

An evaluation of the protective effects of chlorogenic acid on broiler chickens in a dextran sodium sulfate model: a preliminary investigation

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ABSTRACT This study was conducted to investigate the protective effects of chlorogenic acid (CGA) on broilers subjected to dextran sodium sulfate (DSS)-induced intestinal damage. One hundred and forty-four 1-day-old male Arbor Acres broiler chicks were allocated into one of 3 groups with 6 replicates of eight birds each for a 21-d trial. The treatments included: 1) Control group: normal birds fed a basal diet; 2) DSS group: DSS-treated birds fed a basal diet; and 3) CGA group: DSS-treated birds fed a CGA-supplemented control diet. An oral DSS administration via drinking water was performed from 15 to 21 d of age. Compared with the control group, DSS administration reduced 21-d body weight and weight gain from 15 to 21 d, but increased absolute weight of jejunum and absolute and relative weight of ileum ($P < 0.05$). DSS administration elevated circulating D-lactate concentration and diamine oxidase activity ($P < 0.05$), which were partially reversed when supplementing CGA ($P < 0.05$). The oral administration with DSS decreased villus height and villus height/crypt depth ratio, but increased crypt depth in jejunum and ileum ($P < 0.05$).

Compared with the control group, DSS administration increased serum glutathione level and jejunal catalase activity and malonaldehyde accumulation, but decreased jejunal glutathione level ($P < 0.05$). In contrast, feeding a CGA-supplemented diet normalized serum glutathione and jejunal malonaldehyde levels, and increased jejunal glutathione concentration in DSS-administrated birds ($P < 0.05$). Additionally, CGA supplementation reduced ileal malonaldehyde accumulation in DSS-treated birds ($P < 0.05$). DSS challenge increased levels of serum interferon- γ and interleukin-6, jejunal interleukin-1 β , tumor necrosis factor- α , and interleukin-6, and ileal interleukin-1 β and interleukin-6 when compared with the control group ($P < 0.05$). The elevated serum interferon- γ and ileal interleukin-6 levels were normalized to control values when supplementing CGA ($P < 0.05$). The results suggested that CGA administration could partially prevent DSS-induced increased intestinal permeability, oxidative damage, and inflammation in broilers, although it did not improve their growth performance and intestinal morphology.

Key words: chlorogenic acid, intestinal damage, antioxidant status, inflammation, broilers

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INTRODUCTION

The intestinal epithelium represents the largest and most important interface between the host and external environment and plays a fundamental role in maintaining intestinal homeostasis and symbiosis as well as mucosal barrier integrity and function (Odenwald and Turner, 2017; Vancamelbeke and Vermeire, 2017). The

dysfunction of intestinal epithelial barrier would lead to abnormal intestinal structure and function, detrimentally modulate digestive process, and trigger occurrence and development of various infections and diseases, ultimately resulting in poor growth performance and mortality in livestock animals and poultry (Yegani and Korver, 2008; Celi et al., 2017; Oviedo-Rondón, 2019). The immature intestinal barrier in young chicks renders them sensitive and vulnerable to numerous stimuli such as bacterial infections, oxidative factors, environmental stress, and mycotoxins (Murugesan et al., 2015; Mishra and Jha, 2019; Rostagno, 2020; Wickramasuriya et al., 2022). The recent phasing out of antibiotics as growth promoters from broiler diets in China and other countries has actually exacerbated the occurrence of intestinal damage and diseases and resulted in considerable economic loss

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(Cervantes, 2015; Karavolias et al., 2018; Maria Cardinal et al., 2019). The maintenance and optimization of intestinal barrier function, therefore, has important implications for the health and growth performance of broiler chickens. Several experimental models have been established to induce intestinal barrier injury and dysfunction, and to evaluate the effectiveness of dietary modulations in poultry, including pathogenic infections (e.g., *Salmonella*, *Clostridium perfringens*, and coccidiosis), feed deprivation, and stress-inducing agents such as lipopolysaccharide, dexamethasone, and dextran sodium sulphate (DSS), as summarized previously (Gilani et al., 2021). DSS, a polyanionic derivative of dextran, is produced by esterification of dextran with chlorosulphonic acid, and the DSS model of colitis is widely perceived as a generalized model of experimental colitis in rodent animals, mainly due to its similarities with human inflammatory bowel disease in etiology, pathology, and pathogenesis (Solomon et al., 2010; Perše and Cerar, 2012). It has been generally accepted that DSS is toxic to colonic epithelial cells, and therefore could damage epithelial barrier structure and integrity, induce inflammatory cell infiltration and severe mucosal inflammation, and cause microflora imbalance, leading to intestinal barrier failure and bacterial translocation (Solomon et al., 2010; Eichele and Kharbanda, 2017). Aside from inducing colitis, the administration of DSS through drinking water has been also shown to successfully induce small intestinal barrier damage in broiler chickens, as evident by the disrupted intestinal structure, diffuse enteric inflammation, and increased gut permeability (Kuttappan et al., 2015, 2016; Menconi et al., 2015; Simon et al., 2016; Gilani et al., 2017; Murai et al., 2018; Zou et al., 2018; Liu et al., 2021), which, in turn, suggests that DSS may be an ideal model for the establishment of intestinal barrier damage in broiler chickens.

Numerous nutritional interventions have been proposed and implemented to improve gut functionality and maintain intestinal health in practical broiler production, including amino acids, vitamins, feed enzymes, trace minerals, probiotics, prebiotics, acidifiers, and plant extracts (Yegani and Korver, 2008; Adedokun and Olojede, 2019; Alagawany et al., 2021). Among them, the plant-derived feed additives have received increasing attention in animal nutrition due to their multiple biological activities, wide range of sources, lack of residue and drug resistance, low toxicity, easy availability, and environmental friendliness (Zeng et al., 2015; Sugiarto, 2016; Patra et al., 2019; Abdel-Moneim et al., 2020). The chlorogenic acid (CGA), termed as 5-*O*-caffeoylquinic acid, is an ester of caffeic acid with quinic acid and is one of the most important biologically functional polyphenolic compounds found in the human diets (Liang and Kitts, 2015). CGA is abundantly available in a variety of plant species especially in coffee beans, potato tubers, sweet potato leaves, eggplant, artichoke, and sunflower seed kernels (Liang and Kitts, 2015; Santana-Gálvez et al., 2017; Tajik et al., 2017; Lu et al., 2020). The cumulative experimental and clinical evidence has already shown that CGA exhibited a series of

biological properties, such as antioxidant, antimicrobial, antiviral, immunomodulatory, cytoprotective, hypoglycemic, hypocholesterolemic, and antitumor characteristics (Santana-Gálvez et al., 2017; Lu et al., 2020). It has been demonstrated that CGA could effectively mitigate intestinal damage and maintain intestinal barrier integrity and function in a DSS-induced colitis mouse/rat model and in rodent animals subjected to high-fat diet, heavy metal or lipopolysaccharide challenge through restoring the expression of tight junction proteins and myosin light chain kinase, inhibiting inflammatory response, preventing apoptosis of intestinal epithelial cells, and beneficially regulating gut microflora composition and their fermentation (Ruan et al., 2014, 2016; Shin et al., 2015; Vukelić et al., 2018; Xue et al., 2019; Xie et al., 2021). CGA has also been incorporated into diet as a potential feed additive in livestock, poultry, and ruminant. As for weaned piglets, dietary supplementation with CGA at a level of 1,000 mg/kg has been found to enhance growth performance through maintaining antioxidant capacity and intestinal digestion and absorption function, and to improve intestinal barrier function by suppressing mucosal inflammation and cell apoptosis and by maintaining redox status and gut microbiota composition and functionality (Chen et al., 2018a,b,c, 2019). Moreover, a graded supplementation of CGA could improve gut morphology, intestinal barrier function and antioxidant capacity, and intestinal selected bacterial populations in weaned piglets (Zhang et al., 2018). Recently, CGA has been demonstrated to effectively improve growth performance, inhibit small intestine structural damage, improve redox status, prevent damage to ileal mucosal layer construction and tight junctions, and suppress the expression inflammatory cytokines in *Clostridium perfringens*-challenged and/or coccidia-infected broiler chickens (Zhang et al., 2020; Liu et al., 2022a). CGA has also been reported to alleviate heat stress-induced intestinal damage in young hens through suppressing inflammation and improving antioxidant capacity and cecal microbiota composition (Chen et al., 2021). However, little is known about the protective effects of CGA in intestinal damage resulting from the administration of stress-inducing chemicals such as lipopolysaccharide, dexamethasone and DSS in broiler chickens. In this study, a DSS model of intestinal damage was therefore established to investigate the protective effects of CGA on intestinal barrier function in broiler chickens by determining growth performance, and the intestinal permeability, morphology, antioxidant capacity, and cytokine levels, which could provide reference and basis for the establishment of experimental models and future rational utilization of CGA in broiler feed.

MATERIALS AND METHODS

Animal, Diets, and Management

All animal experiments conducted in this study were performed in accordance with the protocol set by the

Institutional Animal Care and Ethics Committee of Nanjing Agricultural University, recognized by the Jiangsu Provincial Department of Science and Technology, P.R. China (SYXK-2017-0007).

A total of one hundred and forty-four 1-day-old male Arbor Acres Plus broiler chicks with a similar initial body weight were distributed in a completely randomized design into 3 groups with 6 replicates (pens) of 8 birds each for a 21-d feeding trial. The three experimental treatments were: 1) Control group: normal birds fed a basal diet; 2) DSS group: DSS-treated birds fed a basal diet; and 3) CGA group: DSS-treated birds fed a basal diet supplemented with 1,000 mg/kg CGA of diet. Birds were orally administrated with DSS (Catalog No. S14048; Molecular weight, 50 KDa; Shanghai Yuanye Bio-Technology Co., Ltd., Shanghai, P.R. China) at a concentration of 2.5% via drinking water for a consecutive 7 d from 15 to 21 d of age according to the method described previously (Simon et al., 2016; Zou et al., 2018). The supplemental CGA was kindly provided by Hunan E.K Herb Co., Ltd. (Changsha, Hunan province, P.R. China) and was separated, extracted, and purified from a traditional Chinese herb, *Eucommia ulmoides* leaves. Its purity was 98.65% when analyzing it with a high-performance liquid chromatography-mass spectrometry system (LCMS-8040; Shimadzu, Kyoto, Japan), using the 5-*O*-caffeoylquinic acid as a standard. The supplemental level of CGA used in the current research was selected according to available findings in both broiler chickens and piglets (Chen et al., 2018a,c; Zhang et al., 2020; Liu et al., 2022a). The corn-soybean meal basal diet offered in mash form was formulated according to the NRC (1994) nutrient requirement recommendations set for broiler chickens during the starter period (0–3 wk), and its ingredient formula and nutrient value on a dry matter basis as well as the premix composition are presented in Table 1. The CGA was initially pre-mixed thoroughly with the premix in a vertical screw mixer (DSH-0.04, Tongxiang Jinzhong Machinery Co., Ltd., Jiaxing, Zhejiang province, P.R. China) until they were homogeneous and afterwards the supplements were added to prepare the complete feed, depending on the treatment. All feed ingredients (except premix) were thoroughly mixed together in a single batch process, which were then equally divided into three halves in order to minimize nutrient variation among these three experimental diets. In this feeding experiment, the birds were reared in clean stainless-steel cages (150 cm × 70 cm × 50 cm) equipped with perforated plastic floors and were allowed free access to feed and drinking water during the whole period of this feeding trial except when necessary feed withdrawal deprivation period was performed for the measurement of body weight. The indoor temperature of the thermostatically controlled chicken house was set at around 33 to 34°C during the initial week after arrival and it was then decreased by 2°C to 3°C at weekly interval. A 23-h light and 1-h dark lighting regime was provided for broiler chickens during the entire experimental period except the initial 3 consecutive days when a 24-h light schedule was performed for

Table 1. Composition and nutrient level of the basal diet.

Ingredients, %	Content
Corn	57.00
Soybean meal	31.50
Corn gluten meal	3.40
Soybean oil	3.10
Limestone	1.20
Dicalcium phosphate	2.00
L-Lysine	0.34
DL-Methionine	0.15
Sodium chloride	0.31
Premix ¹	1.00
Total	100
Calculated nutrient levels	
Apparent metabolizable energy, MJ/kg	12.56
Crude protein, %	21.33
Calcium, %	1.00
Total phosphorus, %	0.68
Available phosphorus, %	0.46
Lysine, %	1.21
Methionine, %	0.50
Methionine + cystine, %	0.86

¹Premix provided per kilogram of diet: vitamin A (transretinyl acetate), 10,000 IU; vitamin D₃ (cholecalciferol), 3,000 IU; vitamin E (all-rac- α -tocopherol), 30 IU; menadione, 1.3 mg; thiamin, 2.2 mg; riboflavin, 8 mg; nicotinamide, 40 mg; choline chloride, 600 mg; calcium pantothenate, 10 mg; pyridoxine-HCl, 4 mg; biotin, 0.04 mg; folic acid, 1 mg; vitamin B₁₂ (cobalamin), 0.013 mg; Fe (from ferrous sulfate), 80 mg; Cu (from copper sulphate), 8.0 mg; Mn (from manganese sulphate), 110 mg; Zn (from zinc oxide), 60 mg; I (from calcium iodate), 1.1 mg; Se (from sodium selenite), 0.3 mg.

adaptation to the environment. The relative humidity of indoor air in the chicken house was maintain at around 70% during the initial three days after arrival at the farm, which was then set at 60 to 65% thereafter.

Sample Collection

One bird was randomly selected from each pen replicate (6 birds from each group and 18 birds in total) at 21 d of age for slaughter and sampling. To avoid possible starvation-induced intestinal damage, a 12 h feed withdrawal period was not done in these sampled flocks. The blood samples were taken from wing vein and collected into a cool and sterile plastic centrifuge tube or an anti-coagulant tube coated with sodium heparin in the morning. The separated plasma and serum samples were collected and equally aliquoted into new sterile Eppendorf tubes and stored at –80°C prior to performing analyses after necessary centrifugation at 4450 × *g* for 15 min at 4°C, using an Eppendorf 5804R refrigerated centrifuge (Hamburg, Germany). After being euthanized by cervical dislocation, the necropsy was immediately performed on birds selected from each pen after the birds were completely dead. The liver, spleen, jejunum (from the end of the duodenum to the Meckel's diverticulum), and ileum (from Meckel's diverticulum to the ileocecal junction) were then excised, trimmed of connective tissues and fat, and weighed to calculate relative organ weight according to the following formula: Relative organ weight (g/kg) = organ weight (g) / final body weight of the individual animal. After dissection the small intestine was separated from the end of the

duodenum to the ileocaecal junction from each euthanized bird, transferred to a chilled stainless-steel tray, and cut into 2 segments (jejunum and ileum), using sterile tweezers and scalpel. After this procedure, a 2-cm-length segment of mid jejunum and ileum was carefully cut, cleaned, gently washed with iced saline solution, and transferred to a 10% neutral buffered formalin solution for histological fixation and subsequent tissue slice staining. The jejunal and ileal segments were then longitudinally opened along its mesenteric border in full length to be converted into a large rectangular mucosal patch after the removal of digesta via through gentle squeezing and washing with phosphate-buffered saline. The intestinal mucosa was carefully and gently scraped and harvested from the everted intestine, pooled, and collected into cryogenic tubes, which was then stored in liquid nitrogen tank at -196°C for further measurement after immediate snap-frozen.

Determination of Growth Performance

At 14 and 21 d of age, all experimental birds were weighed on pen (replicate) basis at early morning after being subjected to a 12-h feed withdrawal period (around 5:30 am) to calculate average body weight and average daily gain (ADG). Also, feed consumption was recorded at weekly intervals to calculate average feed intake (ADFI) and feed conversion ratio (FCR) after adjusting weight of mortalities.

Measurement of Blood Intestinal Permeability-related Indices

The blood diamine oxidase activity and D-lactate level are two sensitive parameters reflecting intestinal permeability (Ducatelle et al., 2018; De Meyer et al., 2019). The serum diamine oxidase activity was quantified with a commercial colorimetric assay kit (Catalog No. A088-1-1, Nanjing Jiancheng Bioengineering Institute, Nanjing, Jiangsu Province, P.R. China), while the measurement of circulating diamine oxidase activity was performed using a visible colorimetric test kit (Catalog No. AAT-13811; AAT Bioquest, Sunnyvale, CA). All measurements were performed in random order strictly following the recommended protocol of the corresponding manufacturer, using a MODEL 680 microplate reader (Bio-Rad Laboratories Inc., Hercules, CA) at different wavelengths.

Histological Measurement

The histological measurement was done according to a method as previously described (Chen et al., 2020). In detail, the fixed intestinal segments were dehydrated in serial alcohol solutions of increasing concentrations, cleared in xylene, and embedded in paraffin wax. The wax blocks were then further processed for section cutting and staining. Six micrometer thickness paraffin sections were prepared and mounted to glass slides by

incubation overnight at room temperature. The slides were then deparaffinized with xylene and rehydrated in graded alcohol and distilled water prior to Hematoxylin & Eosin staining. After staining, the slides were digitally photographed with high resolution camera and the villus height (VH) and crypt depth (CD) of 6 well-preserved villi and crypts were measured and means calculated for each, using a Nikon eclipse 80i microscope equipped with a computer-assisted morphometric system (Nikon Corporation, Tokyo, Japan).

Evaluation of Antioxidant Capacity

After being removed from liquid nitrogen, the jejunum and ileum mucosal scrapings were thawed, cut into small slices, weighed, transferred into sterile tubes, and homogenized with an iced saline solution at a weight/volume ratio of 1: 4 in an ice-cold water bath until no tissue particles were visible, using a motor-driven homogenizer (PRO-PK-02200D, Pro Scientific, Inc., Monroe, CT). Tubes were centrifuged at $4450 \times g$ at 4°C for 15 min and the supernatant was collected and aliquoted into Eppendorf tubes and stored at -80°C for subsequent analysis of antioxidant-related parameters, including superoxide dismutase (SOD), catalase (CAT), reduced form of glutathione (GSH), and malondialdehyde (MDA).

The activities of SOD (Catalog No. A001-1-1) and CAT (Catalog No. A007-1-1), and the levels of GSH (Catalog No. A006-1) and MDA (Catalog No. A003-1) in serum and tissues (jejunal and ileal mucosa) were colorimetrically measured with the commercial assay kits at the different wavelengths following the standardized manual provided by the manufacturer (Nanjing Jiancheng Bioengineering Institute, Nanjing, Jiangsu Province, P.R. China), using a microplate reader (MODEL 680, Bio-Rad Laboratories Inc., Hercules, CA). Briefly, a classic hydroxylamine method was performed to quantify SOD activity (Kono, 1978), and one unit of which was defined as the amount of SOD needed to produce half inhibition of nitrite production rate per milliliter of serum or per milligram protein of tissue samples in 40 min at 37°C . The 5, 5'-dithiobis (2-nitrobenzoic acid) method was adopted for the determination of GSH level (Owens and Belcher, 1965). As for the measurement of CAT activity, the ammonium molybdate method was selected and performed (Góth, 1991), and one unit of CAT activity was defined as the amount of this target enzyme decomposing one micromole hydrogen peroxide per milliliter of serum or per milligram protein of tissue in one min at 37°C . The classic thiobarbituric acid method (Placer et al., 1966) was used to determine MDA accumulation in blood and intestinal mucosa. All results in intestinal mucosal samples were normalized against the corresponding total protein concentration prior to comparison, whose concentration was measured by the Bradford assay method (Kruger, 1994), using the crystalline bovine serum albumin (Sigma-Aldrich, St Louis, MO) as a reference standard.

Quantification of Cytokines

The preparation of intestinal mucosal homogenate for the determination of inflammatory cytokine levels was performed as mentioned above. The chicken-specific enzyme-linked immunosorbent assay kits (CUSABIO Technology LLC, Wuhan, P.R. China) were purchased to measure the concentrations of interleukin-1 β (**IL-1 β** , Catalog No. CSB-E11230Ch, Sensitivity: 0.27 pg/mL), interleukin-6 (**IL-6**, Catalog No. CSB-E08549Ch, Sensitivity: 3.9 pg/mL), interferon- γ (**IFN- γ** , Catalog No. CSB-CSBE08550Ch, Sensitivity: 3.125 pg/mL), and tumor necrosis factor- α (**TNF- α** , Catalog No. CSB-E11231Ch, Sensitivity: 0.27 pg/mL) in plasma and intestinal mucosa of broiler chickens according to the protocols described in manual after necessary dilution with sterile saline of the samples when their absorbance exceeded the range of calibration curve. The intra-assay and inter-assay coefficient of variation of these kits was less than 8% and 10%, respectively. The Bradford method (Kruger, 1994) was performed to determine total protein level in each sample, using the crystalline bovine serum albumin as a reference protein. All acquired results of the small intestinal mucosal samples were adjusted against the total protein level before inter-sample comparisons. The measurements were done in duplicate and the mean value was used for statistical calculation.

Statistical Analysis

Data were analyzed by one-way analysis of variance (ANOVA), using a SPSS statistical software (Ver.22.0 for windows, SPSS Inc., Chicago, IL). A cage (replicate) was the experimental unit for the growth performance data, while an individual bird selected from each replicate was the experimental unit for other measured indices. Differences among groups were tested using Duncan's multiple range test and the level of statistical significance was set at $P < 0.05$. The results are presented as means with their pooled standard errors.

RESULTS

Growth Performance

There was no significant difference in growth performance (ADG, ADFI, or FCR) among treatment groups prior to DSS challenge (1–14 d of age, Table 2). In contrast, an oral administration with DSS (Table 2) via drinking water decreased 21-d average body weight ($P < 0.001$) and ADG from 15 to 21 d ($P < 0.001$) in broiler chickens. However, dietary CGA supplementation did not improve growth performance of DSS-treated birds, as evident by their similar 21-d body weight and ADG to those of DSS challenge group ($P > 0.05$), which were also significantly lower than those of control group ($P < 0.05$).

Table 2. Effects of dietary chlorogenic acid supplementation on growth performance in dextran sodium sulfate-challenged broiler chickens.

Items ¹	Treatments ²			SEM ³	P-value
	CON	DSS	DSS + CGA		
Prior to challenge (1 to 14 days)					
ADG, g/d/bird	27.99	28.17	27.25	0.90	0.918
ADFI, g/d/bird	35.46	34.80	33.57	1.21	0.827
FCR, g/g	1.27	1.23	1.23	0.01	0.400
14-d BW	428.33	430.83	418.00	13.40	0.932
After challenge (15–21 d)					
21-d BW	692.83 ^a	573.83 ^b	578.80 ^b	25.31	<0.001
ADG, g/d/bird	37.79 ^a	20.43 ^b	22.97 ^b	3.94	<0.001

^{a,b}Means within a row with different superscripts are different at $P < 0.05$.

¹ADG = average daily gain; ADFI = average daily feed intake; BW = body weight; FCR = feed conversion ratio.

²CON = nonchallenged broilers fed a basal diet; DSS = dextran sodium sulfate-challenged broilers fed a basal diet; DSS + CGA = dextran sodium sulfate-challenged broilers fed a basal diet supplemented with 1.0 g/kg chlorogenic acid.

³SEM = standard error of the mean (n = 6).

Organ Weight

As indicated in Table 3, DSS treatment reduced absolute weight of jejunum ($P = 0.027$) and decreased both absolute ($P = 0.001$) and relative ($P = 0.019$) weight of ileum in broiler chickens, when compared with their normal counterparts. Dietary supplementation with CGA did not alter absolute weight of jejunum and the absolute and relative weight of ileum due to their insignificant difference between CGA and DSS groups ($P > 0.05$). Moreover, the values of these aforementioned indices in CGA-supplemented group were all lower than those of control group ($P < 0.05$). There was no significant difference in liver or spleen weight, regardless of relative or absolute values ($P > 0.05$).

Table 3. Effects of dietary chlorogenic acid supplementation on organ weight in dextran sodium sulfate-challenged broiler chickens.

Items	Treatments ¹			SEM ²	P-value
	CON	DSS	DSS + CGA		
Liver					
Absolute weight, g	20.30	16.79	19.04	1.08	0.415
Relative weight, g/kg	28.99	29.06	33.13	1.18	0.298
Spleen					
Absolute weight, g	0.66	0.59	0.51	0.03	0.182
Relative weight, g/kg	0.97	1.02	0.89	0.04	0.473
Jejunum					
Absolute weight, g	14.74 ^a	12.37 ^b	11.67 ^b	0.52	0.027
Relative weight, g/kg	21.87	21.41	20.34	0.77	0.747
Ileum					
Absolute weight, g	14.22 ^a	9.79 ^b	8.53 ^b	0.77	0.001
Relative weight, g/kg	20.84 ^a	16.88 ^b	14.94 ^b	0.93	0.019

^{a,b}Means within a row with different superscripts are different at $P < 0.05$.

¹CON = nonchallenged broilers fed a basal diet; DSS = dextran sodium sulfate-challenged broilers fed a basal diet; DSS + CGA = dextran sodium sulfate-challenged broilers fed a basal diet supplemented with 1.0 g/kg chlorogenic acid.

²SEM = standard error of the mean (n = 6).

Table 4. Effects of dietary chlorogenic acid supplementation on intestinal permeability and morphology in dextran sodium sulfate-challenged broiler chickens.

Items	Treatments ¹			SEM ²	P-value
	CON	DSS	DSS + CGA		
Serum					
D-lactate, mmol/L	0.51 ^b	0.75 ^a	0.59 ^{a,b}	0.04	0.040
Diamine oxidase, U/L	10.53 ^b	19.10 ^a	11.54 ^b	1.05	<0.001
Jejunum					
Villus height, μm	1448.43 ^a	1182.13 ^b	1144.74 ^b	37.31	<0.001
Crypt depth, μm	264.08 ^b	294.30 ^a	284.98 ^a	4.76	0.018
Villus height/crypt depth	5.49 ^a	4.17 ^b	3.88 ^b	0.18	<0.001
Ileum					
Villus height, μm	1030.97 ^a	896.66 ^b	953.22 ^b	18.18	0.003
Crypt depth, μm	235.51 ^b	283.69 ^a	259.20 ^{ab}	7.41	0.018
Villus height/crypt depth	4.38 ^a	3.21 ^b	3.71 ^b	0.15	0.001

^{a,b}Means within a row with different superscripts are different at $P < 0.05$.

¹CON = nonchallenged broilers fed a basal diet; DSS = dextran sodium sulfate-challenged broilers fed a basal diet; DSS + CGA = dextran sodium sulfate-challenged broilers fed a basal diet supplemented with 1.0 g/kg chlorogenic acid.

²SEM = standard error of the mean (n = 6).

Intestinal Permeability and Morphology

Compared with the control group (Table 4), DSS administration resulted in an increase in circulating D-lactate concentration ($P = 0.040$) and diamine oxidase activity ($P < 0.001$) in broilers. In contrast, supplementing CGA reversed the elevated blood diamine oxidase activity ($P < 0.05$) in comparison with the DSS-treated birds fed a basal diet, with its value being statistically equivalent to that of control group ($P > 0.05$). Although not statistically different, the serum D-lactate concentration in DSS-challenged birds was numerically reduced when supplementing CGA ($P > 0.05$), and its value in CGA-supplemented group was comparable with that of control group ($P > 0.05$).

The oral administration with DSS through drinking water decreased VH and the ratio between VH and CD, but increased CD in both jejunum and ileum of broiler chickens ($P < 0.05$), when compared with the control group ($P > 0.05$). Dietary supplementation with CGA numerically reduced ileal CD in DSS-treated birds ($P > 0.05$), with its value being similar to that of control group ($P < 0.05$). The DSS-treated birds fed a CGA-supplemented diet exhibited similar jejunal and ileal VH and VH/CD ratio as well as jejunal CD to their DSS-challenged counterparts fed a basal diet only ($P > 0.05$), and these parameters were significantly lower (VH and VH/CD ratio) or higher (CD) than those of normal birds in control group ($P < 0.05$).

Antioxidant Capacity

Compared with the control birds (Table 5), an oral DSS administration elevated serum GSH level ($P = 0.003$) and increased CAT activity ($P = 0.033$) and MDA accumulation in jejunal mucosa ($P < 0.001$), but decreased jejunal mucosal GSH level ($P = 0.031$) in

broiler chickens. In contrast, dietary CGA administration significantly decreased serum GSH level and jejunal mucosal MDA accumulation ($P < 0.05$), but increased GSH concentration in jejunal mucosa ($P < 0.05$), when compared with the DSS-challenged birds given a control diet, with their values being comparable with those of control group ($P > 0.05$). CGA incorporation also numerically reduced jejunal mucosal CAT activity when compared with the DSS group, but this difference did not reach a significant level ($P > 0.05$), with its value in CGA-supplemented group being statistically similar to that of control group ($P > 0.05$). Likewise, dietary CGA supplementation also reduced ileal mucosal MDA accumulation in comparison with the DSS group ($P = 0.046$), and the value of which was intermediate in the control group ($P > 0.05$). However, treatment did not alter SOD activity in serum and tissues (jejunal and ileal mucosa), CAT activity in serum and ileal mucosa, serum MDA accumulation, or GSH level in ileal mucosa ($P > 0.05$).

Inflammatory Cytokines

As illustrated in Table 6, DSS drinking elevated the levels of serum IFN- γ ($P = 0.024$) and IL-6 ($P = 0.019$), jejunal mucosal IL-1 β ($P = 0.017$), TNF- α ($P = 0.009$), and IL-6 ($P = 0.021$), and ileal mucosal IL-1 β ($P = 0.036$) and IL-6 ($P = 0.001$) in broiler chickens in comparison with the control group. Moreover, birds in the control group also exhibited lower concentrations of jejunal mucosal IL-1 β and TNF- α and ileal mucosal IL-1 β than their DSS-treated counterparts given a CGA-supplemented diet ($P > 0.05$), and the values of these aforementioned parameters were comparable between the two DSS-administrated groups, supplemented with

Table 5. Effects of dietary chlorogenic acid supplementation on intestinal permeability and morphology in dextran sodium sulfate-challenged broiler chickens.

Items ¹	Treatments ²			SEM ³	P-value
	CON	DSS	DSS + CGA		
Serum					
SOD, U/mL	222.44	196.68	206.63	4.69	0.068
CAT, U/mL	2.20	3.60	2.27	0.35	0.194
GSH, mg/L	7.12 ^b	11.08 ^a	9.03 ^b	0.53	0.003
MDA, nmol/mL	1.55	2.01	1.97	0.18	0.550
Jejunum					
SOD, U/mg protein	129.92	139.18	144.98	3.08	0.130
CAT, U/mg protein	0.72 ^b	1.80 ^a	1.19 ^{a,b}	0.18	0.033
GSH, mg/g protein	31.11 ^a	22.53 ^b	30.19 ^a	1.53	0.031
MDA, nmol/mg protein	0.54 ^b	1.32 ^a	0.51 ^b	0.10	<0.001
Ileum					
SOD, U/mg protein	130.36	127.62	129.29	2.22	0.891
CAT, U/mg protein	0.83	1.16	0.90	0.16	0.700
GSH, mg/g protein	22.61	22.62	18.90	1.01	0.233
MDA, nmol/mg protein	0.56 ^{ab}	0.68 ^a	0.51 ^b	0.03	0.046

^{a,b}Means within a row with different superscripts are different at $P < 0.05$.

¹CAT = catalase; GSH = reduced glutathione; MDA = malondialdehyde; SOD = superoxide dismutase.

²CON = nonchallenged broilers fed a basal diet; DSS = dextran sodium sulfate-challenged broilers fed a basal diet; DSS + CGA = dextran sodium sulfate-challenged broilers fed a basal diet supplemented with 1.0 g/kg chlorogenic acid.

³SEM = standard error of the mean (n = 6).

Table 6. Effects of dietary chlorogenic acid supplementation on levels of cytokines in plasma and intestinal mucosa of dextran sodium sulfate-challenged broiler chickens.

Items ¹	Treatments ²			SEM ³	P-value
	CON	DSS	DSS + CGA		
Serum					
IFN- γ , pg/mL	20.97 ^b	35.41 ^a	22.49 ^b	2.51	0.024
IL-1 β , pg/mL	310.79	293.70	273.04	8.43	0.193
TNF- α , pg/mL	388.03	411.24	329.59	29.70	0.541
IL-6, pg/mL	10.73 ^b	12.16 ^a	11.19 ^{a,b}	0.23	0.019
Jejunum					
IFN- γ , ng/g protein	1.84	1.85	1.91	0.03	0.676
IL-1 β , ng/g protein	2.33 ^b	7.62 ^a	6.71 ^a	0.87	0.017
TNF- α , ng/g protein	16.66 ^b	34.77 ^a	20.91 ^a	2.75	0.009
IL-6, ng/g protein	122.11 ^b	222.26 ^a	163.23 ^{a,b}	15.74	0.021
Ileum					
IFN- γ , ng/g protein	1.91	1.91	1.91	0.06	0.999
IL-1 β , ng/g protein	3.48 ^b	25.98 ^a	22.24 ^a	3.99	0.036
TNF- α , ng/g protein	15.80	12.28	15.03	2.05	0.784
IL-6, ng/g protein	82.36 ^b	204.17 ^a	111.42 ^b	16.12	0.001

^{a,b}Means within a row with different superscripts are different at $P < 0.05$.

¹IFN- γ = interferon- γ ; IL-1 β = interleukin-1 β ; IL-6 = interleukin-6; TNF- α = tumor necrosis factor- α .

²CON = non-challenged broilers fed a basal diet; DSS = dextran sodium sulfate-challenged broilers fed a basal diet; DSS + CGA = dextran sodium sulfate-challenged broilers fed a basal diet supplemented with 1.0 g/kg chlorogenic acid.

³SEM = standard error of the mean (n = 6).

or without CGA ($P > 0.05$). In contrast, the elevated serum IFN- γ and ileal IL-6 levels were normalized to control values when supplementing a basal diet with CGA ($P < 0.05$). Moreover, dietary incorporation with CGA also numerically decreased serum and jejunal IL-6 levels ($P > 0.05$), with the values of these two parameters being comparable with those of control group ($P > 0.05$). However, there was no significant difference in serum IL-1 β and TNF- α levels, jejunal IFN- γ concentration, or ileal IFN- γ and TNF- α levels among these three experimental groups ($P > 0.05$).

DISCUSSION

DSS administration via drinking water is a classic method to establish experimental murine colitis mode (Solomon et al., 2010; Perše and Cerar, 2012; Eichele and Kharbanda, 2017). After being orally administrated, the soluble chemical toxin, DSS, would directly damage intestinal epithelium and cellular hemostasis and induce severe intestinal inflammation and oxidative injury; the DSS-induced intestinal barrier integrity damage would subsequently lead to entry of luminal bacteria and associated antigens into the mucosa and infiltration of the inflammatory immune cells into the mucosal and submucosal areas, eventually resulting in mortality, diarrhea, decreased feed intake, and weight loss in rodents (Clapper et al., 2007; Eichele and Kharbanda, 2017). Intestinal disorders and damage in commercial broilers are important factors accounting for the productivity losses and mortality (Celi et al., 2017; Oviedo-Rondón, 2019). As for broiler chickens, an oral DSS administration has been shown to

induce histopathological and morphometric changes in the small intestine, cause generalized mild and non-necrotic enteritis, and result in body weight loss, diarrhea and intestinal bleeding in broilers (Menconi et al., 2015; Kuttappan et al., 2016), which, in turn, enable this compound to be useful for intestinal injury modeling in broilers. Simon et al. (2016) observed that an oral administration of 2.5% DSS via drinking water during 10 to 18 d post-hatching reduced 14-d and 21-d body weight in broiler chickens. In the current research, a 7-d consecutive DSS administration at a concentration of 2.5% reduced 21-d average body weight and ADG from 15 to 21 d in broiler chickens, which was in agreement with the findings of Zou et al. (2018) and Simon et al. (2016) in broilers. The decreased body weight and weight gain observed in this study can be partially explained by the reduced small intestinal apical hydrolase activities, acute enteric inflammation, damaged intestinal barrier integrity, increased intestinal permeability, disordered metabolism, and liver dysfunction, as previously reported in broilers, laying hens, and piglets (Lackeyram et al., 2012; Young et al., 2012; Kuttappan et al., 2015, 2016; Menconi et al., 2015; Simon et al., 2016; Murai et al., 2018; Zou et al., 2018; Nii et al., 2020; Liu et al., 2021). In broiler chickens, Zhang et al. (2020) have found that dietary supplementation with 500 mg/kg CGA increased weight gain and feed conversion efficiency in young broiler chickens challenged with *Clostridium perfringens*. Moreover, Liu et al. (2022a) also observed that an administration of CGA at a dosage of 1 g/kg significantly increased ADG and reduced FCR during 14 to 21 d in broilers subjected to coccidia challenge. In this study, however, feeding a CGA-supplemented diet did not improve weight gain and body weight in DSS-treated broilers. The harmful consequences of DSS challenge have been shown to be closely associated with its dosage and molecular weight, animal species and age, and experimental duration (Solomon et al., 2010). Compared with rodents, chickens are more sensitive to DSS challenge, and a single oral 0.75% DSS challenge through drinking water at 3 d of age would even lead to a significantly lower body weight in broiler chicks at 6, 9, and 11 d of age (Menconi et al., 2015). Similarly, Simon et al. (2016) also observed that the addition of 2.5% DSS in drinking water from 11 to 18 d of age significantly decreased body weight in broilers, and their body weight was recovered at 28 d of age. The unchanged weight gain and body weight in DSS-treated birds fed a CGA-supplemented diet may be associated with the concentration of DSS, bird age, and experimental duration.

The colonic damage is a typical pathological feature in DSS-induced colitis model in rodent animals, which is usually characterized by a shortening of colon length (Solomon et al., 2010; Perše and Cerar, 2012; Eichele and Kharbanda, 2017). Aside from the colonic pathological changes, the small intestine is also a target organ in DSS murine mode. Huynh et al. (2019) have observed a reduced small intestine weight in mice subjected to DSS-induced colitis. In this study, the absolute

weight of jejunum and absolute and weight of ileum were reduced when feeding birds a DSS-containing drinking water, indicating that DSS may disturb normal intestinal function. In consistent with the increased intestine weight, an oral DSS administration increased serum D-lactate concentration and diamine oxidase activity in broiler chickens in this research. The D-lactate, mainly generated by intestinal bacteria, is an indigenous products in gut and normally its blood level is maintained at a quite low level; however, an efflux of bacteria and their metabolic products including D-lactate would enter into the circulation when the intestinal mucosa is severely damaged and intestinal permeability is increased (Sun et al., 2001; Levitt and Levitt, 2020). The diamine oxidase is found in various tissues in animal bodies but it is especially active in intestinal mucosa, and this enzyme normally occur in very small amount in blood and its basal plasma level is positively correlated with the maturity and integrity of the intestinal mucosa in animals (Wolvekamp and de Bruin, 1994). These 2 indices are sensitive to reflect intestinal damage and permeability and considered as reliable biomarkers for monitoring intestinal health in poultry (Wang et al., 2015; Ducatelle et al., 2018). The DSS-induced increase in intestinal permeability would account for the elevated circulating D-lactate concentration and diamine oxidase activity since DSS challenge would destroy intestinal barrier integrity and function through directly damaging intestinal epithelium, inducing intestinal inflammation, and disrupting gut microflora composition as reported previously in broiler chickens (Kuttappan et al., 2015; Simon et al., 2016; Murai et al., 2018; Zou et al., 2018, 2019). The increased intestinal permeability resulting from DSS administration has also been reported by Kuttappan et al. (2015) in broiler chickens, as evident by a higher leakage of fluorescein isothiocyanate dextran into serum. Moreover, an increased serum D-lactate level has been also observed in broiler chickens fed a DSS-containing water (Zou et al., 2019). The increased values of these two circulating intestinal permeability-related parameters in this study were in parallel with the simultaneously decreased VH and VH/CD ratio and increased CD in jejunum and ileum, which, in turn, suggested that the toxic chemical, DSS, impaired intestinal morphology. The altered intestinal morphology could be traced to the DSS-induced disrupted intestinal epithelial cell homeostasis, decreased epithelial cell proliferation, and accelerated epithelial cell apoptosis (Tessner et al., 1998; Yuan et al., 2015; Zou et al., 2016). The similar results have also been found previously in broiler chickens (Kuttappan et al., 2015; Menconi et al., 2015; Zou et al., 2018, 2019). The beneficial effects of CGA administration in DSS-induced colitis of rodent animals have been actually reported, which has been demonstrated to be correlated with the inhibition of inflammatory response and oxidative stress as well as its regulation on colonic microbiota composition (Shin et al., 2015; Zhang et al., 2017; Vukelić et al., 2018; Zhang et al., 2019; Wan et al., 2021). In this study, supplementing a CGA-

supplemented diet reversed the elevated serum diamine oxidase activity, and the serum D-lactate concentration and ileal CD were both normalized to control values, which together indicated that dietary CGA incorporation exerted beneficial consequences on intestinal integrity and permeability in broiler chickens. Likewise, Liu et al. (2022a) have reported that dietary CGA administration especially at a level of 1,000 mg/kg significantly reduced blood D-lactate concentration and diamine oxidase activity and improved jejunal and ileal morphology in coccidia-infected broilers. The underlying mechanisms accounting for the beneficial regulatory effects of CGA in intestinal barrier are diverse and may overlap. CGA has been shown to beneficially maintain intestinal barrier integrity and function through suppressing inflammation, improving antioxidant capacity, inhibiting apoptosis of intestinal epithelial cells, and regulating bacterial populations in piglets (Chen et al., 2018a,b,c, 2019; Zhang et al., 2018). Consistently, CGA could attenuate *Clostridium perfringens* challenge-induced intestinal injury by alleviating intestinal oxidative stress and inflammation in broiler chickens (Zhang et al., 2020). Moreover, a recent in vivo study has also shown that dietary CGA supplementation can help to improve intestinal health through beneficially regulating autophagy-mediated nuclear factor erythroid 2-related factor 2 pathway in dexamethasone-challenged broiler chickens (Liu et al., 2022b). In young hens, dietary supplementation with CGA has also been shown to alleviate acute heat stress-induced intestinal damage through inhibiting intestinal inflammation and improving antioxidant status and gut microbial community (Chen et al., 2021).

The inflammation and oxidative stress are two major causes in colitis murine model. Numerous studies have shown that the toxic DSS administration resulted in detrimental colonic inflammation and oxidative stress in rodents by activating/inactivating inflammatory and oxidative signal transduction pathways such as toll-like receptor-4, NOD-like receptor thermal protein domain associated protein 3 inflammasome, and nuclear factor erythroid 2-related factor 2 signaling pathways (Qiu et al., 2020; Mahmoud et al., 2021; Zhou et al., 2022). As for poultry, an oral DSS challenge has been demonstrated to increase serum IL-1 β , TNF- α , and IL-10 levels in broiler chickens (Zou et al., 2018, 2019). The DSS-induced intestinal inflammation response has also been found in laying hens (Nii et al., 2020). Moreover, DSS administration has been reported to disrupt redox balance in piglets, as evident by the increased antioxidant enzyme activities (SOD, CAT, and glutathione peroxidase) and MDA and hydroperoxide levels in colonic mucosa, and the decreased vitamin E concentration in blood (Chen et al., 2007). In this study, the administration of DSS-containing drinking water resulted in inflammation and oxidative damage in broiler chickens. In detail, an oral DSS challenge increased serum GSH level and jejunal mucosal CAT activity and MDA level as well as the levels of inflammatory cytokines in serum (IFN- γ and IL-6) and intestinal

mucosa (IL-1 β , TNF- α , and IL-6), but decreased jejunal mucosal GSH content in broiler chickens, which would partially provide an explanation for the increased intestinal permeability and impaired intestinal morphology in DSS-treated birds observed in this study. It is necessary to mention that DSS actually increases jejunal CAT activity and serum GSH level in this study, and these results could be attributed to the activation of antioxidant defense, which is in agreement with the findings of [Chen et al. \(2007\)](#). In rodents, [Vukelić et al. \(2018\)](#) have found that CGA ameliorated colonic inflammatory response, apoptosis and oxidative stress in experimental colitis model by inactivating pro-inflammatory and apoptotic signaling pathways. Likewise, [Wan et al. \(2021\)](#) reported that CGA supplementation alleviated DSS-induced colitis via inhibiting inflammatory responses and oxidative stress, mainly due to its antioxidant and anti-inflammatory characteristics, resulting an improved gut barrier integrity. In domestic animals, dietary supplementation with CGA could improve intestinal barrier function by suppressing intestinal mucosal inflammation and improving gut antioxidant capacity in weaned pigs ([Chen et al., 2018a,b](#)). We also found an improved redox status and alleviated intestinal mucosal inflammation in DSS-treated birds when feeding a CGA-supplemented diet. Dietary CGA administration normalized serum and jejunal GSH level and jejunal CAT activity, and decreased jejunal and ileal MDA levels. Additionally, the levels of serum IFN- γ and IL-6 in serum and intestinal mucosa in DSS-administrated broilers were also reversed by CGA administration to control values. The improved antioxidant capacity and inflammatory response resulting from CGA supplementation would contribute to the enhanced intestinal barrier integrity and function in DSS-challenged birds. [Zhang et al. \(2020\)](#) have reported that CGA relieved intestinal oxidative injury and inflammation in chickens challenged with *Clostridium perfringens* type A. The CGA-induced improvement in small intestinal antioxidant capacity and immunity has also been found by [Liu et al. \(2022a\)](#) in coccidia-infected broiler chickens. Moreover, [Chen et al. \(2021\)](#) found that CGA ameliorated acute heat stress-induced intestinal damage through inhibiting inflammation and improving antioxidant capacity in young pullets. The underlying mechanism accounting for the in vivo antioxidant and anti-inflammatory functions of CGA are varied and complex. The special polyphenol structure endows CGA with a good free radical scavenging capability, and it could beneficially regulate nuclear factor erythroid 2-related factor 2 pathway to improve redox status in broiler chickens ([Zhao et al., 2019](#); [Liu et al., 2022b](#)). Moreover, the CGA exhibits anti-inflammatory activities by modulating a number of important metabolic pathways, including inactivation of nuclear factor kappa B pathway and subsequent down-regulation of inflammatory cytokine production, and regulation on inflammatory mediators such as cyclooxygenase, nitric oxide, and prostaglandin ([Liang and Kitts, 2015](#)). The composition of intestinal microbiota in poultry is a vital

determinant of intestinal health, and its dysregulation would lead to various harmful consequences, including adverse effects on intestinal immunity and redox status ([Maki et al., 2019](#); [Yadav and Jha, 2019](#)). CGA has been demonstrated to beneficially regulate gut microbiota composition in heat-stressed pullets and weaned piglets ([Chen et al., 2019](#); [Chen et al., 2021](#)), which may also contribute to the improved intestinal immunity and redox status in the current research.

In summary, dietary supplementation with CGA could reduce intestinal permeability in DSS-treated broiler chickens at an early age by alleviating oxidative damage and inflammation in broiler chickens, but feeding a CGA-supplemented diet did not improve their growth performance and intestinal morphology.

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DISCLOSURES

All authors declare no conflicts of interests.

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