli* counts ($P < 0.05$); and (5) dietary supplementation with 100 mg/kg COS significantly increased average daily gain of broilers ($P < 0.05$). It can be concluded that dietary COS supplementation can enhance antioxidant activity, improve meat quality and intestinal mucosal structure, and improve intestinal flora structure to a certain extent in broilers. The optimum supplemental level of COS in broiler diets is 100 mg/kg.

Keywords: chitosan oligosaccharide; broilers; antioxidation; intestinal mucosal structure; intestinal flora

Introduction

Since the early 1950s, antibiotics have played an important role as a novel feed additive in disease prevention and control, animal growth promotion, and feed conversion improvement [1]. However, long-term use of antibiotics in modern animal production has led to numerous negative issues such as resistance transfer, drug residues, and food safety concerns. Developing new feed additives that are pollution-free, residue-free, non-resistance-inducing, and environmentally friendly has become an inevitable trend in today' s animal production industry [2]. Chitosan oligosaccharide (COS) is a type of oligosaccharide and the only alkaline amino-oligosaccharide that exists abundantly in nature. It is a low-molecular-weight product obtained from enzymatic degradation of chitosan, chemically named -(1,4)-2-amino-2-deoxy-D-glucose [3]. Studies have shown that COS possesses multiple biological activities including antibacterial, antioxidant, and immune-enhancing effects, as well as the ability to regulate acid-base balance in the body. It can improve animal growth performance, regulate intestinal microecology, improve intestinal histomorphology, lower blood pressure and lipids, enhance animal immune function, and improve animal product quality [4-5]. Previous reports have extensively studied COS in broiler growth and immune performance, but research on dietary COS supplementation effects on broiler intestinal flora and antioxidant activity remains limited [6-9]. This experiment aimed to investigate the effects of dietary COS supplementation on broiler meat quality, antioxidant activity, intestinal mucosal structure, and intestinal flora, evaluate its effectiveness in broiler diets, and determine the appropriate supplemental level of COS, thereby providing a theoretical basis for guiding the rational application of COS in broiler production.

1.1 Experimental Materials and Animals

COS (purity 85%) was purchased from Leading Bio-Agri Co., Ltd. Experimental animals were Arbor Acres (AA) broilers purchased from Beijing Huadu Broiler Company.

1.2 Experimental Design and Management

This experiment adopted a single-factor completely randomized design. A total of 300 healthy AA male broilers (one-day-old) were randomly divided into 5 groups with 6 replicates per group and 10 birds per replicate, with no significant difference in body weight among replicates ($P > 0.05$). The blank control group was fed a basal diet without antibiotics, the positive control group was fed the basal diet supplemented with 100 mg/kg chlortetracycline, and the experimental groups were fed the basal diet supplemented with 50, 100, and 150

mg/kg COS, respectively. Throughout the experiment, to ensure dietary nutrient levels remained consistent across COS gradient levels, all ingredients used in diet formulation were from the same batch. The experimental period lasted 42 days. The basal diet was formulated according to nutrient levels recommended in the *Arbor Acres Broiler Management Manual* and provided as pellets. The composition and nutrient levels of the basal diet are shown in Table 1 .

The experiment was conducted at the Nankou Pilot Base of the Chinese Academy of Agricultural Sciences. A three-tier cage system was used. Lighting was provided 24 h/day during days 1–7, then reduced to 23 h/day from day 8 onward. House temperature was maintained at 33 °C during days 1–3, then gradually decreased to and maintained at 25 °C from day 4 onward. Birds had free access to feed and water throughout the experimental period. Routine preventive immunization was performed according to standard procedures. Daily observations were made of bird mental state, appetite, and fecal condition, with mortality recorded.

1.3.1 Meat Quality Measurements

pH: A Testo205 portable pH meter was used to measure pH of the left breast and thigh muscle at 45 min post-slaughter. A small incision was made with a scalpel, and the electrode was fully inserted into the muscle. Three different locations were measured on the breast and thigh muscle of each bird, with consistent measurement sites maintained across samples.

Meat Color: At 45 min post-slaughter, a CR-400 automatic colorimeter was used to measure lightness (L*), redness (a*), and yellowness (b*) values of the right breast and thigh muscle. Each sample was measured twice at the same location (rotated 90 degrees between measurements), and the average value was recorded.

Drip Loss: After color measurement, approximately 20 g of right breast and thigh muscle (approximately 5 cm long and 2 cm wide, recorded as W1) was weighed and placed in a self-sealing bag. The bag was inflated with nitrogen to minimize contact between the meat sample and the inner wall of the bag, then suspended in a 4 °C refrigerator. After 24 h, the meat sample was removed and surface juice was gently blotted with pre-prepared filter paper, then the sample was reweighed (recorded as W2). Drip loss was calculated as: Drip loss (%) = 100 × (W1 - W2)/W1.

Cooking Loss: The meat sample used for drip loss measurement was placed back in the self-sealing bag and returned to the 4 °C refrigerator. At 72 h, the sample was removed, surface moisture was gently blotted with pre-prepared filter paper, and the sample was weighed (recorded as W1). The sample was then placed in a self-sealing bag, air was evacuated, and the bag was immersed in an 80 °C water bath for 30 min. The sample was removed from the bag, placed on filter paper for 30 min, cooled to room temperature, then surface moisture was blotted with filter paper and the sample was weighed again (recorded as W2).

Cooking loss was calculated as: Cooking loss (%) = 100 × (W1 - W2)/W1.

1.3.2 Antioxidant Activity Measurements

On days 21 and 42 of the experiment, one bird was randomly selected from each replicate, and 10 mL of blood was collected from the jugular vein into a disposable vacuum tube (containing separation gel and coagulation promoter). After standing at 37 °C for 1 h, serum was prepared by centrifugation at 3,500 r/min for 10 min and used for antioxidant activity measurements. Serum total superoxide dismutase (T-SOD) activity was determined by the xanthine oxidase method, malondialdehyde (MDA) content and total antioxidant capacity (T-AOC) were determined by the thiobarbituric acid method, and glutathione peroxidase (GSH-Px) activity was determined by colorimetric method. All assay kits were purchased from Nanjing Jiancheng Bioengineering Institute and used according to the manufacturer' s instructions.

1.3.3 Intestinal Mucosal Structure Analysis

On the morning of days 21 and 42 before feeding, one bird from each replicate (6 birds per group) was selected and euthanized by jugular vein exsanguination. The abdominal cavity was quickly opened, the jejunum was isolated, and a 2–3 cm segment was rapidly excised, rinsed in physiological saline to remove contents, then fixed in 10% formalin solution. Fixed specimens were processed through water washing, dehydration, and transparency steps, then embedded in paraffin. After cooling and solidification, continuous sections of 5 m thickness were prepared, stained with hematoxylin-eosin, and sealed with resin. Typical fields were selected from paraffin sections, and villous height (V) and crypt depth (C) of the jejunum were measured using a fluorescence inverted microscope and microscopic image analysis system to calculate villous height/crypt depth (V/C). Specific procedures followed the methodology described by Zhou Zhaoying [10].

1.3.4 Intestinal Flora Enumeration

On the morning of day 42 before feeding, one bird was randomly selected from each replicate (6 birds per group), euthanized by jugular vein exsanguination. The abdominal cavity was quickly opened, both ends of the ileum were ligated and cut, the ligated ends were disinfected with alcohol cotton balls, and the segment was placed in a sterilized sample bag with ice packs for cooling. Intestinal flora enumeration was performed within 4 h.

In a laminar flow hood, 0.5 g of ileal content was transferred to a test tube containing 4.5 mL of sterile physiological saline, vortexed for 3–5 min until homogeneous to obtain a 10^{1} dilution. Then 0.5 mL of this diluted bacterial suspension was transferred to an EP tube containing 4.5 mL of sterile physiological saline, vortexed to obtain a 10^{2} dilution. This process was repeated to prepare serial dilutions of 10^{3}, $10^{\ }$, $10^{\ }$, $10^{\ }$, and $10^{\ }$. Three appropriate dilutions were then selected and plated on specific selective media for enumeration

of *Escherichia coli*, *Lactobacillus*, and *Bifidobacterium* colonies after incubation for the appropriate duration. Each dilution was plated in triplicate. Culture conditions are shown in Table 2 .

The colony count was calculated using the formula [11]:
Number of colonies per gram sample = log (average colony count at the same dilution × dilution factor / sample weight in grams).

1.3.5 Growth Performance Measurements

Birds were weighed on days 1 and 42 of the experiment, feed intake was recorded, and average daily gain (ADG), average daily feed intake (ADFI), and feed-to-gain ratio (F/G) were calculated.

1.4 Statistical Analysis

Experimental data were analyzed using SPSS 19.0 software for one-way ANOVA. When significant differences were detected, Duncan' s multiple comparison test was performed. $P < 0.05$ was considered statistically significant, and $0.05 < P < 0.10$ was considered a tendency. Results are expressed as "mean ± standard deviation."

Results

2.1 Effects of Dietary COS on Broiler Meat Quality

As shown in Table 3 , dietary COS supplementation significantly affected the a* value of both breast and thigh muscle ($P < 0.05$). The 50 and 100 mg/kg COS groups showed higher a* values in breast and thigh muscle compared with the blank control group, with the 100 mg/kg COS group being significantly higher than the blank control group ($P < 0.05$). Dietary COS supplementation had no significant effects on pH, L* value, b* value, drip loss, or cooking loss of breast and thigh muscle ($P > 0.05$), but showed a tendency to decrease drip loss in thigh muscle ($0.05 < P < 0.10$).

2.2 Effects of Dietary COS on Serum Antioxidant Activity of Broilers

As shown in Table 4 , during days 1–21, dietary COS supplementation significantly affected serum T-SOD activity ($P < 0.05$). The 50 and 100 mg/kg COS groups showed significantly higher serum T-SOD activity than the blank control group ($P < 0.05$), while the 150 mg/kg COS group showed no significant difference from the blank control group ($P > 0.05$). Dietary COS supplementation had no significant effects on serum MDA content, T-AOC, or GSH-Px activity ($P > 0.05$). During days 22–42, dietary COS supplementation had no significant effect on serum MDA content ($P > 0.05$), but significantly affected serum T-AOC and GSH-Px and T-SOD activities ($P < 0.05$). The 100 mg/kg COS group showed significantly higher serum T-AOC and T-SOD and GSH-Px activities than the blank control group ($P < 0.05$). The 50 mg/kg COS group

showed significantly higher serum T-SOD and GSH-Px activities than the blank control group ($P < 0.05$), while the 150 mg/kg COS group showed no significant differences in serum T-AOC and T-SOD and GSH-Px activities compared with the blank control group ($P > 0.05$).

2.3 Effects of Dietary COS on Jejunal Mucosal Structure of Broilers

As shown in Table 5 , during days 1-21, dietary COS supplementation significantly affected jejunal villous height, crypt depth, and villous height/crypt depth ($P < 0.05$). The 100 and 150 mg/kg COS groups showed significantly higher jejunal villous height than the blank control group ($P < 0.05$), but showed no significant difference compared with the positive control group ($P > 0.05$). The 100 mg/kg COS group and positive control group showed significantly lower jejunal crypt depth than the blank control group ($P < 0.05$). The 100 mg/kg COS group showed significantly higher villous height/crypt depth than the blank control group ($P < 0.05$), with no significant difference from other groups ($P > 0.05$). During days 22-42, dietary COS supplementation significantly affected jejunal villous height and villous height/crypt depth ($P < 0.05$). The 100 mg/kg COS group showed significantly higher jejunal villous height than the blank control group and 150 mg/kg COS group ($P < 0.05$), with no significant difference from the positive control group ($P > 0.05$). No significant differences in jejunal crypt depth were observed among groups ($P > 0.05$). The 100 mg/kg COS group showed significantly higher villous height/crypt depth than the blank control group and 150 mg/kg COS group ($P < 0.05$).

2.4 Effects of Dietary COS on Ileal Flora of Broilers

As shown in Table 6 , dietary COS supplementation significantly affected ileal *Escherichia coli* counts ($P < 0.05$). The 50 and 100 mg/kg COS groups showed significantly lower ileal *E. coli* counts than the blank control group ($P < 0.05$). Dietary COS supplementation had no significant effects on ileal *Bifidobacterium* and *Lactobacillus* counts ($P > 0.05$), but showed a tendency to increase ileal *Bifidobacterium* counts ($0.05 < P < 0.10$).

2.5 Effects of Dietary COS on Growth Performance of Broilers

As shown in Table 7 , dietary COS supplementation significantly affected average daily gain (ADG) of broilers ($P < 0.05$). The 100 mg/kg COS group showed significantly higher ADG than the blank control group ($P < 0.05$), with no significant difference from the positive control group ($P > 0.05$). Dietary COS supplementation had no significant effects on average daily feed intake (ADFI) or feed-to-gain ratio (F/G) ($P > 0.05$), but showed a tendency to decrease F/G ($0.05 < P < 0.10$).

Discussion

3.1 Effects of Dietary COS on Broiler Meat Quality

pH is an important factor affecting meat quality that reflects the rate of post-slaughter glycogen degradation, and either excessively high or low pH can adversely affect meat quality. It has been reported that freshly slaughtered chicken meat has a pH of 6.0–7.0, which reaches a minimum of 5.4–5.6 after approximately 1 h [12]. Meat color, as an important meat quality trait, is a critical indicator for visual meat evaluation and is determined by pigments, myoglobin, and hemoglobin in muscle [13]. The three indicators reflecting meat color are L*, a*, and b* values. Within a certain range, higher a* values indicate better meat quality and freshness [12]. Water-holding capacity refers to the ability of muscle tissue to retain moisture and is commonly measured by drip loss or water loss rate, which shows a negative correlation with water-holding capacity [14]. High drip loss results in substantial fluid loss, severe loss of soluble nutrients and flavor compounds, and dry, tasteless muscle, thereby reducing meat quality [15]. Cooking loss refers to weight reduction during the process of converting raw meat to cooked meat due to moisture loss and other factors, which affects muscle flavor, juiciness, tenderness, processing, and storage functions, reducing economic benefits. The present results showed that, compared with the blank control group, dietary supplementation with 100 mg/kg COS significantly increased the a* value of both breast and thigh muscle and showed a tendency to increase thigh muscle L* value while decreasing thigh muscle drip loss. Li Yang [12] reported that dietary COS supplementation alone significantly increased the a* value of broiler breast and thigh muscle, significantly decreased thigh muscle b* value, and had no significant effects on breast and thigh muscle pH and L* value, which is largely consistent with our results and indicates that dietary COS supplementation improved meat quality to some extent. Song Tao [13] reported that COS supplementation at 200 mg/kg significantly decreased drip loss of Beijing duck muscle but had no obvious effects on meat color and pH, although muscle pH tended to increase with higher dietary COS levels. No clear pattern was observed in our study, with 100 mg/kg being the optimal COS supplemental level. These inconsistent results may be related to experimental materials and rearing environments. Additionally, meat quality is influenced by many factors with complex interrelationships, and the mechanism by which COS affects meat quality remains unclear and warrants further investigation.

3.2 Effects of Dietary COS on Serum Antioxidant Activity of Broilers

Due to their nutritional and physiological characteristics, broilers have relatively high lipid content and are therefore prone to lipid peroxidation reactions, with the resulting peroxides causing certain damage to the body. MDA is a metabolic product of lipid peroxidation in animals, and its level can indirectly reflect the severity of free radical attack on the body, while T-SOD activity can indirectly reflect the body' s ability to scavenge free radicals; MDA content determination is often used in conjunction with T-SOD activity measurement [16]. In

the body' s defense system, T-AOC represents the overall level of enzymatic and non-enzymatic antioxidants and can comprehensively reflect the antioxidant status of animals. Its main function is to maintain the dynamic balance of reactive oxygen species in the internal environment, remove excessive reactive oxygen species, and maintain a relatively stable redox state [17]. GSH-Px is a peroxide-decomposing enzyme widely present in animal bodies that can catalyze the conversion of glutathione (GSH) to oxidized glutathione (GSSG), thereby protecting cell membrane structure and function from interference and damage by peroxides [18]. Zang Min et al. [19] reported through classic Fenton reaction methodology that COS has superoxide anion radical scavenging capacity approaching that of vitamin C, with T-AOC of COS at 192.3 U/mL, similar to ascorbic acid at 214.7 U/mL, further demonstrating the strong antioxidant properties of COS. Our results showed that, compared with the blank control group, dietary supplementation with 100 mg/kg COS significantly increased serum T-SOD activity at 21 days of age and serum T-AOC and T-SOD and GSH-Px activities at 42 days of age, while dietary supplementation with 50 mg/kg COS significantly increased serum T-SOD activity at 21 days of age and serum T-SOD and GSH-Px activities at 42 days of age, but dietary supplementation with 150 mg/kg COS showed no significant differences. These findings indicate that dietary COS supplementation can improve broiler antioxidant activity, but higher supplementation levels are not necessarily better. This is generally consistent with the report by Li Yang et al. [20], who suggested that dietary COS supplementation alone could significantly decrease MDA content and significantly increase T-AOC and T-SOD activity in plasma, breast muscle, and thigh muscle, though their experiment did not determine the optimal COS supplemental level. Long Cimin et al. [21] reported that dietary COS supplementation in late gestation sows significantly increased serum SOD activity in sows and serum GSH-Px activity in newborn piglets. Regarding the antioxidant mechanism of COS, Li Xiaojing [22] reported that the glucosamine chain in the COS molecular structure carries quaternary ammonium ions, and the single electrons provided have good scavenging effects on free radicals, thereby improving the body' s antioxidant capacity. Other studies have reported that COS and its derivatives have strong total reducing capacity and can effectively scavenge hydroxyl radicals and superoxide anions [23-24]. It has also been suggested that COS can provide positive electrons to react with free radicals, converting them into more stable products and terminating free radical chain reactions. Additionally, because COS has a short molecular chain and weak intramolecular hydrogen bonding capability, many active hydroxyl and amino groups are exposed and easily activated, which is beneficial for free radical scavenging [25].

3.3 Effects of Dietary COS on Jejunal Mucosal Structure of Broilers

The small intestine is the main site for nutrient absorption in animals, and its normal structure directly affects nutrient absorption and animal growth and development. Caspary [26] reported that villous height is positively correlated with the contact area between the small intestine and nutrients; higher villous

height indicates more intestinal epithelial cells and greater contact area with nutrients, resulting in stronger nutrient absorption capacity. Crypt depth is negatively correlated with intestinal secretory function; shallower crypts indicate stronger secretory and absorptive functions and higher animal production efficiency [27]. Villous height/crypt depth reflects the functional status of the small intestine; a higher ratio indicates better mucosal integrity, stronger intestinal digestive and absorptive capacity, lower diarrhea rate, and faster animal growth [28]. In our experiment, at both 21 and 42 days of age, dietary supplementation with 100 mg/kg COS significantly increased jejunal villous height and villous height/crypt depth and significantly decreased crypt depth compared with the blank control group, which is consistent with the findings of Wang Xiuwu et al. [29]. Wang Xiuwu et al. [29] reported that COS supplementation significantly increased villous height in both jejunum and ileum and significantly decreased villous width compared with the control group. Tian Juan et al. [30] also reported that dietary COS supplementation at 0.10–0.70% in GIFT tilapia could increase foregut villus length, height, and density to varying degrees. The improvement of intestinal mucosal structure by COS may be attributed to its ability to prevent colonization of pathogenic and putrefactive bacteria on the intestinal mucosa, thereby promoting proliferation of intestinal epithelial cells and facilitating normal development of the animal digestive tract morphology.

3.4 Effects of Dietary COS on Ileal Flora of Broilers

The animal intestine is an important digestive organ and the largest bacterial reservoir, harboring a vast and diverse microbial community. Under normal conditions, the proportions among various microorganisms remain stable, forming a mutually beneficial symbiotic relationship with the host intestine and establishing a dynamic equilibrium that constitutes a stable microecosystem within the animal body [31-33]. Many studies have shown that oligosaccharides can improve broiler intestinal flora by inhibiting harmful bacteria and promoting beneficial bacteria. Liu Weidong et al. [34] reported that mannan oligosaccharide could significantly decrease intestinal *E. coli* counts and increase *Lactobacillus* and *Bifidobacterium* counts. Yuan Ying et al. [35] reported that supplementation of different oligosaccharides at the same level in the basal diet could regulate intestinal microecological balance by increasing cecal *Lactobacillus* and *Bifidobacterium* counts. Zhang Chang et al. [36] reported that dietary fructooligosaccharide supplementation significantly improved the intestinal microenvironment of broilers, significantly increased cecal *Lactobacillus* and *Bifidobacterium* counts, and decreased *E. coli* counts. Our results showed that, compared with the blank control group, dietary supplementation with 50 and 100 mg/kg COS significantly decreased ileal *E. coli* counts, dietary supplementation with 100 mg/kg COS showed a tendency to increase ileal *Bifidobacterium* counts, and had no significant effect on ileal *Lactobacillus* counts. These findings are not entirely consistent with Wang Xiuwu et al. [29], who reported that COS inhibited *E. coli* but also inhibited *Bifidobacterium* and *Lactobacillus*. Research on COS effects on poultry intestinal flora is limited. Lian Guoqi et al. [37]

reported that dietary COS supplementation significantly increased ileal *Lactobacillus* counts and decreased ileal *Streptococcus* counts in Huanjiang mini-pigs, while also significantly increasing cecal *Bifidobacterium* and *Lactobacillus* counts and decreasing cecal *E. coli* and *Streptococcus* counts. Xu Guizhu [38] reported that different COS levels significantly decreased intestinal *E. coli* counts and increased *Lactobacillus* and *Bifidobacterium* counts in Chinese mitten crabs. The different effects of COS on animal intestinal microbiota may be related to COS type and molecular weight. It has been reported that the antibacterial effect of chitooligosaccharides on *E. coli* is related to molecular weight, with decreased antibacterial effects at excessively high molecular weights [39]. Overall, COS has inhibitory effects on *E. coli*, but its effects and mechanisms on *Lactobacillus* and *Bifidobacterium* require further investigation.

Conclusions

1. Dietary supplementation with 100 mg/kg COS in broiler diets can improve broiler meat quality and increase average daily gain throughout the experimental period, while supplementation with 50 and 100 mg/kg COS can significantly enhance broiler antioxidant activity.
2. Dietary supplementation with 100 mg/kg COS can significantly improve jejunal mucosal structure, reduce harmful bacteria counts in the ileum, and show a tendency to increase beneficial bacteria counts.
3. The recommended optimal supplemental level of COS in broiler diets is 100 mg/kg.

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Note: Figure translations are in progress. See original paper for figures.