

Dietary supplementation with chitosan oligosaccharide affects serum lipids and nutrient digestibility in broilers

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Abstract

This research was conducted to determine the effects of supplementation of chitosan oligosaccharide (COS) on the growth performance, blood parameters, carcass traits, fatty acid composition of breast meat, and apparent nutrient digestibility in broiler chickens. A total of 375 one-day-old Ross 308 male chicks were allocated to a control diet (T1) or diets supplemented with 50 ppm (T2) and 100 ppm (T3) of COS. There were five replicates of 25 chicks for each treatment. All the experimental birds were fed a starter (days 1 - 4), grower (days 15 - 28), and finisher diet (days 29 - 42). No differences were detected among treatments for live weight, gain, feed intake or feed conversion, except that feed intake was depressed in T3 during the grower period. Serum total protein and albumin levels did not differ among the treatments. Serum total cholesterol, low density lipoproteins (LDL), very low density lipoproteins (VLDL) and triglyceride concentrations were reduced significantly by supplementation with COS, whereas the high density lipoprotein (HDL) concentration was significantly lower only for T2 relative to T1. The dressing percentage was significantly higher for T2 and T3 than for T1. Fatty acid composition of the breast meat was unaffected by the treatments. The digestibility of the diet and some of its constituents was affected in a graduated manner by the addition of COS. Thus, supplementation of broiler diets with COS improved carcass yield and had a hypolipidemic effect in improving the serum lipid profile.

Keywords: carcass, digestibility, hypolipidemic effect, performance

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Introduction

Since 1 January 2006, antibiotics have been prohibited as feed additives in the European Union and other countries, including Turkey. This prohibition has prompted further studies on alternative feed additives with similar effects. Prebiotics are defined as an undigested dietary supplement that promotes growth and acts as an antibiotic alternative that affects the health status of the supplemented individual favourably (Gibson & Roberfroid, 1995; Tufan *et al.*, 2015). Various oligosaccharides are accepted as prebiotics. One of these compounds is chitosan oligosaccharide (COS), which is the second most common carbohydrate polymer in nature after cellulose. Chitosan oligosaccharide is obtained from chitin by chemical and enzymatic hydrolysis of polychitosan and has low molecular weight compared with chitin and chitosan. Its viscosity is also low, and it can be dissolved easily in water (Xiaofeng *et al.*, 2017). Chitosan oligosaccharide is antimicrobial, antidiabetic, antitumoral, antifungal, antioxidant, and hypocholesterolemic.

Some studies examined the use of COS as a feed additive for poultry (e.g. Gao, *et al.*, 2001; Lee *et al.*, 2002; Huang *et al.*, 2007; Wang, 2008; Tufan *et al.*, 2015). It enhances the immune function and scavenges free radicals when used as a supplement for broilers (Huang *et al.*, 2005; Kim & Rajapakse, 2005; Huang *et al.*, 2007; Tufan *et al.*, 2015; Xiaofeng *et al.*, 2017). There have been reports that dietary supplementation with COS might increase blood serum high-density lipoprotein (HDL) cholesterol and decrease the abdominal fat content, thus improving the quality of meat from broiler chickens (Wang *et al.*, 1998; Tang *et al.*, 2005; Zhou *et al.*, 2009). Li *et al.* (2007) reported that dietary supplementation with COS increased nutrient digestibility in broiler chickens.

To date, there have been variable outcomes from studies that examined the effects of dietary supplementation on growth performance of broiler chickens. Zhou *et al.* (2009) and Wang *et al.* (2003) reported that COS supplementation had a positive effect on growth performance. However, other studies failed to detect this favourable effect (Razdan & Petterson, 1994; Kobayashi *et al.*, 2006a; Keser *et al.*, 2011). Therefore, this study was conducted to determine the effects of chitosan oligosaccharide supplementation on growth, serum protein and lipid fractions, carcass yield, breast meat fatty acid profile, and nutrient digestibility in broiler chickens.

Material and Methods

This research was approved by the Ethics Committee of the Faculty of Veterinary Medicine, Kafkas University, 2010-35. Three hundred and seventy-five one-day old male broiler chicks (Ross 308) were randomly allotted to a control diet (T1) or diets supplemented with 50 ppm (T2) and 100 ppm (T3) COS (GlycoBio Co., Dalian, China). There were five replicates of 25 chicks for each treatment. The study was conducted in three feeding periods, namely a starter period for the first 14 days, a grower period from day 15 to day 28, and a finisher period from day 29 to day 42. The chicks were fed corn and soybean-based diets that were formulated according to NRC (1994) recommendations (Table 1). The dry matter, deacetylation degree and molecule weight of the COS, as declared by the supplier, were 5.0 - 6.5, 90⁺%, 85⁺%, and ≤1000 Da, respectively. The chicks had ad libitum access to feed and water.

The chicks were housed on wood shavings in an automatically ventilated room. For the first three days, the temperature of the room was maintained at 33 ± 1 °C. Then the temperature was gradually decreased until the last two weeks of the study, when it was kept at 22 ± 1° C. During the first three days of the study, light was provided for 23 hours a day, and then the lighting schedule allowed for 16 hours of light and 8 hours of darkness each day. Light intensity was approximately 20 lux at bird level throughout the experimental period. The chicks were vaccinated against Newcastle disease and infectious bronchitis at hatching and on day 14, and against infectious bursal disease on day 15. The birds were monitored daily for wellbeing and mortality.

The chicks were weighed biweekly. The average daily weight gain was calculated by dividing the difference between the final weight and the initial weight by fourteen. Feed intake was also determined biweekly by deducting the remaining feed from the total feed given during the fourteen-day period. Daily average feed consumption was calculated by dividing average daily feed intake by the total number of birds in the replicate. Feed conversion ratio was calculated by dividing the average daily feed consumption by average daily weight gain.

At the end of the experiment (day 42), five birds from each replicate were randomly selected and a blood sample was drawn from their *Vena subcutanea ulnaris* (25 blood samples per treatment). The samples were centrifuged at 3000 g for 10 minutes, and the serum was stored at -20 °C for subsequent analyses. Five birds were randomly chosen from each replicate and slaughtered to determine the carcass yield and the weights of the internal organs. The carcasses were cut into parts according to Jones (1984). Samples of the breast meat were taken from the middle third of the muscle on both sides of the birds. They were kept at -20 °C until analysed for their fatty acid contents.

On days 39 and 40, excreta samples were collected from each replicate, mixed to homogenize, placed in a plastic container, and kept frozen at -20 °C until they were analysed. All chemical analyses of the diets and excreta used AOAC (2000) recommended procedures. For the nutrient analyses, the excreta samples were thawed at room temperature. Crude protein content was determined using the wet excreta. The remaining excreta samples were dried at 65 °C in a forced-air oven and crushed with a porcelain mortar and pestle to a dust-like consistency. Nutrient digestibility was estimated using acid insoluble ash (AIA) as an indigestible marker (Ergün *et al.*, 2017). Thus,

$$\text{digestibility} = 100 - \frac{\text{AIA in feed} \times \text{Nutrient in excreta}}{\text{AIA in excreta} \times \text{Nutrient in feed}} \times 100$$

The total protein, albumin, total cholesterol, HDL, low-density lipoprotein (LDL), and triglyceride contents of the serum samples were determined with a HumaStar 600 autoanalyser (HUMAN Gesellschaft für Biochemica und Diagnostica mbH, Wiesbaden, Germany). The amount of very low density lipoprotein (VLDL) was calculated according to Edwards *et al.* (1999).

Table 1 Composition and nutrient content of basal diets fed to broilers during the starter, grower and finisher phases of the study

Ingredient, %	Starter phase	Grower phase	Finisher phase
Wheat	20.13	29.95	39.73
Yellow corn	27.03	19.60	10.21
Soybean meal, 44% CP	23.94	17.73	14.24
Full fat soybean	15.00	15.00	20.00
Poultry meal	4.00	5.00	5.00
Vegetable oil	3.10	5.04	6.00
Sunflower meal, 28% CP	1.67	2.56	-
Meat-bone meal	2.00	2.00	2.00
Limestone	0.62	0.66	0.68
Dicalcium phosphate	0.62	0.47	0.45
Salt	0.30	0.30	0.30
Vitamin/mineral premix ¹	0.30	0.30	0.25
DL-methionine	0.40	0.36	0.37
L-lysine	0.21	0.32	0.26
Sodium bicarbonate	0.30	0.32	0.31
Choline chloride	0.10	0.10	-
Mycobond	0.10	0.10	0.10
Salgard	0.10	0.10	-
Roviyo enzyme	0.10	0.10	0.10
Analysed nutritive value			
ME, kcal/kg ⁵	3010	3111	3200
Dry matter, %	93.20	93.47	93.67
Crude protein, %	24.10	21.90	20.08
Ether extract, %	10.94	13.67	13.59
Crude fibre, %	4.99	3.66	5.94
Crude ash, %	5.41	6.09	6.68
Estimated calcium content, %	1.00	0.90	0.90
Estimated phosphorus content, %	0.78	0.76	0.75

¹ Vitamin A: 6.35 mg, vitamin D₃: 3.39 mg, vitamin E: 40 mg, vitamin K₃: 1.6 mg, vitamin B₁: 12 mg, vitamin B₂: 32 mg, niacin: 24 mg, calcium D-Pantothenate: 72 mg, vitamin B₆: 20 mg, vitamin B₁₂: 64 mg, D-Biotin: 80 mg, folic acid: 8 mg, vitamin C: 400 mg, manganese: 42 mg, iron: 33.6 mg, zinc: 33.6 mg, copper: 3.6 mg, cobalt: 0.80 mg, iodine: 0.40 mg, selenium: 0.72 mg, molybdenum: 0.41 mg.

Mycobond: sodium aluminosilicate; Salgard: propionic acid 170 000 ml, ammonium propionate 80 000 ml; ammonium formate 170 000 ml, formic acid 330 000 ml; Roviyo enzyme: Wheatzyme 80 000 mg, phytase 20 000 mg

Concentrations of individual fatty acids of breast meat were analysed with a gas chromatograph (Agilent Technologies, Santa Clara, California, USA) equipped with an auto sampler following the methods described by Paquot (2013). Chromatographic separations were accomplished with an Optima delta-6 capillary column (0.25 mm i.d. x 60 m, film thickness 0.25 µm). Analysis was carried out with nitrogen as the carrier gas. The column temperature ranged from an initial temperature of 50 °C, held for 1 minute, then ramped from 50 °C to 300 °C at 3 °C per minute, and held for 5 minutes. The detector temperature was 250 °C. The separated components were identified by retention time of matching standard fatty acid methyl esters. The external standard method was used. Quantitative determination was carried out based on peak area integration.

Data were analysed by one-way ANOVA with SPSS 16.0 (SPSS Inc., Chicago, Illinois, USA). Duncan's multiple comparison test was used to compare the treatment means ($P < 0.05$). The results are presented as mean \pm standard error.

Results and Discussion

Mortality rates in T1, T2, and T3 were 11.2%, 4.8% and 8.8%, respectively. The Chi square value testing independence of treatments and mortality was 3.44 ($P > 0.10$).

No differences were detected among the treatments for live weight, average live weight gain, average feed intake and feed conversion ratio during the starter, grower and finisher periods, except that feed intake was depressed in T3 during the grower period. In aggregate, no significant differences were detected among treatments for the entirety of the study (Table 2).

Table 2 Effects of chitosan oligosaccharide supplementation of diets on growth performance of broiler chickens

Parameters	Treatments			P-value
	Control diet	50 ppm COS	100 ppm COS	
Initial weight, g	37.06 \pm 0.04	37.02 \pm 0.05	37.02 \pm 0.05	0.783
Starter period (days 0 - 14)				
Live weight, g	383.26 \pm 7.90	387.00 \pm 4.69	391.04 \pm 12.04	0.823
Average live weight gain, g/d	24.73 \pm 1.24	25.00 \pm 0.73	25.29 \pm 1.93	0.825
Average feed intake, g/d	27.64 \pm 0.66	27.68 \pm 0.46	27.90 \pm 0.36	0.875
Feed conversion	1.12 \pm 0.03	1.11 \pm 0.02	1.10 \pm 0.02	0.344
Grower period (days 15 - 28)				
Live weight, g	1403.16 \pm 20.40	1435.58 \pm 17.24	1397.04 \pm 31.42	0.490
Average live weight gain, g/d	72.85 \pm 2.26	74.90 \pm 1.36	71.86 \pm 3.49	0.224
Average feed intake, g/d	108.58 \pm 3.65 ^a	106.66 \pm 4.66 ^{ab}	101.40 \pm 2.98 ^b	0.031
Feed conversion	1.49 \pm 0.04	1.43 \pm 0.05	1.41 \pm 0.04	0.208
Finisher period (days 29 - 42)				
Live weight, g	2666.04 \pm 27.41	2804.82 \pm 30.41	2718.14 \pm 68.19	0.140
Average live weight gain, g/d	90.20 \pm 5.72	97.80 \pm 5.61	94.36 \pm 6.51	0.173
Average feed intake, g/d	181.64 \pm 3.27	189.38 \pm 5.96	187.64 \pm 9.33	0.195
Feed conversion	2.03 \pm 0.14	2.01 \pm 0.06	2.06 \pm 0.04	0.652
Overall (days 1 - 42)				
Average live weight gain, g/d	62.59 \pm 1.43	65.90 \pm 1.63	63.84 \pm 3.64	0.140
Average feed intake, g/d	105.94 \pm 0.68	107.90 \pm 0.82	105.66 \pm 3.26	0.223
Average feed conversion	1.55 \pm 0.04	1.52 \pm 0.01	1.52 \pm 0.02	0.234

^{a,b} Within a row, mean values with a common superscript did not differ at $P = 0.05$
COS: chitosan oligosaccharide

Serum total protein and albumin concentrations were not different among the groups (Table 3). Total cholesterol, LDL, VLDL, and triglyceride concentrations were lower in T2 and T3 than the control group. The HDL concentration was lower in T2 than the control ($P < 0.05$).

Table 3 Effects of chitosan oligosaccharide supplementation of diets for broiler chicken on certain serum constituents

Serum constituents	Treatments			P-value
	Control diet	50 ppm COS	100 ppm COS	
Total protein, g/dL	3.42 ± 0.23	3.20 ± 0.31	2.69 ± 0.19	0.107
Albumin, g/dL	0.71 ± 0.06	0.56 ± 0.05	0.61 ± 0.04	0.106
Total cholesterol, mg/dL	68.47 ± 3.50 ^a	53.20 ± 4.06 ^b	53.90 ± 2.48 ^b	0.003
HDL, mg/dL	41.18 ± 2.06 ^a	34.80 ± 2.22 ^b	35.69 ± 1.57 ^{ab}	0.052
LDL, mg/dL	20.36 ± 1.60 ^a	12.92 ± 1.85 ^b	12.34 ± 0.92 ^b	0.001
VLDL, mg/dL	6.94 ± 0.42 ^a	5.51 ± 0.42 ^b	5.86 ± 0.21 ^b	0.019
Triglycerides, mg/dL	34.68 ± 2.08 ^a	27.56 ± 2.11 ^b	29.28 ± 1.07 ^b	0.020

^{a,b} Within a row, mean values with a common superscript did not differ at $P=0.05$

COS: chitosan oligosaccharide; HDL: high density lipoprotein, LDL: low density lipoprotein, VLDL: very low density lipoprotein

Live weight at slaughter and the weights of abdominal fat, heart, spleen, and gizzard of the chicks did not differ among treatments (Table 4). The dressing percentages of the birds and their wing weight differed among the treatments ($P < 0.05$ and $P < 0.001$, respectively) with T2 and T3 being greater than T1. Breast weights of the birds that were received the intermediate level of COS were higher than those of either T1 or T3 ($P < 0.01$). Leg weights of the birds in T2 exceeded those of T1 ($P < 0.01$) but were not significantly different from T3. The liver weights of the birds at slaughter decreased in a graduated manner with T3 being significantly less than T1 ($P < 0.05$).

Table 4 Effects of chitosan oligosaccharide supplementation of diets fed to broiler chickens on carcass traits

Traits	Treatments			P-value
	Control diet	50 ppm COS	100 ppm COS	
Slaughter live weight, g	2654.10 ± 38.37	2756.16 ± 34.83	2683.88 ± 36.48	0.135
Dressing percentage	70.24 ± 0.69 ^b	73.05 ± 0.94 ^a	72.48 ± 0.50 ^a	0.021
Breast weight, g	634.46 ± 12.68 ^b	689.25 ± 12.00 ^a	652.83 ± 13.00 ^b	0.010
Leg weight, g	775.02 ± 14.19 ^b	842.62 ± 12.61 ^a	810.15 ± 13.87 ^{ab}	0.003
Wing weight, g	210.27 ± 4.91 ^b	235.96 ± 4.07 ^a	238.96 ± 3.47 ^a	0.000
Abdominal fat weight, g	18.97 ± 1.85	16.07 ± 1.02	18.48 ± 1.81	0.398
Heart weight, g	14.54 ± 0.51	13.51 ± 0.44	14.40 ± 0.56	0.302
Liver weight, g	67.57 ± 2.67 ^a	61.92 ± 1.94 ^{ab}	60.17 ± 1.42 ^b	0.036
Spleen weight, g	3.24 ± 0.18	3.23 ± 0.20	3.24 ± 0.14	0.998
Gizzard weight, g	41.08 ± 2.12	38.67 ± 1.13	38.07 ± 1.34	0.369

^{a,b} Within a row, mean values with a common superscript did not differ at $P=0.05$

COS: chitosan oligosaccharide

No significant differences were detected among treatments in the individual fatty acid content, total saturated fatty acids (SFA), total monounsaturated fatty acids (MUFA) and total polyunsaturated fatty acids (PUFA) contents of breast (Table 5).

Table 5 Effects of chitosan oligosaccharide supplementation of diets on fatty acid composition of breast meat from broilers

Fatty acids, %	Treatments			P-value
	Control diet	50 ppm COS	100 ppm COS	
Myristic acid	0.31 ± 0.01	0.33 ± 0.01	0.32 ± 0.01	0.196
Pentadecylic acid	0.09 ± 0.00	0.08 ± 0.01	0.10 ± 0.00	0.009
Palmitic acid	14.15 ± 0.18	14.61 ± 0.23	14.50 ± 0.16	0.230
Margaric acid	0.20 ± 0.01	0.21 ± 0.01	0.22 ± 0.05	0.382
Stearic acid	5.35 ± 0.09	5.39 ± 0.12	5.35 ± 0.11	0.942
Arachidic acid	0.03 ± 0.00	0.04 ± 0.00	0.03 ± 0.00	0.208
Heneicosylic acid	0.31 ± 0.01	0.30 ± 0.01	0.30 ± 0.01	0.705
Lignoceric acid	0.05 ± 0.01	0.02 ± 0.01	0.03 ± 0.09	0.180
Palmitoleic acid	1.44 ± 0.11	1.44 ± 0.09	1.53 ± 0.10	0.765
Oleic acid	28.31 ± 0.36	28.63 ± 0.34	28.79 ± 0.35	0.609
Eicosenoic acid	0.22 ± 0.00	0.23 ± 0.00	0.22 ± 0.00	0.282
Gadoleic acid	0.60 ± 0.03	0.54 ± 0.03	0.55 ± 0.02	0.260
Nervonic acid	0.17 ± 0.01	0.17 ± 0.02	0.17 ± 0.01	0.962
Linoleic acid	41.57 ± 0.49	40.98 ± 0.55	40.89 ± 0.46	0.577
Eicosadienoic acid	0.22 ± 0.01	0.21 ± 0.01	0.21 ± 0.01	0.582
γ-linolenic acid	4.68 ± 0.08	4.61 ± 0.07	4.53 ± 0.04	0.285
α-linolenic acid	0.06 ± 0.01	0.05 ± 0.01	0.06 ± 0.01	0.202
Cervonic acid	0.05 ± 0.01	0.04 ± 0.02	0.01 ± 0.01	0.130
Σ SFA	20.48 ± 0.21	20.97 ± 0.30	20.83 ± 0.20	0.340
Σ MUFA	30.73 ± 0.43	31.01 ± 0.41	31.26 ± 0.43	0.681
Σ PUFA	46.58 ± 0.56	45.89 ± 0.62	45.70 ± 0.49	0.513

COS: chitosan oligosaccharide

SFA: myristic acid, pentadecylic acid, palmitic acid, margaric acid, stearic acid, arachidic acid, heneicosylic acid, and lignoceric acid

MUFA: palmitoleic acid, oleic acid, eicosenoic acid, gadoleic acid, and nervonic acid

PUFA: linoleic acid, eicosadienoic acid, γ-Linolenic acid, α-Linolenic acid, and cervonic acid

Birds in T1 had greater digestibility of dry matter and crude fat than those in T3 ($P < 0.05$) (Table 6). Organic matter digestibility was higher for birds in T1 than those in T2 and T3 ($P < 0.05$).

Table 6 Effects of chitosan oligosaccharide supplementation on the digestibility of dry matter, organic matter and crude fat in broiler chicks

Nutrient	Treatments			P-value
	Control diet	50 ppm COS	100 ppm COS	
Dry matter	96.15 ± 0.24 ^a	95.52 ± 0.12 ^{ab}	95.03 ± 0.34 ^b	0.025
Organic matter	82.42 ± 1.20 ^a	78.21 ± 0.62 ^b	77.57 ± 1.47 ^b	0.023
Crude fat	86.68 ± 1.04	83.79 ± 0.43	83.15 ± 1.35	0.066

^{ab} Within a row, mean values with a common superscript did not differ at $P = 0.05$

COS: chitosan oligosaccharide

Discussion

Supplementation of 50 and 100 ppm COS to the ration had no detectable effects on final live weight, average live weight gain, average feed intake and average feed conversion of broilers compared with unsupplemented birds over the 42-day feeding period. However, supplementation with COS produced numerical increases in the final live weights of the birds (5.21% and 1.95% for T2 and T3, respectively). Such an increase could be economically profitable, if the effect were real. However, the current study lacked statistical power to detect effects of this magnitude. However, taken together with the results from other studies in which the addition of COS did not cause any difference to the live weight in broiler chickens (Huang *et al.*, 2007; Keser *et al.*, 2011), supplementation with COS cannot be advised based on the expectation of altered performance. In some studies, it was observed that chitosan has high viscosity and decreased feed intake and weight gain. Chitosan may have caused increased viscosity in the digestive system, which decreased the rate of passage of the digesta and the birds therefore consumed less feed (Razdan *et al.*, 1996; Razdan & Petterson, 1997). However, in this study, the lack of differences between treatments in feed intake might be associated with the low molecule weight and low viscosity of the COS that was used (Razdan & Petterson, 1996; Razdan *et al.*, 1997; Chae *et al.*, 2005; Kim & Rajapakse, 2005).

The results of this study also suggest that the addition of COS to broiler rations did not affect protein metabolism. Similar to this study, the addition of COS to broiler rations at various rates did not change serum concentrations of protein (Zhou *et al.*, 2009; Keser *et al.*, 2011) and albumin (Zhou *et al.*, 2009). However, Li *et al.* (2007) found that adding COS to broiler rations at rates of 50 and 100 mg/kg increased serum protein levels significantly.

The results of the present study show that COS supplementation had a hypocholesterolemic effect in boiler chickens. Similar effects were reported (Razdan & Petterson, 1994; 1996; Razdan *et al.*, 1991; Osman *et al.*, 2010). In contrast, adding chitosan to broiler rations did not alter serum HDL-cholesterol levels significantly (Keser *et al.*, 2011; Razdan & Petterson, 1994; Razdan & Petterson, 1996; Razdan *et al.*, 1997). Other researchers found that COS supplementation decreased serum triglyceride concentration significantly (Li *et al.*, 2007; Zhou *et al.*, 2009). The reasons for the decrease in serum lipid fractions may be associated with increased viscosity of the digesta in the intestine and a coincident decrease in lipid absorption (Razdan & Petterson 1994; Razdan *et al.*, 1997). Further evidence indicated decreased lipase activity in the small intestine (Kobayashi *et al.*, 2002) and increased cholesterol in the faeces as a result of chitosan binding bile acids (Razdan & Petterson 1996; Chiang *et al.*, 2000; Zheng & Zhu, 2003). There is a positive relationship between high serum triglyceride concentration and liver fat, and high serum triglyceride concentration may cause the condition commonly known as fatty liver. Low serum triglyceride content is thus desired for the continuity of the health of broiler chickens. In the current study, supplementing the broiler ration with 50 and 100 ppm COS produced significant decreases in LDL, VLDL, and triglyceride concentrations. Similar to these results, it was observed that adding chitosan (Osman *et al.*, 2010) and COS (Keser *et al.*, 2011) to broiler rations reduced serum LDL concentration significantly. These decreases may be interpreted as indicating better health conditions for the broilers and for the people who eat them ultimately.

In this study, supplementation of the broiler diet with 50 and 100 ppm COS did not affect the weights of abdominal fat, hearth, spleen, and gizzard. Abdominal fat weight is an indicator of total body fat (Fortin *et al.*, 1983; Sonaiya, 1985) and less accumulation of abdominal fat is desired. Similar to the present results, Khambualai *et al.* (2008) found no difference in abdominal fat in broilers that were fed chitosan. However, there were several reports that supplementation of broiler rations with chitosan or COS decreased abdominal fat (Kobayashi *et al.*, 2002; Kobayashi *et al.*, 2006b; Zhou *et al.*, 2009). Also similar to the present results, Zhou *et al.* (2009) found that chito-oligosaccharide supplementation did not affect spleen weight. Khambualai *et al.* (2008) found that chitosan supplementation did not affect heart weight in broilers. There were contradictory results of the effects of COS supplementation on the liver weight of broilers. Because liver weight in T3 was significantly lower than in T1, and T2 was intermediate, the present results suggest that the COS supplementation effect is dose dependent. However, Zhou *et al.* (2009) reported that adding COS to broiler rations increased the liver weight depending on the amount of COS. Wang *et al.* (2003) reported that supplementation of COS to broiler rations decreased the liver weight. Finally, there were reports of no differences in the liver weight of broilers as a function of COS supplementation (Kobayashi *et al.*, 2006b; Kobayashi *et al.*, 2006a; Khambualai *et al.*, 2008).

Live weight at slaughter was unaffected by COS supplementation, but the dressing percentage was increased, leading to the expectation that one or more components of the carcass would be heavier in the supplemented birds. Increased wing weight was observed in both T2 and T3 relative to T1. In addition, T2 significantly increased breast weight compared with T1 and T3, and T2 significantly increased leg weight compared with T1. Taken together, these results might suggest an intermediate optimum level of COS supplementation. Zhou *et al.* (2009) reported that adding COS to rations did not have a significant effect on

breast weight. Likewise, Khambualai *et al.* (2008) failed to detect a numerical increase in breast weight that resulted from chitosan supplementation.

In the current study, the effects of COS supplementation for broilers on the fatty acid profile of breast meat and on total SFA, MUFA, and PUFA concentrations were minimal. Unlike these results, Zhou *et al.* (2009) reported that adding COS to broiler rations decreased the SFA content and increased the MUFA and PUFA contents of the breast muscle.

Supplementation of the rations for broilers with COS decreased the digestibility of organic matter and dry matter in a dose-dependent manner. It also tended to decrease the digestibility of fat. The observed decreases in digestibility may be because of the increased viscosity of the digesta, slowing peristaltic movement of the intestines, binding fat micelle of the digesta, and suppressing enzymatic activity. Razdan and Petterson (1994) reported that chitosan increased the viscosity of the digesta, decreased the motility of the intestines, and inhibited the release of digestive enzymes, all affecting digestion negatively. However, Li *et al.* (2007) found that adding 50 or 100 ppm COS to broiler rations did not affect dry matter digestion. Further, Lim *et al.* (2006) found that supplementation of broiler rations with 100 ppm COS increased the digestibility of dry matter. This latter result was confirmed by Wang *et al.* (2008) with 0.05%, 0.10%, and 0.15% COS supplementation of goose rations.

Conclusion

Supplementation of broiler rations with 50 and 100 ppm COS had a favourable hypolipidemic effect on the birds without materially compromising their survival, performance, and serum fatty acid profile. Dressing percentage was also increased with modest effects on the weights of some carcass components. Digestibility of dietary dry matter, organic matter and crude fat may also be altered in a dose-dependent manner. Thus, COS might be useful as an alternative and natural feed additive in feeding broiler chickens.

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Authors' Contributions

TT and CA made equal contributions at every stage of this research.

Conflict of Interest Declaration

The authors declare no conflict of interest.

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