Effects of dietary supplementation with chlorogenic acid on growth performance, antioxidant capacity, and hepatic inflammation in broiler chickens subjected to diquat-induced oxidative stress

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ABSTRACT This study was conducted to investigate the protective effects of chlorogenic acid (**CGA**) on broilers subjected to (**DQ**)-induced oxidative stress. In experiment 1, one hundred and ninety-two male one-day-old Ross 308 broiler chicks were distributed into 4 groups and fed a basal diet supplemented with 0, 250, 500, or 1,000 mg/kg CGA for 21 d. In experiment 2, an equivalent number of male one-day-old chicks were allocated to 4 treatments for a 21-d trial: 1) Control group, normal birds fed a basal diet; 2) DQ group, DQ-challenged birds fed a basal diet; and 3) and 4) CGA-treated groups: DQ-challenged birds fed a basal diet supplemented with 500 or 1,000 mg/kg CGA. The intraperitoneal DQ challenge was performed at 20 d. In experiment 1, CGA administration linearly increased 21-d body weight, and weight gain and feed intake during 1 to 21 d ($P < 0.05$). CGA linearly and/or quadratically increased total antioxidant capacity, catalase, superoxide dismutase, and glutathione peroxidase activities, elevated glutathione level, and reduced malondialdehyde accumulation in serum, liver, and/or jejunum ($P < 0.05$). In experiment 2, compared with the control group, DQ challenge reduced body weight ratio ($P < 0.05$), which was reversed by CGA administration ($P < 0.05$). DQ challenge increased serum total protein level, aspartate aminotransferase activity, and total bilirubin concentration ($P < 0.05$), which were normalized when supplementing 500 mg/kg and/or 1,000 mg/kg CGA ($P < 0.05$). DQ administration elevated hepatic interleukin-1β, tumor necrosis factor-α, and interleukin-6 levels ($P < 0.05$), and the values of interleukin-1β were normalized to control values when supplementing CGA ($P < 0.05$). DQ injection decreased serum superoxide dismutase activity, hepatic catalase activity, and serum and hepatic glutathione level, but increased malondialdehyde concentration in serum and liver ($P < 0.05$), and the values of these parameters (except hepatic catalase activity) were reversed by 500 and/or 1,000 mg/kg CGA. The results suggested that CGA could improve growth performance, alleviate oxidative stress, and ameliorate hepatic inflammation in DQ-challenged broilers.

Key words: chlorogenic acid, diquat, oxidative stress, inflammation, broilers

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INTRODUCTION

The modern genetic selection toward fast growth rate, high feed efficiency, and the lean and large breast muscles has led to significant welfare problems in commercial broiler chickens and renders them particularly susceptible to oxidative stress (Lee et al., 2019; Hartcher and Lum, 2020). In practical production, the intensively reared broilers are continuously and unavoidably exposed to numerous oxidative stimuli, including high environment temperature, oxidized oils and fats, pathogens, mycotoxins, heavy metals, and other toxic and hazardous substances, which would induce oxidative stress and cause oxidative damage (Estévez, 2015; Surai et al., 2019). The oxidative damage has been actually recognized as a generalized mechanism responsible for the harmful consequences of multiple stress factors in poultry and livestock (Estévez, 2015; Bacou et al., 2021; Hao et al., 2021). Available literature has overwhelmingly shown that oxidative stress arising from different sources would compromise growth performance, disturb cellular antioxidant defenses, affect nutrient digestion and absorption, impair intestinal mucosal barrier function and integrity, cause local and systemic inflammation, disrupt gut microbiota balance, and even degrade quantity and quality of meat products in broilers (Estévez, 2015; Lauridsen, 2019; Mishra and Jha, 2019).

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A variety of oxidative stress models have been established and applied to better understanding and revealing the underlying mechanisms involved in oxidative damage on domestic animals and the action models of cellular antioxidant defense, as well as to evaluate the effectiveness of different antioxidants (Bacou et al., 2021; Cottrell et al., 2021). These models can be generally divided into environment-induced oxidative stress (e. g., heat stress; Münzel and Daiber, 2018), nutritional oxidative distress (e. g., oxidized fats and oils, and mycotoxin-contaminated feed; Bacou et al., 2021), and chemical-inducing oxidative stress (e. g., hydrogen peroxide, dexamethasone, and diquat [**DQ**]) (Koch and Hill, 2017). After being administered, the toxic chemical DQ, a potent redox cycler, is readily converted to a free radical in the presence of molecular oxygen, resulting in the generation of highly reactive superoxide anions and other redox products, which subsequently lead to oxidative stress, lipid peroxidation, and cell damage by disturbing the fragile balance between the oxidant and antioxidant processes (Jones and Vale, 2000; Magalhães et al., 2018). The intraperitoneal DQ administration is widely employed to mimic oxidative stress in livestock and poultry, and it has been demonstrated that DQ challenge reduced growth performance, impaired antioxidant capacity, caused liver and intestine dysfunction, led to systematic inflammatory response, induced mitochondrial dysfunction, and disrupted intestinal barrier integrity in piglets (Mao et al., 2014; Zheng et al., 2017; Cao et al., 2018, 2019; Liu et al., 2019; Guo et al., 2020) and broilers (Chen et al., 2020; Li et al., 2020a; Chen et al., 2021b).

Chlorogenic acid (**CGA**), also known as 5-*O*-caffeoylquinic acid, is an ester of caffeic acid with quinic acid, which is naturally found in a variety of different plant species and possesses multiple biological functions, such as antioxidant, antimicrobial, antiviral, anti-inflammatory, antidiabetic, and anticancer activities (Liang and Kitts, 2015; Hussain et al., 2016; Tajik et al., 2017; Li et al., 2020b; Lu et al., 2020). The special phenolic structure endows CGA with good free radical scavenging activities, and it could efficiently scavenge different free radicals and effectively inhibit cellular lipid peroxidation and beneficially regulate cellular membrane stability (Liang and Kitts, 2015). The experimental and clinical evidence has confirmed its in vivo and in vitro antioxidant effects through its direct antioxidant activity and/or regulation on signal transduction pathways involved in cellular antioxidant defense (Baeza et al., 2014; Hao et al., 2015; Shi et al., 2016; Rebai et al., 2017; Bao et al., 2018; Shi et al., 2018; Wei et al., 2018). The antioxidant characteristic of CGA enables its usage as a promising and green antioxidant in animal feed. It has been shown that dietary supplementation with CGA could improve growth performance and intestinal mucosal antioxidant capacity in piglets by elevating antioxidant enzyme activities, preventing lipid peroxidation, and activating antioxidant signaling pathways (Chen et al., 2018a,b; Zhang et al., 2018). In broilers, dietary CGA supplementation has been reported to enhance growth performance, alleviate inflammatory response, prevent gut damage, improve intestinal mucosal barrier function, and ameliorate oxidative injury in broilers challenged with *Clostridium perfringens* or coccidia (Zhang et al., 2020; Liu et al., 2022a). However, little was known about the antioxidant function of CGA in oxidatively stressed broilers. This study was, therefore, conducted to evaluate the protective effects of CGA on broilers subjected to DQ challenge-induced oxidative stress, and the findings would provide reference and theoretical basis for the relief of oxidative stress and rational application of CGA in broiler feed.

MATERIALS AND METHODS

Animal, Diets, and Management

The animal experiment procedures were carried out in compliance with the experimental protocols approved by the Institutional Animal Care and Ethics Committee of Nanjing Agricultural University, P.R. China.

In experiment 1, a total of 192 male one-day-old Ross 308 broiler chicks with an initial body weight of 44.4 ± 0.3 g were randomly assigned to one of four groups with 6 replicates (cages) of 8 birds each for a 21-d feeding trial. Birds were given a corn-soybean meal basal diet supplemented 0 (Control group), 250, 500, and 1,000 mg/kg of CGA, respectively. The basal diet was formulated according to the nutritional recommendations of broiler chickens (National Research Council, 1994) during the starter phase (0−3 wk). The ingredient composition and nutrient specifications of basal diet are presented in Table 1. The four experimental diets were prepared consecutively using the same batch of feed ingredients, and were offered in mash form ad libitum at all times except when necessary feed withdrawal was performed prior to determination of growth performance or euthanasia. The different supplemental levels of CGA were chosen according to previous studies conducted on piglets (Chen et al., 2018b; Zhang et al., 2018). The used CGA in this study was kindly gifted by Hunan E.K Herb Co., Ltd. (Changsha, Hunan province, P.R. China), which was prepared from *Eucommia ulmoides* leaves, a traditional Chinese medicine, after necessary extraction, separation, and purification process. The purity of this CGA was found to be 98.65%, when determining it with a high-performance liquid chromatography-mass spectrometry system (LCMS-8040; Shimadzu, Kyoto, Japan), using the 5-*O*-caffeoylquinic acid (Catalog No. c3878-1g, Sigma-Aldrich, St Louis, MO) as a standard chemical to build a calibration curve. The CGA powder with a good fluidity was evenly mixed with corresponding premix in a vertical screw mixer for 10 min (DSH-0.04, Tongxiang Jinzhong Machinery Co., Ltd., Jiaxing, Zhejiang province, P.R. China) prior to preparing the complete feed. After arrival at farm, all chickens reared in wire cages equipped with plastic floors and water nipples (8 birds each cage, 150 cm (length) × 70 cm (width) × 50 cm (height)) were provided with sufficient formulated feed

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

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[2]	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, %	0.90

[1]The basal diet composition in experiment 1 and experiment 2 is the same.

[2]Premix provided per kilogram of diet: vitamin A (transretinyl acetate), 10,000 IU; vitamin D_3 (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 B_{12} (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.

and tap water under a 23-h light and 1-h dark lighting schedule during whole phase of this feeding trial, and the indoor temperature of thermostatically controlled chicken house was decreased from 33 to 34°C at weekly intervals (2°C−3°C per week). The ambient relative humidity was initially set at approximately 70%, and it was then maintained at 60 to 65% thereafter.

In experiment 2, an equivalent number of one-day-old male Ross 308 broiler chicks (192 in total) with similar hatching weight were allocated into one of 4 treatments with 6 replicates of 8 birds each. Treatments included 1) Control group, normal birds fed a basal diet; 2) DQ group, DQ-challenged birds given a basal diet; 3) and 4) CGA I and II groups, DQ-challenged birds fed a basal diet supplemented with 500 mg/kg or 1,000 mg/kg CGA. This feeding experiment lasted for 21 d. The DQ challenge was performed at 20 days of age through an intraperitoneal administration of 20 mg/mL DQ solution (diquat dibromide monohydrate; Sigma-Aldrich) at a dosage of 1 mL/kg of body weight after weighing according to our previous findings (Chen et al., 2020, 2021b), while the control birds were intraperitoneally administrated with an equivalent amount of vehicle solution, 0.86% physiological saline. All other experimental and animal management procedures in experiment 2 conformed to those of experiment 1.

Sample Collection

In experiment 1, one bird from each replicate (cage) was selected at random and weighed after a 12-h feed deprivation at 21 days of age. The blood samples were collected from wing vein, and the serum was then harvested and aliquoted into clean tubes after centrifugation at 4,450 × *g* for 15 min at 4°C, which was immediately frozen and stored at −20°C until analysis. After blood was taken, birds were euthanized by cervical dislocation and necropsy was done. The right left lobe of liver was excised, collected individually in cryogenic tubes, and then stored in liquid nitrogen for subsequent determination. The jejunum (from the end of the duodenum to the Meckel's diverticulum) was dissected from the connective tissues and fat, placed in cold stainless-steel tray, and opened along the mesenteric border. The jejunal mucosal samples were collected from individual bird, placed into cryogenic tubes, pooled, immediately snap-frozen in liquid nitrogen, and stored in a liquid nitrogen tank until analysis.

In experiment 2, an equivalent number of birds (6 birds from each group and 24 birds in total) were randomly selected for sampling over a 24-h period postadministration of DQ. The blood collection, serum preparation, and slaughter procedures were performed as mentioned above in experiment 1. After necropsy, liver, spleen, and jejunum were excised, immediately washed in ice-cold saline, surface-dried with filter paper, and then their fresh weight was measured to calculate absolute weight and relative weight according to the following formula: relative organ weight (g/kg) = absolute organ weight/terminal body weight. After being placed on a chilled stainless-steel tray, a portion of right lobe of liver from each individual bird was dissected from the remaining fresh liver, chopped with scissors, and then collected into sterile and clean tubes, which was immediately snap-frozen in liquid nitrogen and stored at −80°C for later measurement.

Determination of Growth Performance

In experiment 1, all broiler chickens were weighed by cage (replicate) at 21 days of age after being subjected to a 12-h feed restriction with free access to water, and feed consumption was recorded for each replicate on the same occasions to calculate average body weight, average daily gain (**ADG**), and average feed intake (**ADFI**), and feed conversion ratio (**FCR**) was calculated by dividing total feed consumed by body weight gain, with the weight of mortalities and culls being included. In experiment 2, birds were weighed on cage basis at both 20 and 21 days of age to calculate average body weight and body weight ratio (**BWR**) (the ratio between 21-d body weight and 20-d body weight).

Measurement of Serum Biochemical Indices

The blood samples collected in experiment 2 were used to determine serum biochemical indices, including total bilirubin (Catalog No. C019-1-1), total protein (Catalog No. A045-4-1), albumin (Catalog No. A028-2-1), total cholesterol (Catalog No. A111-1-1), triglyceride

(Catalog No. A110-2-1), and glucose (Catalog No. F006-1-1) concentrations, and the activities of transaminase activities (aspartate aminotransferase (Catalog No. C010-1) and alanine aminotransferase (Catalog No. C009-2)), using the corresponding commercially-available kits supplied by Nanjing Jiancheng Bioengineering Institute (Nanjing, P.R. China). The assay was performed strictly following manufacturer's protocol and guidelines, using a microplate reader (MODEL 680, Bio-Rad Laboratories Inc., Hercules, CA).

Preparation of Homogenate

The liquid nitrogen-frozen liver and jejunal mucosal scrapings were thawed, finely chopped with scissors, weighed into sterile tubes, diluted with normal cold saline solution at an appropriate weight/volume ratio (1: 9 and 1: 4 for liver and jejunal mucosa, respectively), and then homogenized in an iced water bath with a motor-driven homogenizer (PRO-PK-02200D, Pro Scientific, Inc., Monroe, CT) at a moderate speeds for approximately 30 s until reaching complete dissolution. After that the homogenate was centrifuged at 4450 × g at 4°C for 15 min, and the supernatant was carefully collected and equally aliquoted into five portions with Eppendorf tubes, which were then stored in liquid nitrogen tank until subsequent measurement.

Quantification of Hepatic Cytokine Level

The chicken-specific enzyme-linked immunosorbent assay kits purchased from CUSABIO Technology LLC (Wuhan, P.R. China) were employed to measure pro-inflammatory cytokine concentrations in collected liver samples in experiment 2, including interleukin-1β (**IL-1β**, 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). The determination was done in compliance with standardized protocol provided by manufacturer after recommended dilution with saline. All results were normalized against corresponding total protein level prior to statistical analysis and inner comparison, which was measured using a Bradford assay reagent (Nanjing Jiancheng Bioengineering Institute).

Assay of Antioxidant-Related Parameters

The prepared hepatic and jejunal mucosal homogenate as well as serum samples were used to perform the measurement of antioxidant-related indices. The activities of total antioxidant capacity (**T-AOC**, Catalog No. A015), superoxide dismutase (**SOD**, Catalog No. A001-1-1), catalase (**CAT**, Catalog No. A007-1-1), and glutathione peroxidase (**GSH-Px**, Catalog No. A005-1-2), and the concentrations of glutathione (**GSH**, Catalog No. A006-1) and malondialdehyde (**MDA**, Catalog No. A003-1) were colorimetrically determined with the commercially-available kits following the recommended manual provided with each kit (Nanjing Jiancheng Bioengineering Institute), using a microplate reader at different wavelengths. The T-AOC is a sensitive parameter reflecting overall cellular endogenous antioxidant capability in body, and its activity was detected with a spectrometric method in which the ferric 2,4,6 tripyridyl-S-triazine complex could be reduced to produce the blue ferrous 2,4,6 tripyridyl-S-triazine complex in the presence of cellular antioxidants (Benzie and Strain, 1996). The serum and tissue SOD activity was quantified with the classical hydroxylamine method (Kono, 1978), and one unit of its activity was defined as the amount of enzyme that would produce 50% inhibition of nitrite generation from hydroxylamine in each milliliter of serum or milligram protein of tissue samples (liver or jejunal mucosa) in 40 min at 37°C. The measurement of CAT activity was done using an ammonium molybdate method (Góth, 1991), whose activity was defined as the enzyme quantity that could catalyze one micromole hydrogen peroxide decomposition per milliliter of serum or per milligram protein in 1 min at 37°C. The determination of GSH-Px activity and GSH level were both performed with 5, 5′-dithiobis (2-nitrobenzoic acid) method as described previously (Owens and Belcher, 1965), and one unit of GSH-Px activity was defined the amount of this enzyme required to deplete one micromole GSH in one hundred microliter of serum or one milligram of tissue protein in 5 min at 37°C. A rapid aqueous acid extraction thiobarbituric acid method (Placer et al., 1966) was adopted to determine MDA concentration, a sensitive lipid peroxidation marker, and its accumulation level was expressed as nmol/mL and nmol/mg protein in serum and tissue samples, respectively.

Statistical Analysis

Data were analyzed by one-way analysis of variance, using SPSS statistical software (Ver.22.0 for windows, SPSS Inc., Chicago, IL). A pen was the experimental unit for growth performance data, while an individual bird selected from each pen was the experimental unit for other measured parameters. Orthogonal polynomial contrasts were also employed to determine the linear and quadratic effects of supplemental CGA levels in experiment 1. Differences among treatments were examined by Duncan's multiple range test, with significant difference being set at $P < 0.05$. The results were presented as means with their pooled standard errors.

RESULTS

Effects of Supplemental Levels of CGA on Growth Performance in Broilers

In experiment 1 (Table 2), feeding a CGA-supplemented diet linearly increased ADFI ($P = 0.006$) in broiler chickens during 1 to 21 days of age. Birds fed a

Table 2. Effects of graded levels of dietary chlorogenic acid supplementation on growth performance of broiler chickens in experiment 1.

	Chlorogenic acid level (mg/kg)					*P* values		
Items[1]	0	250	500	1,000	SEM[2]	ANOVA	Linear	Quadratic
BW (g)	670.12	712.59	730.76	739.95	11.95	0.167	0.036	0.471
ADG (g/d)	29.80	31.83	32.69	33.11	0.57	0.171	0.038	0.462
ADFI (g/d)	41.22[b]	43.62[ab]	47.08[a]	45.28[ab]	0.69	0.011	0.006	0.079
FCR (g/g)	1.39	1.37	1.44	1.37	0.02	0.339	0.893	0.372

[1]ADFI, average daily feed intake; ADG, average daily gain; BW, body weight; FCR, feed conversion ratio.
[2]SEM, standard error of the mean (n = 6).
[a-b]Means within a row with different superscripts are different at $P < 0.05$.

basal diet supplemented with 500 mg/kg CGA had a higher ADFI than their counterparts in the control group ($P = 0.011$), and there was no significant difference in ADFI among CGA-supplemented groups ($P > 0.05$). The 21-d BW and ADG during 1 to 21 d were linearly increased by CGA supplementation ($P < 0.05$), but their differences did not reach statistical significance ($P > 0.05$). Likewise, dietary supplementation with CGA did not alter FCR in broiler chickens during 1 to 21 d ($P > 0.05$).

Effects of Supplemental Levels of CGA on Antioxidant Capacity in Broilers

In experiment 1 (Table 3), dietary CGA supplementation quadratically increased T-AOC activity ($P = 0.004$) and GSH concentration ($P = 0.008$), and linearly increased CAT activity ($P < 0.001$) in serum. Birds in 250 and 500 mg/kg CGA-supplemented groups exhibited a higher serum T-AOC activity than their counterparts in the control and 1,000 mg/kg CGA-treated group ($P = 0.032$), and there was no significant difference between these 2 CGA-supplemented groups ($P > 0.05$). Dietary supplementation with CGA, irrespective of its dosage, increased serum CAT activity ($P = 0.004$), and the highest value was observed in 1,000 mg/kg CGA-supplemented group, which was also significantly higher that of 250 mg/kg CGA-supplemented group ($P < 0.05$), with its value being intermediate in 500 mg/kg CGA group ($P > 0.05$). Likewise, a higher serum GSH level was observed in birds receiving a basal diet supplemented with 500 mg/kg CGA ($P = 0.011$), when compared with the control and other 2 CGA-treated groups. However, there was no significant difference in serum SOD activity, GSH-Px activity, or MDA accumulation level ($P > 0.05$).

The CGA administration linearly and quadratically increased T-AOC activity and GSH level, and quadratically elevated GSH-Px activity in the liver of broiler chickens ($P < 0.05$). Compared with the control group and other CGA-supplemented groups, feeding a CGA-supplemented diet at a level of 1,000 mg/kg increased hepatic T-AOC activity ($P < 0.001$). Moreover, birds in

Table 3. Effects of graded levels of dietary chlorogenic acid supplementation on antioxidant status of broiler chickens in experiment 1.

	Chlorogenic acid level (mg/kg)					*P* values		
Items[1]	0	250	500	1000	SEM[2]	ANOVA	Linear	Quadratic
Serum								
T-AOC (U/mL)	7.86[b]	12.33[a]	11.89[a]	8.90[b]	0.67	0.032	0.612	0.004
SOD (U/mL)	184.43	152.04	201.99	172.77	10.68	0.432	0.877	0.942
GSH-Px (U/mL)	251.89	261.22	272.75	265.19	5.81	0.670	0.349	0.489
CAT (U/mL)	3.90[c]	5.05[b]	5.23[ab]	6.28[a]	0.25	0.004	<0.001	0.897
GSH (mg/L)	4.21[b]	4.51[b]	5.60[a]	4.00[b]	0.20	0.011	0.753	0.008
MDA (nmol/mL)	1.45	1.76	1.52	1.83	0.15	0.793	0.530	0.990
Liver								
T-AOC (U/mg protein)	0.65[b]	0.49[b]	0.56[b]	2.18[a]	0.15	<0.001	<0.001	<0.001
SOD (U/mg protein)	149.92	157.60	160.07	151.04	3.41	0.692	0.856	0.250
GSH-Px (U/mg protein)	25.36[b]	28.29[b]	33.42[a]	24.10[b]	1.08	0.004	0.861	0.002
CAT (U/mg protein)	3.22	3.26	2.80	3.17	0.28	0.941	0.818	0.780
GSH (mg/g protein)	4.23[b]	5.13[b]	7.28[a]	5.21[b]	0.32	0.001	0.022	0.004
MDA (nmol/mg protein)	0.38	0.49	0.48	0.45	0.02	0.370	0.338	0.154
Jejunum								
T-AOC (U/mg protein)	1.03[b]	1.28[a]	1.30[a]	1.37[a]	0.04	0.013	0.003	0.207
SOD (U/mg protein)	130.38[b]	138.51[ab]	154.57[a]	158.19[a]	4.04	0.034	0.005	0.751
GSH-Px (U/mg protein)	15.83	19.33	14.10	14.15	0.91	0.135	0.192	0.323
CAT (U/mg protein)	0.61[a]	0.53[a]	0.33[b]	0.29[b]	0.04	0.006	0.001	0.739
GSH (mg/g protein)	15.05	12.73	14.86	12.67	0.59	0.318	0.347	0.957
MDA (nmol/mg protein)	0.33[a]	0.12[b]	0.13[b]	0.16[b]	0.03	0.033	0.046	0.029

[1]CAT, catalase; GSH, reduced form of glutathione; MDA, malondialdehyde; GSH-Px, glutathione peroxidase; SOD, superoxide dismutase; T-AOC, total antioxidant capacity.
[2]SEM, standard error of the mean (n = 6).
[a-c]Means within a row with different superscripts are different at $P < 0.05$.

500 mg/kg CGA-treated group exhibited the highest hepatic GSH-Px activity ($P = 0.004$) and GSH level ($P = 0.001$) in comparison with the control group and other 2 CGA-supplemented groups. However, the graded levels of dietary CGA supplementation did not affect hepatic SOD activity, CAT activity, or MDA concentration ($P > 0.05$).

The CGA supplementation linearly increased T-AOC and SOD activities, linearly decreased CAT activity, and linearly and quadratically decreased MDA level in jejunal mucosa ($P < 0.05$). The administration with CGA, regardless of its supplemental level, increased T-AOC activity ($P = 0.013$) and decreased MDA concentration ($P = 0.033$) in jejunal mucosa to similar values, when compared with the control group. Additionally, dietary supplementation with 500 mg/kg and 1,000 mg/kg CGA equally increased SOD activity ($P = 0.034$) and reduced CAT activity ($P = 0.006$) in jejunum of broiler chickens, but a similar effect was not found when its supplemental dosage was 250 mg/kg ($P > 0.05$). Neither jejunal GSH-Px activity nor GSH level was altered when feeding birds a CGA-supplemented diet ($P > 0.05$).

Effects of CGA on Growth Performance in DQ-Challenged Broilersroilers

In experiment 2, there was no significant difference in 20-d BW among 4 groups prior to DQ challenge (Figure 1, $P > 0.05$). Compared with the control group, an abdominal DQ injection numerically reduced 21-d BW, but this difference did not reach statistical significance ($P > 0.05$). Likewise, dietary CGA administration also resulted in a numerically increased 21-d BW in DQ-challenged birds ($P > 0.05$). The DQ challenge decreased BWR of broiler chickens ($P < 0.001$), when compared with the control group. In contrast, feeding a CGA-supplemented diet, irrespective of its dosage, increased BWR in DQ-challenged birds in comparison with their counterparts receiving a basal diet only ($P < 0.05$), but its value in 1,000 mg/kg CGA-administrated group was still lower than that of control group ($P < 0.05$).

Effects of CGA on Organ Weight and Serum Biochemical Parameters in DQ-Challenged Broilers

As presented in Table 4, there was no significant difference in liver, jejunum, or spleen weight, irrespective of absolute or relative weight ($P > 0.05$). Compared with the control group (Table 5), DQ challenge increased circulating total protein level ($P = 0.005$), aspartate aminotransferase activity ($P = 0.042$), and total bilirubin concentration ($P = 0.003$) in broiler chickens, and the values of total protein and total bilirubin levels were equally normalized to control values when supplementing 500 mg/kg and 1,000 mg/kg CGA ($P < 0.05$). Moreover, dietary supplementation with CGA at a level of 1,000 mg/kg reduced serum aspartate aminotransferase activity in DQ-treated birds ($P < 0.05$), but a similar effect was not found when its supplemental dosage was 500 mg/kg ($P > 0.05$).

Effects of CGA on Antioxidant Status in DQ-Challenged Broilers

The DQ challenge decreased SOD activity (Table 6, $P = 0.027$) and GSH level ($P = 0.045$), but increased MDA accumulation ($P = 0.004$) in serum of broiler chickens when compared with the control group. In contrast, supplementing 500 mg/kg and 1,000 mg/kg CGA equally reduced serum MDA level in DQ-treated birds when compared with their challenged counterparts ($P < 0.05$), with their values being comparable with those of control group ($P > 0.05$). Dietary administration with 500 mg/kg and 1,000 mg/kg CGA increased serum GSH level and SOD activity in DQ-challenged broiler chickens, respectively, when compared with DQ group ($P < 0.05$). Neither serum GSH-Px nor CAT activity was altered by treatment ($P > 0.05$).

An abdominal DQ challenge reduced CAT activity ($P = 0.034$) and GSH level ($P = 0.030$), but increased MDA concentration ($P = 0.001$) in liver of broiler chickens in comparison with the control group. The generation of hepatic MDA in DQ-treated birds was inhibited when feeding them with CGA, regardless of its administrated dosage ($P < 0.05$). Moreover, CGA

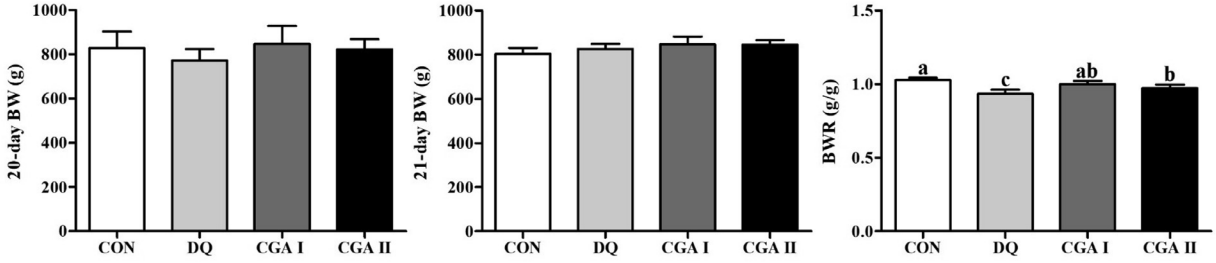

Figure 1. Effects of dietary chlorogenic acid supplementation on growth performance broiler chickens subjected to diquat-induced oxidative stress in experiment 2. The column and its bar represented the means value and standard error (n = 6), respectively. BW, body weight; BWR, the ratio between 21-d BW and 20-day BW; CON, normal broilers fed a basal diet; DQ, diquat-challenged broilers fed a basal diet; CGA I, diquat-challenged broilers fed a basal diet supplemented with 500 mg/kg chlorogenic acid; CGA II, diquat-challenged broilers fed a basal diet supplemented with 1,000 mg/kg chlorogenic acid; SEM, standard error of the mean (n = 6). [a-c]Means within a row with different superscripts are different at $P < 0.05$.

Table 4. Effects of dietary chlorogenic acid supplementation on organ weight of broiler chickens subjected to diquat-induced oxidative stress in experiment 2.

Items[1]	Treatment				SEM[2]	*P* value
	CON	DQ	CGA I	CGA II		
Liver						
Absolute weight (g)	21.82	22.11	22.73	20.92	0.61	0.791
Relative weight (g/kg)	26.39	28.90	26.88	25.44	0.81	0.510
Jejunum						
Absolute weight (g)	17.12	16.03	17.30	16.84	0.44	0.764
Relative weight (g/kg)	20.80	20.70	20.64	20.44	0.50	0.996
Spleen						
Absolute weight (g)	0.79	0.75	0.77	0.72	0.03	0.860
Relative weight (g/kg)	0.97	0.98	0.92	0.87	0.04	0.816

[1]CON, normal broilers fed a basal diet; CGA I, diquat-challenged broilers fed a basal diet supplemented with 500 mg/kg chlorogenic acid; CGA II, diquat-challenged broilers fed a basal diet supplemented with 1,000 mg/kg chlorogenic acidDQ, diquat-challenged broilers fed a basal diet.

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

supplementation at a level of 1,000 mg/kg also increased hepatic GSH level in DQ-treated birds when compared with their challenged counterparts ($P < 0.05$), with its value being comparable with the control group ($P > 0.05$). Dietary CGA administration also numerically increased hepatic CAT activity, but this difference did not reach statistical significance ($P > 0.05$). However, dietary treatment did not affect hepatic SOD or GSH-Px activity in broiler chickens ($P > 0.05$).

Effects of CGA on Hepatic Cytokine Levels in DQ-Challenged Broilers

Compared with normal birds (Table 7), DQ challenge elevated hepatic IL-1β ($P = 0.002$), TNF-α ($P = 0.031$), and IL-6 ($P = 0.045$) levels in broiler chickens. The elevated hepatic IL-1β level was normalized to a control value when supplementing a basal diet with 500 mg/kg or 1,000 mg/kg CGA ($P < 0.05$). Although not significantly different, an administration with CGA also numerically reduced hepatic TNF-α and IL-6 levels in DQ-challenged birds ($P > 0.05$). However, there was no significant difference in hepatic IFN-γ level among the four groups ($P > 0.05$).

DISCUSSION

The beneficial effects of dietary CGA supplementation on growth performance of domestic animals under normal physiological conditions have been predominantly reported in weaned piglets. Chen et al. (2018b) found that an addition of 250 mg/kg and 500 mg/kg CGA did not alter ADFI, ADG, or FCR in weaned piglets during a 14-d feeding trial, but a higher supplemental level of CGA (1,000 mg/kg) improved ADG and FCR of piglets during the same experimental period. Similarly, Zhang et al. (2018) have reported that CGA supplementation at levels ranging from 250 mg/kg to 1,000 mg/kg linearly increased body weight and linearly and quadratically increased ADG of piglets in a 28-d experiment. In experiment 1, an improved growth performance was found in normal broiler chickens fed a CGA-supplemented diet according to the linearly elevated 21-d body weight and ADG and ADFI during 1 to 21 d. The improved growth performance of CGA-treated birds under normal physiological conditions observed in experiment 1 could be partially explained by the simultaneously improved antioxidant capacity. In other studies, the increased nutrient digestibility and digestive enzyme activities (Chen et al., 2018b), enhanced growth hormone secretion (Wu et al., 2018), improved intestinal integrity and barrier function (Chen et al., 2018a; Chen et al., 2023), superior immune function (Zhang et al., 2020), and the improved intestinal microbiota composition (Zhang et al., 2018; Chen et al., 2021a) have been reported to contribute to the improved growth in poultry and swine, but whether these beneficial effects also account for the improved growth performance in experiment 1 still need further investigation.

The toxic DQ administration would result in excessive generation of free radicals and acute oxidative stress (Li et al., 2020a), impair digestive function (Yuan et al., 2007), alter nutrient metabolism and allocation (Lv et al., 2012), destroy structure and function of organs (Mao et al., 2014), induce systemic inflammatory

Table 5. Effects of dietary chlorogenic acid supplementation on serum biochemical parameters of broiler chickens subjected to diquat-induced oxidative stress in experiment 2.

Items[1]	Treatment				SEM[2]	*P* value
	CON	DQ	CGA I	CGA II		
Total protein (g/L)	30.74[b]	40.32[a]	34.09[b]	33.43[b]	1.08	0.005
Albumin (g/L)	15.79	15.92	14.13	16.42	0.39	0.188
Glucose (mmol/L)	13.37	15.14	14.77	13.64	0.38	0.300
Total cholesterol (mmol/L)	5.14	5.80	4.90	6.19	0.19	0.051
Triglyceride (mmol/L)	0.58	0.52	0.48	0.56	0.02	0.505
Alanine aminotransferase (U/L)	7.64	7.68	8.38	7.27	0.16	0.076
Aspartate aminotransferase (U/L)	13.22[b]	18.50[a]	15.60[ab]	13.06[b]	0.80	0.042
Total bilirubin (μmol/L)	10.27[b]	15.44[a]	6.42[b]	6.40[b]	1.08	0.003

[1]CON, normal broilers fed a basal diet; DQ, diquat-challenged broilers fed a basal diet; CGA I, diquat-challenged broilers fed a basal diet supplemented with 500 mg/kg chlorogenic acid; CGA II, diquat-challenged broilers fed a basal diet supplemented with 1,000 mg/kg chlorogenic acid.

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

[a-b]Means within a row with different superscripts are different at $P < 0.05$.

Table 6. Effects of dietary chlorogenic acid supplementation on antioxidant capacity of broiler chickens subjected to diquat-induced oxidative stress in experiment 2.

	Treatment					
Items[1]	CON	DQ	CGA I	CGA II	SEM[2]	*P* value
Serum						
SOD (U/mL)	225.18[a]	177.33[b]	204.27[ab]	227.35[a]	6.98	0.027
GSH-Px (U/mL)	612.04	576.05	565.36	594.94	13.48	0.653
CAT (U/mL)	2.67	1.07	2.13	2.11	0.23	0.081
GSH (mg/L)	8.85[a]	6.49[b]	8.08[a]	7.22[ab]	0.32	0.045
MDA (nmol/mL)	1.07[b]	1.65[a]	0.94[b]	0.72[b]	0.10	0.004
Liver						
SOD (U/mg protein)	264.38	263.86	276.71	255.98	4.59	0.482
GSH-Px (U/mg protein)	85.82	82.89	79.44	88.41	2.16	0.523
CAT (U/mg protein)	10.69[a]	7.50[b]	9.30[ab]	8.53[ab]	0.41	0.034
GSH (mg/g protein)	24.10[a]	13.32[b]	19.09[ab]	23.92[a]	1.54	0.030
MDA (nmol/mg protein)	0.86[b]	1.39[a]	0.91[b]	1.02[b]	0.06	0.001

[1]CAT, catalase; CON, normal broilers fed a basal diet; DQ, diquat-challenged broilers fed a basal diet; CGA I, diquat-challenged broilers fed a basal diet supplemented with 500 mg/kg chlorogenic acid; CGA II, diquat-challenged broilers fed a basal diet supplemented with 1,000 mg/kg chlorogenic acid; GSH-Px, glutathione peroxidase; GSH, reduced form of glutathione; MDA, malondialdehyde; SOD, superoxide dismutase.

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

[a-b]Means within a row with different superscripts are different at $P < 0.05$.

response (Guo et al., 2020), and damage intestinal barrier function (Cao et al., 2018, 2019), ultimately leading to inferior growth performance in animals. In broiler chickens, Chen et al. (2020) found that DQ administration induced a rapid loss of body weight during a 24-h post challenge. Additionally, Chen et al. (2021b) also reported that an abdominal DQ challenge reduced weight gain and body weight change rate during 24 h after injection, which was in agreement with this finding. Although body weight was similar among groups in experiment 2, dietary administration with CGA, regardless of its dosage, increased BWR of DQ-treated birds, indicating that CGA was able to reverse body weight loss in birds subjected to DQ-induced oxidative stress. In a dexamethasone-induced oxidative stress model, Liu et al. (2022b) recently noted that CGA administration at a level of 500 mg/kg was effective in improving weight gain of oxidatively stressed broilers, which has been shown to be linked with its in vivo antioxidant characteristic. The improvement in growth performance has also been reported in broiler chickens under heat stress-induced oxidative stress, when supplementing 1,000 mg/kg of CGA-enriched extract from *Eucommia ulmoides* leaves to a basal diet (Zhao et al., 2019). The improved growth performance of DQ-treated birds receiving CGA may be correlated with the alleviated oxidative damage and inflammatory response in broiler chickens as discussed in the following context, which have been also demonstrated in broilers subjected to heat stress (Zhao et al., 2019), immunological stress (Liu et al., 2022a), and toxic chemicals (Chen et al., 2021a).

Table 7. Effects of dietary chlorogenic acid supplementation on the levels of hepatic inflammatory cytokines in broiler chickens subjected to diquat-induced oxidative stress in experiment 2 (ng/g protein).

	Treatment					
Items[1]	CON	DQ	CGA I	CGA II	SEM[2]	*P* value
IFN-γ	1.38	1.47	1.72	1.58	0.06	0.158
IL-1β	20.65[b]	46.31[a]	22.85[b]	13.57[b]	3.58	0.002
TNF-α	32.24[b]	64.60[a]	42.87[ab]	43.96[ab]	4.12	0.031
IL-6	304.88[b]	342.35[a]	336.51[a]	318.69[ab]	5.41	0.045

[1]CON, normal broilers fed a basal diet; CGA I, diquat-challenged broilers fed a basal diet supplemented with 500 mg/kg chlorogenic acid; CGA II, diquat-challenged broilers fed a basal diet supplemented with 1,000 mg/kg chlorogenic acid; DQ, diquat-challenged broilers fed a basal diet; IFN-γ, interferon-γ; IL-1β, interleukin-1β; IL-6, interferon-6; TNF-α, tumor necrosis factor-α. [2]SEM, standard error of the mean (n = 6).

[a-b]Means within a row with different superscripts are different at $P < 0.05$.

As for domestic animals under normal physiological conditions, Chen et al. (2018a,b) have observed that CGA supplementation at a level of 1,000 mg/kg elevated GSH-Px and CAT activities and inhibited MDA generation in serum and small intestinal mucosa of piglets. Consistently, Zhang et al. (2018) also observed an improvement of antioxidant status in weaned piglets given graded levels of CGA, as evident by the linearly and/or quadratically increased antioxidant enzyme activities (GSH-Px, CAT, SOD, and T-AOC) and decreased MDA accumulation in serum and/or intestinal mucosa. In agreement with these findings, dietary supplementation with different levels of CGA linearly and/or quadratically increased T-AOC, CAT, and GSH-Px activities as well as GSH concentration in serum and/or liver of broiler chickens under normal physiological conditions in experiment 1. Moreover, CGA supplementation also linearly elevated T-AOC and SOD activities, linearly reduced CAT activity, and linearly and quadratically inhibited MDA accumulation in jejunal mucosal scrapings in experiment 1. These results together indicated that CGA could improve antioxidant capacity of broiler chickens. The chemical structure of CGA consists of an aromatic ring and an alicyclic ring, with a conjugated chain and five hydroxyl groups attached to the 2 rings (Saqib et al., 2016). The phenolic CGA has been shown to efficiently scavenge reactive free radicals in vitro, such as superoxide anions, hydroxyl radicals, 1,1-diphenyl-2-picrylhydrazyl radicals, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic

acid) radicals, etc., although its scavenging ability varies greatly (Liang and Kitts, 2015). The radical adduct formation and hydrogen atom transfer are two potential mechanisms accounting for the in vitro antioxidant activity of CGA in acidic and neutral environment, while the sequential proton loss electron transfer is the likely antioxidative mechanism of CGA with extremely high rate in basic environment (Tošović et al., 2017). Therefore, these beneficial consequences of CGA on antioxidant capacity of broilers in experiment 1 can be partially explained by its direct antioxidant properties mainly due to its phenolic structure (Shin et al., 2017). Additionally, CGA and its isomers have been shown to directly interact with Kelch-like erythroid cell-derived protein 1/nuclear factor erythroid 2-related factor 2 signaling due to their structure-specific interactions (Liang and Kitts, 2015; Liang et al., 2019), eventually leading to the activation of this important antioxidant signal pathway and up-regulated expression of its downstream antioxidant genes (Boettler et al., 2011; Bao et al., 2018; Shi et al., 2018; Han et al., 2019), which, in turn, may provide another explanation for the improved redox status of broiler chickens in experiment 1. It is necessary to mention that variation trend of CAT activity in serum and liver was completely opposite to that of jejunal mucosa in experiment 1. There was no clear explanation for this puzzling observation, but it may be correlated with variation in the adsorption and metabolism of CGA among different organs and tissue-specific antioxidant response in broiler chickens.

The detrimental consequences of DQ is generally presumed to depend on its capacity to undergo a single electron addition and redox cycling to produce unstable free radicals such as superoxide anion radical, hydrogen peroxide and hydroxyl radical, ultimately leading to devastating effects to cells, including oxidative stress, cellular membrane damage, mitochondrial injury, cellular homeostasis imbalance and inflammation, apoptosis and even death, when the cellular antioxidant protective systems are overwhelmed (Burk et al., 1995; Jones and Vale, 2000; Magalhães et al., 2018; Azad et al., 2021; Jin et al., 2021). The liver is responsible for the detoxification of a variety of xenobiotics in vertebrate animals and, therefore, is a major target of DQ (Magalhães et al., 2018). In broiler chickens, available studies have reported that an abdominal DQ administration disrupted antioxidant defense, caused mitochondrial dysfunction, and resulted in excessive apoptosis in liver (Chen et al., 2020, 2021b). In a model of DQ-induced acute oxidative stress, Li et al. (2020a) have observed that DQ challenge led to compromised hepatic antioxidant status of chicken embryos, as evident by the elevated generation of hepatic nitric oxide and protein carbonyl. In agreement with these findings, DQ administration also induced oxidative stress in broiler chickens in experiment 2. In detail, the toxic DQ treatment significantly decreased serum SOD activity, hepatic CAT activity, and GSH levels in serum and liver, and increased MDA accumulations in serum and liver. The antioxidant activities of CGA have been substantially demonstrated in different oxidative stress models, in either cell culture experiments or model animal experiments. The protective effects of CGA and its isomers against oxidative stress resulting from different stimuli have been reported in different cell types including hepatocytes through its direct antioxidant activities, maintenance of cellular antioxidant defense, improvement of mitochondrial function, and regulation on signaling pathways involved in antioxidant defense (Pavlica and Gebhardt, 2005; Kim et al., 2012; Li et al., 2012; Park, 2013; Baeza et al., 2014; Cha et al., 2014; Liang et al., 2019). In rodent animals, an orally administered CGA has been shown to alleviate streptozotocin −nicotinamide generated oxidative stress in rats by inhibiting the generation of thiobarbituric acid reactive substances and lipid hydroperoxides and by elevating GSH, vitamin C, vitamin E, and ceruloplasmin concentrations in the blood (Karthikesan et al., 2010). Moreover, CGA administration could also effectively mitigate hepatic and intestinal oxidative stress in rodent animals by activating nuclear factor erythroid 2-related factor 2 and upregulating its target antioxidant gene expression (Shi et al., 2016; Zhou et al., 2016a; Shi et al., 2018; Wei et al., 2018; Xue et al., 2019). As for broiler chickens, dietary administration with CGA has been reported to improve intestinal antioxidant status in oxidatively stressed broilers induced by dexamethasone through regulating autophagy-mediated nuclear factor erythroid 2-related factor 2 pathway (Liu et al., 2022b). A CGA-enriched extract from *Eucommia ulmoides* leaves has also been found to alleviate heat stress-induced oxidative stress in broiler chickens by activating antioxidant defense system (Zhao et al., 2019). In this study, dietary CGA supplementation partially relieved DQ-challenge induced oxidative stress in serum and liver of broiler chickens. To be specified, CGA elevated serum SOD activity and serum and hepatic GSH level, but decreased MDA accumulations in both serum and liver of DQ-treated broiler chickens, and it could be explained by its antioxidant characteristics and regulation on the different signaling pathways involved in cellular antioxidant defense, as summarized previously (Liang and Kitts, 2015). The hepatic antioxidant effects of CGA have also been found in a local broiler chickens subjected to cadmium chloride-induced oxidative stress (Shi et al., 2021). The regulatory effects of CGA on cellular mitochondrial respiratory function, redox status, and biosynthesis may also contribute to the elevated antioxidant capacity in broiler chickens subjected to DQ challenge-induced oxidative stress, since mitochondria are a major source of endogenous reactive oxygen species and their dysfunctions would disrupt cellular antioxidant defense system (Zhou et al., 2016a,b; Tsai et al., 2018; Kong et al., 2019).

In consistent with a previous finding (Chen et al., 2021b), DQ injection elevated circulating total protein and total bilirubin concentrations and aspartate aminotransferase activity of broiler chickens in experiment 2, indicating that DQ challenge impaired normal homeostasis and function of liver and caused its dysfunction,

which would be correlated with DQ-induced oxidative damage, inflammatory response, and accelerated apoptosis (Chen et al., 2020; Li et al., 2020a; Chen et al., 2021b). Although its absorption rate varies greatly among different animal species, CGA can be absorbed in the stomach and small intestine and then rapidly enter into liver via portal vein system in mammals, and therefore, liver plays an important role in the transport and metabolism of absorbed CGA in animal body (Olthof et al., 2001; Lafay et al., 2006a, b; Farah et al., 2008). CGA has been found to reduce circulating activities of alanine transaminase and aspartate transaminase and total bilirubin level in mice subjected to arsenic-induced oxidative stress (Dkhil et al., 2020). Moreover, CGA administration also decreased serum alanine aminotransferase and aspartate aminotransferase activities in mice subjected to ethanol-induced oxidative stress, and the ameliorative effects of CGA on liver oxidative injury was correlated with its modulation on gut-liver axis homeostasis (Zhu et al., 2022). In experiment 2, dietary CGA supplementation partially or totally reversed circulating total protein level, aspartate aminotransferase activity, and total bilirubin concentration in DQ-administrated birds in this study, suggesting that CGA protected against hepatic oxidative damage in broilers, which may be associated with the simultaneously improved antioxidant capacity and alleviated inflammatory response of DQ-treated broilers chickens. An in vitro cell culture study has shown that pre-treatment with CGA effectively protected human HepG2 cells against tert-butylhydroperoxide-induced oxidative damage by increasing cell viability through the maintenance cellular antioxidant defense (Baeza et al., 2014). Moreover, CGA could also protect against ischemia/reperfusion-induced hepatic oxidative injury in rats through the enhancement of antioxidant defense systems and inhibition of inflammatory response (Yun et al., 2012).

Aside from inducing oxidative stress, an abdominal DQ challenge caused hepatic inflammation through increasing levels of hepatic pro-inflammatory cytokines (e. g., IL-1β, TNF-α, and IL-6), which was in agreement with the finding of Zheng et al. (2013), who reported that an intraperitoneal injection with DQ upregulated mRNA expression levels of hepatic IL-6 and TNF-α in weaned piglets. Similarly, Guo et al. (2020) found that DQ challenge increased relative mRNA expression of inflammatory factors in the small intestine, liver, and kidney of piglets by activating nuclear factor-kappa B signaling pathway. In the current research, CGA administration, regardless of its dosage, partially normalized hepatic levels of pro-inflammatory cytokines in broiler chickens subjected to DQ challenge. The anti-inflammatory effects of CGA have been reported in broiler chickens challenged with either *Clostridium perfringens* type A or coccidia according to the decreased circulating pro-inflammatory cytokine concentrations and downregulated expression levels of pro-inflammatory cytokines in small intestinal mucosa (Zhang et al., 2020; Liu et al., 2022a). In pullets, CGA also ameliorated acute heat stress-induced oxidative stress and inflammatory response (Chen et al., 2021a). In an in vivo study, an administration with CGA has been reported to alleviate carbon tetrachloride-induced oxidative stress and subsequent inflammatory response in the liver of male Sprague-Dawley rats through activation of nuclear factor erythroid 2-related factor 2 and inactivation of toll-like receptor 4 and NOD-like receptor thermal protein domain associated protein 3 signaling pathways (Shi et al., 2013, 2016; Shi et al., 2018). The improved redox status could partially at least contribute to the alleviated hepatic inflammation in broiler chickens since oxidative stress could initiate and aggravate cellular inflammatory process (Lauridsen, 2019). The anti-inflammatory effect of CGA may be also due to its regulation on signaling pathways involved in various inflammatory processes, including toll-like receptor 4/ nuclear factor-kappa B, NOD-like receptor thermal protein domain associated protein 3, extracellular signal-regulated kinases, c-Jun N-terminal kinases, and signal transducer and activator of transcription signaling pathways (Shi et al., 2013; Park et al., 2015; Shi et al., 2018; Vukelić et al., 2018; Zeng et al., 2020).

In summary, the graded levels of dietary CGA supplementation could improve growth and antioxidant capacity of broiler chickens under normal physiological status, and could effectively protect against DQ-induced oxidative stress in broiler chickens through maintaining growth performance and redox status in serum and liver, and alleviating hepatic inflammatory response.

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DISCLOSURES

All authors approve the submission of this manuscript and declare no conflict of interest.

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