

Chitosan oligosaccharide supplementation alleviates stress stimulated by in-feed dexamethasone in broiler chickens

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ABSTRACT This experiment was conducted to investigate the effect of dietary chitosan oligosaccharide (COS) on growth performance, nutrient digestibility, jejunal morphology, gene expression, and plasma antioxidant enzymes in male broiler chickens under experimentally induced stress via in-feed dexamethasone (DEX). On day 3 after hatching, male broiler chicks were assigned to 2 diets supplemented with COS at 0 or 1 g/kg in a randomized complete block design and fed to day 27 after hatching. Birds were pooled within each diet (0 or 1 g/kg COS) to equalize the average BW and fed 2 diets supplemented with 0 or 1 g/kg DEX, within each dietary COS, from day 20 to 27 after hatching. This resulted in a 2 × 2 factorial arrangement of treatments with 2 levels each of COS and DEX, 8 replicate cages of 7 birds per cage. On day 27 after hatching, birds were weighed and euthanized, and samples were collected. Dietary COS decreased ($P < 0.05$) DEX-induced effects (interaction; $P < 0.05$) on BW, BW gain, and gain:feed. Dietary COS

supplementation attenuated the DEX effects (interaction; $P < 0.05$) on villus height, crypt depth, villus height to crypt depth ratio, and ileal digestibility of dry matter and energy. The DEX-induced effect of relative mRNA expression of jejunal mucosa IL-6, IL-10, and claudin-1 was reduced by dietary COS supplementation (interaction; $P < 0.05$). Responses (interaction; $P < 0.05$) in the activity of plasma superoxide dismutase, catalase, and glutathione peroxidase to COS and DEX were similar to those observed with the relative mRNA expression. Chitosan oligosaccharide supplementation increased ($P < 0.05$) the mRNA expression of IL-8 and occludin. In conclusion, dietary COS decreased the DEX-induced effect by improving growth performance, nutrient digestibility, jejunal morphology, gene expression, and plasma antioxidant enzymes in broiler chickens. This implies that dietary COS may be useful for ameliorating the negative effect of stress on gut health in broiler chickens.

Key words: broiler chickens, chitosan oligosaccharide, dexamethasone, gene expression, stress

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INTRODUCTION

A multitude of stressors exists in the rearing of broiler chickens including immunological challenge, oxidative stress, and transportation. Stress has many detrimental effects on broiler chickens; for example, it reduces growth performance, increases susceptibility to disease, and impairs immune function (Lin et al., 2006a, b). It has been reported that the hypothalamus-pituitary-adrenal axis is activated (Siegel, 1980) when an animal is under stress; thus, there is secretion of glucocorticoid from the adrenal gland. A plethora of glucocorticoids is a hallmark of stress (Chrousos and Gold, 1992).

Dexamethasone (DEX) is a synthetic glucocorticoid used as an immunosuppressive agent. It has been used to induce oxidative stress and to investigate stress responses in poultry species (Gao et al., 2010; Njagi et al., 2012). Dietary antioxidants could have positive effects and benefits in preventing oxidative stress and relieving the immunosuppression induced by DEX. In previous studies, glucocorticoids have proven to be involved in the alteration of redox balance in poultry (Lin et al., 2004; Mujahid et al., 2006), as well as modulating immune function and enteric mucosal integrity (Lin et al., 2000).

In the past, oxidative stress and immune suppression were controlled by using antibiotic growth promoters in broiler chickens (Williams et al., 2001). Currently, specific dietary supplementation can offer a viable and practical alternative to antibiotics that alleviate these stressors in broiler chickens. Previous studies on poultry and swine have found that chitosan oligosaccharide (COS) improves growth performance (Huang et al., 2005), enhances

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immune function (Xiao et al., 2013), and increases antioxidant properties (Niu et al., 2013).

In the current experiment, DEX was administered to broiler chickens diet to induce stress. There are limited studies in broiler chickens that evaluate the effect of COS on antioxidant enzymes and immune response. This finding may provide a useful evidence for the application of COS in diets to mitigate immunological stress and improve antioxidant activity in broiler chickens. We therefore hypothesized that dietary COS supplementation will not mitigate the stress-induced effect by in-feed dexamethasone in broiler chickens. Our objective was to investigate the effect of dietary COS supplementation on anti-oxidative function and immune response induced by in-feed DEX supplementation.

MATERIALS AND METHODS

The Purdue University Animal Care and Use Committee (West Lafayette, IN) approved the experiment protocol.

Chitosan and Dexamethasone Materials

Chitosan oligosaccharide supplement used in the present experiment was purchased from Qingdao Yunzhou Biochemistry Co. Ltd. (Jimo, Qingdao, China). They were derived from shrimp shells, and the degree of deacetylation was greater than 90%. Dexamethasone was purchased from Alfa Aesar (Tewksbury, MA). Both materials were in powdered form.

Birds and Diets

A total of 288 male broiler chicks (Cobb 500; Cobb-Vantress Inc., Siloam Springs, AR) were used in this study. The birds were maintained in cages in an environmentally controlled room. All birds received a basal diet consisting of antibiotic-free corn-soybean meal based diet meeting the nutritional requirements of poultry recommended by National Research Council (1994). Four diets were formulated by supplementing a portion of the basal diet with 2 levels of COS (0 or 1 g/kg) and 2 levels of DEX (0 or 1 mg/kg) (Table 1).

Experimental Procedure and Design

On day 3 after hatching, 288 birds were individually tagged and weighed. All birds were randomly assigned to 2 experimental diets (basal diet supplemented with COS at 0 or 1 g/kg diet). The optimum dietary concentration of COS for broiler chickens was determined to be 1 g/kg (Osho and Adeola, 2019). There were 16 replicate cages of 0 g COS/kg and 16 replicate cages of 1 g COS/kg diet with 9 birds per cage. On day 20 after hatching, all birds were weighed and birds were re-randomized within each of 0 or 1 g COS/kg diet to equalize the average BW of the birds before administration of in-feed DEX. In each of the 0 or 1 g COS/kg, birds in 8 replicate cages of 7 birds per cage were fed diet

Table 1. Ingredients and nutrient composition of experimental diets, g/kg as-fed basis.

Ingredients, g/kg	Control	COS (1 g/kg)
Corn	538.1	528.1
Soybean meal (48.5% CP)	345.2	345.2
Soybean oil	45.0	45.0
Monocalcium phosphate ¹	17.0	17.0
Limestone (38% Ca)	15.5	15.5
Salt	4.0	4.0
Vitamin–mineral premix ²	3.0	3.0
DL-Methionine	3.5	3.5
L-Lysine.HCl	3.0	3.0
Threonine	0.7	0.7
Chromic oxide premix ³	25	25
COS premix ⁴	0	10
DEX premix ^{5,6}	0	0
Total	1,000	1,000
Calculated nutrients and energy content		
CP, g/kg	220	220
ME, kcal/kg	3,115	3,112
Ca, g/kg	9.7	9.7
P, g/kg	7.3	7.3
Non-phytate P, g/kg	4.8	4.8
Ca: Total phosphorus	1.3	1.3

¹Contained 16% Ca, 21% P.

²Supplied the following per kilogram of diet: vitamin A, 5,484 IU; vitamin D₃, 2,643 IU; vitamin E, 11 IU; menadione sodium bisulfite, 4.38 mg; riboflavin, 5.49 mg; D-pantothenic acid, 11 mg; niacin, 44.1 mg; choline chloride, 771 mg; vitamin B₁₂, 13.2 µg; biotin, 55.2 µg; thiamine mononitrate, 2.2 mg; folic acid, 990 µg; pyridoxine hydrochloride, 3.3 mg; I, 1.11 mg; Mn, 66.06 mg; Cu, 4.44 mg; Fe, 44.1 mg; Zn, 44.1 mg; Se, 300 µg.

³Prepared as 1 g of chromic oxide added to 4 g of ground corn.

⁴1 g of chitosan oligosaccharide (COS) (Qingdao Yunzhou Biochemistry Co. Ltd., Jimo, Qingdao, China) added to 9 g of ground corn.

⁵Prepared as 0.01 g of dexamethasone (DEX) (Alfa Aesar, Maryland) added to 9.99 g of ground corn.

⁶Dexamethasone premix was added at the expense of corn to supply 0, 1 mg DEX/kg diet.

supplemented with either 0 or 1 mg DEX/kg diet. This resulted in a 2 × 2 factorial arrangement of treatments with 2 levels of COS at 0 or 1 g/kg and 2 levels of DEX with 8 replicate cages of 7 birds per cage.

Growth Performance and Nutrient Utilization

Individual BW of birds and feed intake were determined at day 20 and day 27 after hatching. Final BW, feed intake (FI), and feed efficiency for each weigh period were measured. On day 27 after hatching, all birds were euthanized and ileal digesta were collected from the distal two-thirds of ileum excised from each bird, and contents were gently flushed with distilled, deionized water and pooled within cage (Kluth et al., 2005). The samples were stored at -20°C before further analyses. Apparent ileal digestibility of DM, N, and energy were calculated using the index method as described by Olukosi et al. (2007).

Apparent ileal digestibility was calculated using the formula:

$$\text{Apparent ileal digestibility, \%} = 100 \times [1 - (\text{Cr}_i/\text{Cr}_o \times \text{N}_o/\text{N}_i)]$$

where C_i and C_o were the concentration of chromium in the diet or ileal digesta, respectively, while N_i and N_o were the concentration of nutrient or energy in the diet or ileal digesta, respectively (Olukosi et al., 2007).

Chemical Analysis

Pooled ileal digesta samples were dried in a forced-air oven for 1 wk at 56°C. Diets and dried ileal digesta were finely ground using an electric coffee grinder and thereafter analyzed for DM and gross energy. The concentration of DM in diets and ileal digesta was measured by drying at 105°C for 24 h in a forced-air oven (The Precision Scientific Co., Chicago, IL; method 934.01; [AOAC International, 2006](#)). Gross energy was determined using an isoperibol bomb calorimeter (Parr 6200, Parr Instruments, Moline, IL) standardized with benzoic acid. Chromic oxide was used as an indigestible marker. Diet and ileal digesta chromium concentrations were determined according to the procedures of [Fenton and Fenton, \(1979\)](#).

Jejunal Morphology and Total RNA Extraction and Reverse Transcription

Jejunum tissues were excised (10-cm mid jejunum) from one bird per cage (median weight bird) on day 27 after hatching and processed as previously described ([Osho et al., 2019](#)). The tissues were gently flushed with cold sterile saline solution to remove intestinal contents and immediately placed in 10% neutral buffered formalin. Tissue sections (4 mm) were prepared and stained with hematoxylin and eosin by the Purdue Histology and Phenotyping Laboratory and were examined using a light microscope (National Optical and Scientific Instruments, Inc., Schertz, TX). Villus height and crypt depth were measured from 4 well-oriented villi per slide. Villus height was measured as the distance from the tip of the villus to the crypt mouth, whereas crypt depth was measured from the base of the villi to the submucosa. Villus height to crypt depth ratio was calculated by dividing the villus height by crypt depth.

Total RNA was extracted from the jejunum using TRIzol reagent (Invitrogen) following the manufacturer's protocol and processed as previously described ([Osho and Adeola, 2019](#)). The concentration of the extracted RNA was determined using a NanoDrop spectrophotometer (ND-1000; NanoDrop Products) at an optical density of 260 nm, whereas RNA purity was verified by measuring absorbance at an optical density of 260/280. From each sample, 2 µg of total RNA was reverse-transcribed into cDNA using the MMLV reverse transcription system (Promega), and cDNA was then diluted 1:10 with nuclease-free water (Ambion) and stored at -20°C until use.

Quantitative Real-Time PCR Analysis

Expression levels of IL-6, IL-8, IL-10, TNF- α , Muc-2, and Claudin-1 genes were analyzed in the jejunum by quantitative real-time PCR. It was performed with Bio-Rad iCycler with the FastStart SYBR green-based mix (Life Technologies). The PCR programs for all genes were designed as follows: 10 min at 95°C; 40 cycles of 95°C for 30 s, primer-specific annealing temperature

for 30 s, and 72°C for 30 s; followed by melting curve analysis. The primer sequences used in this study are similar to those described by [Osho and Adeola, 2019](#). Primer specificity and efficiency (90–100%) were verified. Samples were analyzed in duplicate, and a difference lesser than or equal to 5% was acceptable. Relative gene expression was calculated using the $2^{-\Delta\Delta C_t}$ method ([Livak and Schmittgen, 2001](#)) with normalization against the housekeeping gene, glyceraldehyde 3-phosphate dehydrogenase (GAPDH), as described in a previous study ([Tan et al., 2014](#)).

Analysis of Plasma Samples

Blood samples (2 mL) were taken via cardiac puncture and kept on ice until plasma was separated by centrifugation for 10 min at 1,500 rpm. Plasma samples were stored at -80°C until assayed. The corresponding assay kits (MyBiosource, Inc., San Diego, CA) for plasma superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) concentration were determined according to the instructions of the manufacturer. Samples were added in duplicate to check intra-assay variability. The interassay and intra-assay coefficients of variations were less than 10%.

Statistical Analysis

Post-DEX-challenge data were analyzed using the MIXED procedure of SAS as a 2 × 2 factorial consisting of 2 levels of COS (0 or 1 g/kg) and 2 levels of DEX (0 or 1 mg/kg) and evaluated for main effects and interactions. Statistical significance was defined at a probability of $P \leq 0.05$ and cage was the experimental unit.

RESULTS AND DISCUSSION

Broiler chickens are usually subjected to several potentially immunosuppressive stimuli during their lifetime ([Galha et al., 2008](#)). Dexamethasone is an analog of the glucocorticoid secreted when animals are under stress. Glucocorticoids act by decreasing the population of both B and T lymphocytes, promoting a considerable immunosuppression. Hence, administration of DEX is a satisfactory way to mimic stress and provide a way to study the effects of stress conditions on enteric mucosa integrity. In the present study, broiler chickens were visibly depressed, less active, and diarrheic during the immunological stress mimicked by in-feed DEX, in accordance with previous reports ([Lin et al., 2004](#); [Gao et al., 2008](#)).

The effect of in-feed DEX and COS supplementation on growth performance is summarized in [Table 2](#). In the group of birds fed diet supplemented with 0 g/kg COS, dietary DEX at 1 mg/kg decreased average BW gain by 75%. However, in birds fed diet supplemented with 1 g/kg COS, dietary DEX at 1 mg/kg decreased average BW gain by 49%, thus resulting in a COS × DEX interaction ($P < 0.05$). In-feed DEX decreased ($P < 0.05$) feed intake (FI), whereas COS

Table 2. Growth performance, ileal digestibility, and jejunal morphology of broiler chickens fed diets containing chitosan oligosaccharide (COS) concentration at 0 or 1 g/kg with or without dexamethasone (DEX).^{1,2}

Item	0, g/kg COS		1 g/kg COS		SEM	<i>P</i> -value ³		
	No-DEX	DEX	No-DEX	DEX		COS	DEX	COS × DEX
Day 20 to 27								
Day 20 BW, g/bird	750	750	750	750	21.2	0.85	0.95	0.85
Day 27 BW, g/bird	1,127	841	1,226	993	11.4	<0.01	<0.01	0.03
Gain, g/bird	378	91	477	243	18.8	<0.01	<0.01	0.02
Feed intake, g/bird	619	399	708	526	30.6	<0.01	<0.01	0.55
Gain:feed, g/kg	627	236	674	487	20.2	<0.01	<0.01	<0.01
Jejunal morphology ⁴								
VH, μm	926	742	902	862	34.1	0.07	<0.01	0.02
CD, μm	76	144	74	101	7.9	0.01	<0.01	0.02
VH/CD	12.3	5.55	12.2	8.65	0.621	0.02	<0.01	0.02
Ileal digestibility								
DM, %	65.1	58.2	67.2	63.7	0.68	<0.01	<0.01	0.02
Energy, %	69.6	62.8	71.7	68.0	0.70	<0.01	<0.01	0.04
IDE, kcal/kg	3,561	3,142	3,655	3,375	37	<0.01	<0.01	0.07

¹On day 0 after hatching, birds were divided into 2 groups with 16 replicates each, and were fed control diet and COS supplemented diets, each with an initial body weight of 61 g/bird. The final BW on day 13 after hatching was 707 and 720 g/bird for birds fed control diet and COS supplemented diet, respectively. The feed intake was 857 and 879 g/bird for birds fed control and COS. The feed efficiency was 755 g/kg and 750 g/kg for birds fed control diet and COS-supplemented diets, respectively.

²Data are least squares means of 8 replicate cages with 7 birds per cage.

³*P*-value according to main effects of COS, DEX, and a COS × DEX interaction.

⁴CD, crypt depth; IDE, ileal digestible energy; VH, villus height.

supplementation increased ($P < 0.05$) FI. Chitosan oligosaccharide supplementation decreased the DEX-induced effect (interaction; $P < 0.05$) on gain to feed ratio. It has been established that decreased FI is a primary cause of reduced growth rate in broiler chickens. The result of reduced FI agrees with the report from Sapolsky et al. (2000), who observed that DEX reduced appetite. In broiler chickens, one of the most recognizable effects of glucocorticoid treatment on growth performance is a drastic reduction in BW gain (Puvadolpirod and Thaxton, 2000a), which was observed in the current experiment.

It is well known that glucocorticoid plays an important role in reduced anabolic and enhanced catabolic processes (Virden and Kidd, 2009). Perhaps, stressed broiler chickens used up more energy to adapt to the stress condition and less energy for growth. Meanwhile, supplementation with COS relieved the inhibitory effect of in-feed DEX on growth performance of broiler chickens, suggesting a potentially important role for COS to inhibit the adverse effects of immune stress in broiler chickens. This is similar to a previous report (Zhang et al., 2011), which showed that dietary COS supplementation remarkably prevented the decrease in BW gain caused by immune stress.

Dietary COS supplementation attenuated the DEX effects (interaction; $P = 0.02$) on villus height, crypt depth, and villus height to crypt depth ratio in the mid-jejunum (Table 2). The structure of the intestinal mucosa can reveal some information on the absorptive ability of intestine to nutrients and is always associated with the performance of animals. The ratio of villus height to crypt depth reflects the comprehensive ability for intestinal nutrient absorption and function. The results indicate that glucocorticoids may decrease nutrient absorption, by

regulating the non-specific absorption of jejunum in broilers. Broiler chickens fed with COS-supplemented diet showed improved gut morphology in the mid-jejunum, regardless of the DEX treatment, indicating that supplementing COS exerted a positive role in controlling immunological stress. Few data are available on the effects of COS on gut morphology during DEX challenge. However, low molecular weight COS may enhance intestinal morphology through cell proliferation as shown in mice (Torzsas et al., 1996).

Dietary COS supplementation ameliorated DEX effects (interaction; $P < 0.05$) on ileal digestibility of DM and energy (Table 2). Previous research has described the physiology of stress in chickens (Siegel, 1985). However, descriptions of digestion and metabolism inherent to stress in chickens are limited. By considering the many effects of immunological stress on metabolism and broiler performance, we speculate that the potential exists for a reduction in the detrimental effects of stress. Puvadolpirod and Thaxton, (2000b) observed that broiler chickens given adrenocorticotrophic hormone had significantly lower nutrient digestibility than broilers in the non-stressed control group. These researchers concluded that the reduction in digestion of nutrient was most likely due to an increase in feed passage rate in the presence of the stressor because birds treated with adrenocorticotrophic hormone displayed polydipsia and polyuria during and after stress. Similarly in the present study, we observed poor absorption of nutrients, which could be as a result of decreased villus height in birds fed diet supplemented with DEX.

The jejunal mucosa gene expression of cytokines is presented in Table 3. Dexamethasone-induced effect on relative mRNA expression of jejunal mucosa on IL-6,

Table 3. Relative gene expression⁵ of cytokines, tight junction proteins in jejunal mucosa, plasma antioxidant enzymes of broiler chickens fed diets containing chitosan oligosaccharide (COS) concentration at 0 or 1 g/kg with or without dexamethasone (DEX).^{1,2}

Item	0, g/kg COS		1 g/kg COS		SEM	<i>P</i> -value ³		
	No-DEX	DEX	No-DEX	DEX		COS	DEX	COS × DEX
Jejunal mucosa								
IL-6	1.00	4.74	0.88	2.20	0.621	<0.01	0.02	0.03
IL-8	1.00	0.72	3.80	1.31	0.753	0.04	0.08	0.15
IL-10	1.00	7.27	2.91	3.04	1.504	0.44	0.04	0.05
Occludin	1.00	0.90	3.37	2.11	0.551	<0.01	0.23	0.30
Claudin-1	1.00	0.47	3.10	2.55	0.585	<0.01	<0.01	0.02
TNF- α	1.00	0.68	3.51	2.45	0.653	<0.01	0.30	0.57
MUC-2	1.00	1.53	0.59	1.38	0.332	0.40	0.05	0.70
Antioxidant enzymes ⁴								
SOD, U/mL	174.08	25.23	231.99	110.53	1.567	<0.01	<0.01	<0.01
CAT, U/mL	45.05	15.49	70.77	34.06	0.753	<0.01	<0.01	<0.01
GPx, U/mL	22.49	7.51	42.40	15.18	0.665	<0.01	<0.01	<0.01

¹Data were least squares means of 8 replicate cages with 7 birds per cage.

²*P*-value according to main effects of COS, DEX, and a COS × DEX interaction.

³*P*-value according to main effects of COS, DEX, and a COS × DEX interaction.

⁴CAT, catalase; GPx, glutathione peroxidase; IL, interleukin; MUC2, mucin 2; SOD, superoxide dismutase; TNF- α , tumor necrosis factor α .

⁵Relative gene expression ($2^{-\Delta\Delta C_t}$) was calculated with Glyceraldehyde-3-phosphate dehydrogenase as the endogenous control.

IL-10, and claudin-1 was ameliorated by dietary COS supplementation (interaction; $P < 0.05$). Chitosan oligosaccharide decreased the mRNA expression of IL-8, TNF- α , and occludin, whereas DEX decreased the expression of MUC-2. Glucocorticoids inhibit many of the initial events in an inflammatory response that impact both the innate and adaptive immune responses (Busillo et al., 2011). Thus, the primary anti-inflammatory action of glucocorticoids is to repress a plethora of proinflammatory genes encoding cytokines and chemokines, to resolve the inflammatory process and restore homeostasis. Interestingly, we found increased mRNA expression of IL-10 in birds that were fed diets supplemented with DEX. Interleukin-10 is known to be an inhibitor of proinflammatory cytokines; it is possible that the beneficial effect of DEX partly results from upregulation of IL-10-producing cells.

Glucocorticoids can inhibit inflammation by abrogating the activity of transcription factors (such as nuclear factor- κ B and activator protein-1) that controls the production of proinflammatory cytokines by interacting with glucocorticoid-responding elements when bound to glucocorticoid receptors (Dejager et al., 2014). Glucocorticoids affect toll-like receptor signaling pathways and promote T-helper type 2 responses by increasing the production of interleukin (IL) 4 and IL-10 (Brunetti et al., 1995; Kawabe et al., 2012). The epithelial tight junction complex includes the proteins claudin, occludin, and ZO-1. Some tight junction proteins such as claudin-1 and occludin are important in the assembly and maintenance of tight junctions (Amasheh et al., 2009). In chronically stressed and repeatedly corticosterone-injected rats, a significant reduction in claudin-1 and occludin protein expression was observed (Zheng et al., 2013). The mechanism underlying downregulation of these tight junction proteins remains largely unknown. A higher expression of

claudin-1 within the tight junction was observed in the challenge group fed COS compared with broiler chickens fed diet without COS. The mechanism of the activity of reversible tight junction opening, induced by COS, is known to be involved in the translocation of transmembrane protein JAM-1 (a transmembrane tight junction protein).

Dietary COS supplementation mitigated the DEX-induced effect ($P < 0.05$) on the activity of plasma superoxide dismutase, catalase, and glutathione peroxidase (Table 3). In previous studies by Gao et al., (2010), glucocorticoids have been proven to be involved in the altered redox balance in poultry. The maintenance of redox balance depends on the production and extinguishing of reactive oxygen species. The antioxidative systems, responsible for the quenching of reactive oxygen species, consist of an enzymatic system and non-enzymatic antioxidants. The enzyme defense system consists of SOD, CAT, and GPx. Superoxide dismutase provides the efficient dismutation of superoxide radicals into less toxic hydrogen, whereas CAT and GPx reduce hydrogen peroxide into oxygen and water (Ahmad et al., 2012).

Indeed, COS has shown beneficial effects in relieving oxidative stress induced by certain drugs or under various physiological and pathophysiological conditions (Anandan et al., 2013). Previous studies showed that supplementation of COS in diets resulted in the improvement of serum GPx, SOD, and CAT activities in piglets (Li et al., 2013) and serum SOD activity in beef cattle (Li et al., 2015). In agreement with those findings, the current results showed that dietary supplementation with COS increased plasma SOD, GPx, and CAT activities, which are representative enzymatic antioxidants in broiler chickens. A well-known function of COS is its ability to mitigate stress and improve antioxidant activity (Fenga et al. 2008). One possible mechanism that

explains this potential could be that COS and its derivatives react with free radicals due to the active hydroxyl and amino groups present on their chains. The hydroxyl and amino group in COS can serve as hydrogen donors to the proxy radicals and react with unstable free radicals, hence protecting cells from damage (Fenga et al. 2008). Indeed, in our study, COS supplementation in diets had preprotective effects against oxidative stress induced by DEX in broiler chickens, suggesting that COS might contribute to the enhancement of anti-oxidative functions.

In conclusion, the results of our study indicate that stress mimicked by in-feed DEX treatment could significantly increase BW loss, induce oxidative stress, and suppress immune function. However, dietary COS improved growth performance and immune function in broiler chickens, especially in the presence of stress, which can be partially ascribed to the ability of COS to decrease catabolism and oxidative injury of tissues. Inclusion of COS in broiler diet was effective in improving growth performance by improving villus structure, sustaining a balanced intestinal barrier function, and decreasing stress response. Therefore, COS supplementation may be a potential agent to relieve oxidative stress in immunosuppressed broiler chickens.

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