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Chlorogenic acid mitigates avian pathogenic *Escherichia coli*-induced intestinal barrier damage in broiler chickens via anti-inflammatory and antioxidant effects
CHLOROGENIC ACID AND BROILER CHICKENS

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ABSTRACT

This study was conducted to investigate the protective effects of chlorogenic acid (**CGA**) on intestinal health in broilers challenged with avian pathogenic *Escherichia coli* (**APEC**). One hundred and eighty one-day-old male broiler chicks were divided into three groups with six replicates of ten chicks each for a 21-day trial. The birds in the control and APEC groups were fed a basal diet, while birds in the CGA-treated group received a basal diet supplemented with 1000 mg/kg of CGA. At 14 days, birds in the APEC and CGA groups were administered with an APEC suspension Compared with the APEC group, CGA incorporation decreased mortality and cecal *Escherichia coli* colonies in bacterially challenged broilers ($P < 0.05$). Additionally, CGA reduced the relative weight of the heart, liver, kidney, gizzard, proventriculus, and intestine, as well as serum triglyceride level and alanine aminotransferase activity in APEC-challenged broilers ($P < 0.05$). Supplementing CGA reduced the concentrations of interferon-γ, tumor necrosis factor-α, interleukin-1β, and/or interleukin-6 in serum, duodenum, jejunum, and/or ileum in APEC-challenged broilers presumably through the inactivation of the toll-like receptor 4/myeloid differentiation factor 88 pathway ($P < 0.05$). CGA administration reduced serum diamine oxidase activity and d-lactate and endotoxin concentrations, but increased the ratio between villus height and crypt depth in duodenum and jejunum of APEC-infected chickens, accompanied by the restored intestinal expression of tight junction proteins (claudin-1, claudin-2, occludin, and zonula occludens-1) and genes involved in apoptosis (B cell lymphoma-2 associated X protein, B cell lymphoma-2, and cysteine-requiring aspartate protease 9) ($P < 0.05$). Additionally, CGA increased superoxide dismutase, glutathione peroxidase, and catalase activities, and glutathione levels in serum and intestinal mucosa, but inhibited the accumulation of intestinal malondialdehyde in APEC-challenged broilers possibly via activating the nuclear factor-erythroid 2-related factor-2/heme oxygenase-1 pathway ($P < 0.05$). The results suggested that CGA alleviated APEC-induced intestinal damage in broilers by inhibiting inflammation and oxidative stress. However, its potential application in practical poultry production is contingent upon both its efficacy and cost-effectiveness.

Introduction

The bacteria, *Escherichia coli* (***E. coli***), are a diverse and ubiquitous group of species that comprise both commensal nonpathogenic and pathogenic strains. They predominantly colonize in the lower intestinal tracts of humans, mammals, livestock, poultry, and other animals, and can be released into the environment through waste water and manure (Fleckenstein and Kuhlmann, 2019; Yehia et al., 2023). These enteric bacteria have been implicated in a variety of clinical outcomes and can cause a range of diseases in humans and other animal species, from self-limiting diarrhea to life-threatening systemic diseases (Mirsepasi-Lauridsen et al., 2019; Riley et al., 2020). In poultry, the avian pathogenic *E. coli* (**APEC**) is one of the major pathogens in practical production, and it can cause diverse local and systemic infections in

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broiler chickens, laying hens, breeding chickens, turkeys, and other poultry species (Nawaz et al., 2024; Watts and Wigley, 2024). It has been reported that APEC is prevalent across all chicken production systems and age groups, with an average incidence rate ranging from 9.52 % to 36.73 %; notably, the infection rates of APEC peak during summer, and young chickens exhibit higher susceptibility than adults (Kathayat et al., 2021). Generally, the APEC infection has been shown to account for at least half of mortality cases reported in young chickens (Kathayat et al., 2021; Swelum et al., 2021). Avian colibacillosis, characterized by a diverse array of multiple organ lesions including airsacculitis, pericarditis, perihepatitis, peritonitis and other clinical and pathological manifestations, is the most common disease caused by APEC infection, which can induce high mortality, lead to growth retardation, cause postmortem carcass condemnation, and increase the monetary costs of medication, ultimately threatening poultry production and public health and resulting in severe economic loss (Guabiraba and Schouler, 2015; Hu et al., 2021; Nawaz et al., 2024). Traditionally, APEC infections have been traditionally prevented and controlled by large amounts and various types of antibiotics, leading to the occurrence and prevalence of antibiotic-resistant *E.coli*, and this has raised the public's concern about the transfer of antibiotic-resistant gene and the presence of antibiotic-resistant zoonotic bacteria through the food chain (Roth et al., 2019; Sargeant et al., 2019; Zhang et al., 2020b; Christensen et al., 2021). However, the withdrawal of antibiotic growth promoters from animal feeding has caused an increase in the incidence of APEC infection along with its complication and substantial economic losses in poultry production (Maria Cardinal et al., 2019; Fancher et al., 2020). It is, therefore, imperative and urgent to develop and apply new strategies to prevent and control APEC from the research perspective of animal nutrition, with an emphasis on limiting the transmission of antibiotic resistance from food animals to humans. Among these strategies, the natural plant extracts have gained the limelight, mainly owing to their characteristics and advantages of abundant availability, multiple biological activities, high effectiveness and efficiency, environmental friendliness and healthiness, absence of residue and toxicity, no drug resistance, and little side-effects (Abdel-Moneim et al., 2020; Veiga et al., 2020; Kuralkar and Kuralkar, 2021; Pliego et al., 2022; Rafeeq et al., 2023). While promising, the use of natural plant extracts in animal feed encounters several challenges. Existing extraction methods for plant-derived bioactive compounds remain inefficient, demanding extended processing durations and substantial solvent quantities while producing limited yields (Ameer et al., 2017). These technical limitations, combined with the high production costs and chemical instability of plant-derived extracts, substantially restrict their broader implementation in animal nutrition.

Chlorogenic acid (**CGA**), a quinic acid ester of caffeic acid, is now designated and termed as 5-*O*-caffeoylquinic acid, although its old chemical nomenclature (3-*O*-caffeoylquinic acid) is still prevalent to date due to the old numbering system of the International Union of Pure and Applied Chemistry (Tajik et al., 2017; Lu et al., 2020; Xue et al., 2023; Dai et al., 2024; Nguyen et al., 2024). This natural polyphenolic compound is one of the most abundant and beneficially functional polyphenols present in the seeds, roots, and leaves in a variety of plants such as coffee beans, *Lonicerae flos, Eucommia ulmoides* leaves, potato tubers, sweet potato leaves, and eggplant (Wang et al., 2019; Li et al., 2020; Lu et al., 2020). As a natural polyphenol, the cumulative evidence from the *in vitro* studies and clinical trials have revealed that CGA exhibited antimicrobial, antiviral, antioxidant, anti-inflammatory, glucose- and cholesterol-lowering, metabolic and immune regulatory, anti-tumoral, and multiple organ-protective properties and bioactivities (Tajik et al., 2017; Lu et al., 2020; Singh et al., 2023; Dai et al., 2024; Nguyen et al., 2024). Previous studies in swine and poultry have demonstrated that dietary administration of CGA can improve growth performance and reproductive performance, digestive function, metabolism, antioxidant capacity, immunity, intestinal integrity and barrier function, gut microflora composition, and meat quality (Chen, 2018a, b, c, 2019; Zhang et al., 2018; Zhao et al., 2019; Liu et al., 2023b; Xie et al., 2023; Bi et al., 2024; Dai et al., 2024). It has also been shown to effectively alleviate heat stress, oxidative stress, and immunological stress in model studies (Chen et al., 2021; Liu et al., 2022b, 2023a; Chen et al., 2023; Zhang et al., 2022; Tan et al., 2023; Zha et al., 2023, 2024; Liu et al., 2024; Song et al., 2024). *In vitro* experimental findings have shown that CGA can efficaciously inhibit the growth of a variety of pathogenic Gram-positive bacteria (e.g. *Streptococcus pneumoniae* and *Staphylococcus aureus*) and Gram-negative bacteria (e.g. *Salmonella* typhimurium and *E.coli*) by increasing cellular membrane permeability, disrupting membrane barrier, depleting intracellular reactive oxygen species, exhausting intracellular potential, disturbing metabolism, and inducing the release of cytoplasmic macromolecules (Lou et al., 2011; Li et al., 2014; Lee and Lee, 2018; Su et al., 2019; Le at al., 2022; Feng et al., 2023). Consistent with these findings, CGA has been demonstrated to improve growth performance, alleviate inflammatory response and oxidative stress, and prevent intestinal damage in *Eimeria*- and *Clostridium perfringens*-challenged broiler chickens (Zhang et al., 2020c; Liu et al., 2022a; Lv et al., 2024). However, until recently, little was known about the protective effects of CGA against APEC infections. According to the bioactivities of CGA, the current research was therefore designed and conducted to systematically investigate the potential beneficial effects of CGA on the intestinal health status of the APEC-infected broiler chickens by evaluating their growth performance, inflammatory response, antioxidant capacity, and intestinal integrity and barrier function. The results of this study will provide valuable insights for effectively preventing and controlling APEC challenges in the commercial broiler chicken production.

Materials and methods

Animals, diets, and management

The animal feeding experiment, sampling, and bacterial gavage in this study were conducted in accordance with guidelines and protocols approved by the Institutional Animal Care and Ethics Committee of Nanjing Agricultural University, Nanjing, Jiangsu, P.R. China.

One hundred and eighty one-day-old male broiler chicks with similar initial weight (43.25 ± 0.14 g; Arbor Acres Plus strain) were used in a 21-day feeding trial in this study. The birds were randomly assigned to one of the three groups, with each group consisting of six replicates (cages) of ten chicks each. The birds in the control and APEC challenge groups were fed a basal diet, while their counterparts in the CGA-treated group received a basal diet supplemented with 1000 mg/kg CGA of diet. The CGA, purchased from a commercial company (Hunan E.K Herb Co., Ltd., Changsha, Hunan, P.R. China), was extracted from a traditional Chinese herb, *Eucommia ulmoides* leaves, using ethyl acetate as an organic solvent. Its purity, determined by the liquid chromatography-mass spectrometry, was 98.84 %. The administration dosage of CGA used in this research was based on previous studies (Chen et al., 2023; Liu et al., 2023b; Zha et al., 2023, 2024). The APEC infection procedure was performed as described previously (Tan et al., 2024). At 14 days of age, birds in the APEC and CGA groups were orally gavaged with 1 mL of bacterial solution suspended in the sterilized bacterial culture medium, Luria-Bertani (**LB**) broth (Hope Bio-Technology Co., Ltd., Qingdao, Shandong, P.R. China; Catalog No. HB0128) at a concentration of 0.78×10^8 colony forming units per milliliter, while the unchallenged chickens were received an equivalent volume of LB broth only. The used pathogenic *E. coli* O_{78} strain (CVCC1553) was kindly gifted by Prof. Xue of the Joint International Research Laboratory of Animal Health and Food Safety of the Ministry of Education, Nanjing, Jiangsu, P.R. China. The lyophilized bacteria (*E. coli* O_{78}) were inoculated and activated on MacConkey agar plates (Hope Bio-Technology Co., Ltd., Qingdao, Shandong, P.R. China; Catalog No. HB8458) before determining viable counts on the LB agar plates. The composition and nutrient levels of the corn-soybean meal basal diet, as well as its exact vitamin and trace

mineral concentrations, are presented in the Table 1. All three experimental mash diets were prepared and processed together in a single batch and then divided into three equal amount of portions. The CGA-supplemented diet was manufactured by mixing the corresponding basal diet with CGA powder until homogeneous using a double-shaft paddle mixer. All chickens were reared in stainless steel cages (150 cm × 70 cm × 50 cm) covered with plastic mesh that were placed in a temperature-controlled house under a light schedule of 23 h light and 1 h darkness except the initial three days after arrival when a 24-h continuous light period was maintained. The temperature of the chicken house was initially controlled at 33°C to 34°C at the beginning of this experiment and then gradually decreased by 2°C to 3°C each week until reaching 25°C, which was maintained thereafter. The ambient relative humidity was initially adjusted to approximately 70 % and subsequently maintained within the range of 60 % to 65 % throughout the experimental period. All birds were allowed free access to feed and drinking water during the experimental period, except when feed restriction was implemented for the determination of growth performance during the specific period.

Sample collection

One chicken was randomly selected from each replicate and individually weighed at 21 days of age. Feed withdrawal was not employed for these sampled chickens to avoid potential intestinal barrier damage caused by feed deprivation. Blood samples were drawn from the wing vein and centrifuged at 4000 × g for 15 min at 4°C. The separated sera were refrigerated and stored at −80°C until analysis. After blood collection, the chickens were then euthanized by cervical dislocation and immediately eviscerated after blood collection. The heart, liver, thymus, spleen, bursa of Fabricius, lung, kidney, gizzard, proventriculus, duodenum, jejunum, and ileum were carefully excised, removed from the adjacent tissues and internal contents, rinsed in a chilled phosphate buffer solution, blotted on filter paper, and weighed separately to calculate relative organ weight. This was done using the following formula: relative organ weight (g) = absolute organ weight (g)/live weight of chicken (kg). A 2-cm segment was gently excised from the middle of each intestine (duodenum, jejunum, and ileum) and fixed in 10 % neutral-buffered formalin for subsequent histological examination after the clearance of connective mesentery and removal of luminal contents. The remaining intestine was then everted on a stainless tray and the exposed mucosa was carefully and gently scraped off with a sterile glass slide. The mucosal scrapings were evenly mixed, divided into three portions, snap frozen, and immediately stored in liquid nitrogen until assays were performed. Additionally, cecal content was also aseptically sampled from each bird for the microbial test.

Table 1
Composition and nutrient levels of the basal diet.

Ingredients, %	Content
Corn	58.00
Soybean meal	30.00
Corn gluten meal	4.00
Soybean oil	3.00
Limestone	1.20
Dicalcium phosphate	2.00
L-Lysine	0.30
DL-Methionine	0.20
Sodium chloride	0.30
Premix[1]	1.00
Total	100.00
Calculated nutrient levels	
Apparent metabolizable energy, MJ/kg	12.78
Crude protein, %	21.11
Calcium, %	0.99
Total phosphorus, %	0.69
Available phosphorus, %	0.46
Lysine, %	1.15
Methionine, %	0.55
Methionine + cystine, %	0.90
Analyzed nutrient levels[2]	
Crude protein, %	20.97
Calcium, %	0.95
Total phosphorus, %	0.70
Lysine, %	1.12
Methionine, %	0.53

[1] 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 sulfate), 60 mg; I (from calcium iodate), 1.1 mg; Se (from sodium selenite), 0.3 mg.
[2] Results are the average values of triplicate measurements.

Growth performance

All chickens were weighed on day of *E.coli* challenge (14 days) and at end of this study (21 days) on a replicate basis after a 12-h feed deprivation period (water remained available during the feed withdrawal period) to calculate average body weight (**BW**) and average daily gain (**ADG**). The feed consumption was recorded by subtracting the residual feed from total feed offered during each period to determine the average daily feed intake (**ADFI**) and feed conversion ratio (**FCR**). The mortality of each group during the infection period (15 to 21 days) was also calculated.

Cecal microflora counts

Approximately 0.2 g of the aseptically collected cecal content was diluted with a 2-mL sterile saline solution followed by homogenization with vigorous vortex mixing. The homogenized suspension was then serially diluted from $10^{\text{ to }3}$ to 10^{-6} and 100 μL of each diluted sample was subsequently spot on MacConkey agar plates (Hope Bio-Technology Co., Ltd., Qingdao, Shandong, P.R. China), which was incubated at 37°C for 24 h. The number of colony-forming units was counted at least twice per plate and expressed as a logarithmic value per gram of the cecal content in each chicken.

Serum biochemical parameters

The serum biochemical indices, including total protein (Catalog No. A045-4-2), albumin (Catalog No. A028-2-1), triglyceride (Catalog No. A110-1-1), total cholesterol (Catalog No. A111-1-1), and glucose (Catalog No. 154-1-1) levels, as well as transaminase activities (aspartate aminotransferase (Catalog No. C010-2-1) and alanine aminotransferase (Catalog No. C009-2-1), were colorimetrically determined with the corresponding kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, P.R. China) on a microplate reader (MODEL 680, Bio-Rad Laboratories Inc., Hercules, CA, USA), following the manufacturer's instructions.

Preparation of mucosal homogenate

The intestinal mucosal scrapings were thawed from liquid nitrogen and diluted in cold saline solution. They were then homogenized using a motor-driven homogenizer (PRO-PK-02200D, Pro Scientific, Inc., Monroe, CT, USA) at high speed for approximately 30 s in an ice-cold water bath. To prevent localized heating, there was a 30-s interval between each homogenization. The resulting homogenate was then centrifuged for 15 min at 5000 × g in a refrigerated centrifuge and the supernatant was collected and aliquoted into Eppendorf tubes, which were stored at −80°C for further analysis.

Cytokines in blood and intestinal mucosa

The concentrations of cytokines in both serum and intestinal mucosa were quantified with an enzyme-linked immunosorbent assay, including interleukin-1β (**IL-1β**, sensitivity < 1.0 pg/mL, catalog No. CK-EN60036), interferon-γ (**IFN-γ**, sensitivity < 0.1 pg/mL, catalog No. CK-EN60027), tumor necrosis factor-α (**TNF-α**, sensitivity < 0.1 pg/mL, catalog No. CK-EN60161), interleukin-6 (**IL-6**, sensitivity < 0.1 pg/mL, catalog No. CK-EN60041), and interleukin-10 (**IL-10**, sensitivity < 0.1 pg/mL, catalog No. CK-EN60031). The chicken-specific enzyme-linked immunosorbent assay kits were purchased from Hongsheng Co., Ltd. (Nanjing, Jiangsu, P.R. China) and all measurements were performed using a microplate reader after necessary dilution, strictly following the protocols provided by the manufacturer.

Intestinal permeability-related biomarkers

The levels of d-lactate and endotoxin and diamine oxidase activity in the blood are commonly used as sensitive and useful indicators of intestinal permeability in broiler chickens (Gilani et al., 2021). d-lactate concentration was colorimetrically determined with a microplate reader and a commercial assay kit (AAT Bioquest, Sunnyvale, CA, USA; Catalog No. AAT-13811) based on a standard curve. The circulating endotoxin level was quantitatively detected by the limulus amebocyte lysate pyrogen test kit (Xiamen Houshiji, Ltd., Fujian, P.R. China; Catalog No. EC80545S). The activity of diamine oxidase was measured spectrophotometrically for absorbance using a reagent kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, Jiangsu, P.R. China; Catalog No. A088-1-1). A unit of diamine oxidase activity was defined as the amount of enzyme required to catalyze the oxidation of 1,4-diaminobutane under standard assay conditions within a 10-min incubation period at 37°C.

Histological examination

The formalin-fixed intestinal segments were dehydrated in graded ethanol, transparentized with xylene, and embedded in paraffin wax. The 5-μm-thick sections were then cut using a rotary microtome. After being baked overnight at 37°C, the sections were deparaffinized in a xylene-ethanol series, dehydrated in an ethanol-xylene series, and washed in deionized water prior to hematoxylin and eosin staining. An optical microscope equipped with a digital camera (Nikon ECLIPSE 80i; Nikon Corporation, Tokyo, Japan) was used to take digital photographs of the stained sections. The acquired images were then quantitatively analyzed using ImageJ software. The examinations of villus height (**VH**) and crypt depth (**CD**) were performed on at least six random areas with well-preserved villi in each specimen.

Antioxidant capacity

In the current research, we measured various antioxidant-related parameters, including total antioxidant capacity (**T-AOC**, catalog No. A015-1-1), superoxide dismutase (**SOD**, catalog No. A001-1-2), glutathione peroxidase (**GSH-Px**, catalog No. A005-1-2), catalase (**CAT**, catalog No. A007-1-1), malondialdehyde (**MDA**, catalog No. A003-1-2), and glutathione (**GSH**, catalog No. A006-2-1), in both serum and intestinal mucosa. These measurements were performed using commercially available assay kits purchased from Nanjing Jiancheng Bioengineering Institute in Nanjing, P.R. China. The T-AOC activity reflects the overall cellular antioxidant capacity and was assessed using a spectrometric method (Haida and Hakiman, 2019). This method measures the reduction of the substrate ferric 2,4,6 tripyridyl-S-triazine complex by cellular antioxidants, resulting in the formation of a blue ferrous 2,4,6 tripyridyl-S-triazine complex, which can be measured by changes in absorbance. SOD activity was determined using the hydroxylamine method (Kono, 2022), and one unit of SOD activity was defined as the amount of enzyme required to achieve 50 % inhibition of nitrite generation from hydroxylamine per milliliter of serum or milligram of tissue protein during a 40-min incubation at 37°C. The CAT activity was measured using the ammonium molybdate method (Farman and Hadwan, 2021), with enzyme activity defined as the amount required to catalyze the decomposition of 1 μmol hydrogen peroxide per minute at 37°C, either per milliliter of serum or per milligram of protein. The classic 5, 5′-dithiobis (2-nitrobenzoic acid) method was used to quantify GSH-Px activity and GSH concentration, as described previously (Giustarini et al., 2014). One unit of GSH-Px activity was calculated as enzyme quantity needed to oxidize one micromole of GSH within a 5-min period at 37°C, measured either per one hundred microliters of serum or per milligram of protein from the intestinal mucosa. MDA, an end product of lipid peroxidation, was determined using the thiobarbituric acid method (Ghani et al., 2017). All measurements were carried out following the standard protocol provided by the manufacturer.

RNA isolation and gene expression

The total RNA was extracted from the intestinal mucosal scrapings using a commercial RNA extraction kit (Accurate Biology, Hunan, P.R. China; Catalog No. AG21024). The quality and quantity of the extracted RNA were assessed by measuring the ratio of the optical densities at 260 nm and 280 nm using a NanoDrop ND-1000 UV spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). The integrity of the RNA was verified by electrophoresis on a 1.5 % (w/v) agarose gel stained with Ultra GelRed (Vazyme Biotech Co., Ltd., Nanjing, Jiangsu, P.R. China; Catalog No. GR501-01). The qualified RNA was then reverse-transcribed into complementary DNA using 5X All-In-One RT MasterMix (abmGood, Zhenjiang, Jiangsu, P.R. China; Catalog No. G592). The polymerase chain reaction (**PCR**) was performed on a QuantStudio™ 5 Real-Time PCR System (Applied Biosystems, Life Technologies, CA, USA) with a TOROGreen® qPCR Master Mix (TOROIVD, Shanghai, P.R. China; Catalog No. QST-100) under the following thermocycling program: a 30-s pre-run at 95°C, followed by 40 cycles of denaturation at 95°C for 5 s, and an annealing step at 60°C for 30 s. The primer sequences for the reference gene and target genes are presented in Table 2 (*IFN-γ, TNF-α, IL-1β, IL-6, IL-10*, toll-like receptor 4 (***TLR4***), myeloid differentiation factor 88 (***MyD88***), nuclear factor-erythroid 2-related factor-2 (***Nrf2***), heme oxygenase-1 (***HO-1***), superoxide dismutase 1 (***SOD1***), glutathione peroxidase 1 (***GPX1***), claudin-1 (***CLDN1***), claudin-2 (***CLDN2***), occludin (***OCLN***), zonula occludens-1 (***ZO-1***), cysteine-requiring aspartate protease 3 (***Caspase 3***), cysteine-requiring aspartate protease 9 (***Caspase 9***), B cell lymphoma-2 associated X protein (***Bax***), B cell lymphoma-2 (***Bcl-2***), and *β-actin*). The relative mRNA abundance of the target genes was determined using the classic $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001).

Statistical analysis

The data acquired were analyzed using the Statistical Package for the Social Sciences Packet Program (ver. 22.0 for Windows, SPSS Inc., Chicago, USA) The experimental unit for all growth performance data was the pen, while an individual chicken selected from each replicate was the experimental unit for other indices. The Levene's test and Shapiro-Wilk test were used to assess the homogeneity of variances and the normality of the response variables, respectively. For normally distributed data, one-way ANOVA and Tukey's post hoc test were used to determine statistical significance and perform pairwise comparisons. A two-tailed *P* value of less than 0.05 was considered statistically significant, while a *P* value between 0.05 and 0.1 was considered a trend (Schumm et al., 2013; Wood et al., 2014). The results were presented as means and their pooled standard errors for data in tables and as means and their corresponding standard errors for data in figures.

Table 2
Sequences of primers for polymerase chain reaction.

Gene[1]	Gene Bank ID	Primer sequence, sense/antisense
TLR4	NM_001030693.1	AGGCACCTGAGCTTTTCCTC
		TACCAACGTGAGGTTGAGCC
MyD88	NM_001030962.1	ATCCGGACACTAGAGGGAGG
		GGCAGAGCTCAGTGTCCATT
IFN-γ	NM_205149.1	CACTGACAAGTCAAAGCCGC
		ACCTTCTTCACGCCATCAGG
IL-1β	NM_204524.1	GTACCGAGTACAACCCCTGC
		AGCAACGGGACGGTAATGAA
TNF-α	NM_204267	TGTGTATGTGCAGCAACCCGTAGT
		GGCATTGCAATTTGGACAGAAGT
IL-6	HM179640	AAATCCCTCCTCGCCAATCT
		CCCTCACGGTCTTCTCCATAAA
IL-10	NM_001004414.2	CGCTGTCACCGCTTCTTCA
		TCCCGTTCTCATCCATCTTCTC
Nrf2	NM_205117.1	GATGTCACCCTGCCCTTAG
		CTGCCACCATGTTATTCC
HO-1	NM_205344.2	GTCGTTGGCAAGAAGCATCC
		GGGCCTTTTGGGCGATTTTC
SOD1	NM_205064.2	CCGGCTTGTCTGATGGAGAT
		TGCATCTTTTGGTCCACCGT
GPX1	NM_001277853.3	GACCAACCCGCAGTACATCA
		GAGGTGCGGGCTTTCCTTTA
OCLN	NM_205128.1	AGTTCGACACCGACCTGAAG
		TCCTGGTATTGAGGGCTGTC
CLDN1	NM_001013611.2	AAGTGCATGGAGGATGACCA
		GCCACTCTGTTGCCATACCA
CLDN2	NM_001277622.1	CCTGCTCACCCTCATTGGAG
		GCTGAACTCACTCTTGGGCT
ZO-1	XM_015278981.1	ACAGCTCATCACAGCCTCCT
		TGAAGGGCTTACAGGAATGG
Caspase 3	XM_015276122.2	ACAGCAAGCGAAGCAGTTTT
		TCACCTCTGAAAAGGCTGGT
Caspase 9	XM_424580.5	TATGGTGGAGGACATGCAGA
		AATATTGGGAAGGCCTGCTT
Bcl-2	NM_205339.2	ATCGTCGCCTTCTTCGAGTT
		ATCCCATCCTCCGTTGTCCT
Bax	XM_015274882	GTACGTCAATGTGGTCACCC
		TGGGATAATGCTGGGGTTGA
β-actin	NM_205518.1	TTGGTTTGTCAAGCAAGCGG
		CCCCCACATACTGGCACTTT

[1] *Bax*, B cell lymphoma-2 associated X protein; *Bcl-2*, B cell lymphoma-2; *Caspase 3*, cysteine-requiring aspartate protease 3; *Caspase 9*, cysteine-requiring aspartate protease 9; *CLDN1*, claudin-1; *CLDN2*, claudin-2; *GPX1*, glutathione peroxidase 1; *HO-1*, heme oxygenase-1; *IFN-γ*, interferon-γ; *IL-1β*, interleukin-1β; *IL-6*, interleukin-6; *IL-10*, interleukin-10; *MyD88*, myeloid differentiation factor 88; *Nrf2*, nuclear factor-erythroid 2-related factor-2; *OCLN*, occludin; *SOD1*, superoxide dismutase 1; *TLR4*, toll-like receptor 4; ZO-1, zonula occludens-1.

Results

Growth performance

There were no statistically significant differences (Table 3) in the growth performance (ADG, ADFI, BW, and FCR) of broiler chickens among the three treatment groups prior to APEC challenge during the initial 14 days ($P > 0.05$). However, an oral gavage with APEC caused sharp reductions in ADG, ADFI, and BW compared to the control group, along with a significant increase in mortality rate ($P < 0.05$). However, when compared with the control group, an oral gavage with APEC caused sharp reductions in ADG, ADFI, and BW, accompanied by a higher mortality rate ($P < 0.05$). Interestingly, the FCR remained unchanged in the presence of the APEC challenge ($P > 0.05$). In contrast to these results, the incorporation of CGA resulted in an increase FCR and a decrease in mortality of broiler chickens during days 15 to 21 ($P < 0.05$) when compared with the APEC challenge group. However, CGA supplementation did not exert any significant effect on ADFI, ADG, or BW in those birds administered with *E. coli* ($P > 0.05$).

Table 3
Effects of dietary chlorogenic acid supplementation on the growth performance of avian pathogenic *Escherichia coli*-challenged broiler chickens.

Items	Treatments[1]			SEM[2]	*P*-values	
	CON	APEC	CGA		CON vs *E. coli*	CGA vs *E. coli*
Prior to challenge (1 to 14 days)						
Average daily gain (g/d)	23.98	23.91	24.68	0.27	0.922	0.277
Average daily feed intake (g/d)	29.66	30.74	32.51	0.70	0.508	0.349
Feed conversion ratio (g/g)	1.24	1.28	1.32	0.02	0.244	0.592
14-day body weight (g)	379.17	377.83	389.17	3.89	0.897	0.258
After challenge (15 to 21 days)						
Average daily gain (g/d)	68.93	60.26	62.91	1.30	0.007	0.380
Average daily feed intake (g/d)	103.38	88.10	96.79	2.32	0.009	0.135
Feed conversion ratio (g/g)	1.50	1.46	1.54	0.02	0.326	0.041
21-day body weight (g)	861.67	799.62	829.50	10.72	0.034	0.277
Mortality (%)	0.00	20.00	0.00	2.91	0.006	0.006

[1] CON, normal broilers fed a basal diet; APEC, avian pathogenic *Escherichia coli*-challenged broilers fed a basal diet; CGA, avian pathogenic *Escherichia coli*-challenged broilers fed a basal diet supplemented with 1.0 g/kg of chlorogenic acid.
[2] SEM, standard error of the mean ($n = 6$).

Cecal E. coli colonies

As indicated in Fig. 1, the bacterially challenged birds had a higher number of *E. coli* colonies in the cecal content than that of their counterparts in the control group ($P < 0.05$). The addition of CGA to a basal diet decreased cecal *E. coli* colonies in broiler chickens subjected to an APEC challenge in comparison with those challenged birds fed a basal diet only ($P < 0.05$).

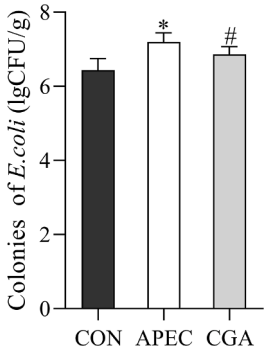

Fig. 1. Effects of dietary chlorogenic acid supplementation on the number of *Escherichia coli* colonies in the cecal content of avian pathogenic *Escherichia coli*-challenged broiler chickens The columns with error bars represent the mean ± standard error of measurement for each experimental group ($n = 6$). CON, normal broilers fed a basal diet; APEC, avian pathogenic *Escherichia coli*-challenged broilers fed a basal diet; CGA, avian pathogenic *Escherichia coli*-challenged broilers fed a basal diet supplemented with 1.0 g/kg of chlorogenic acid; CFU, colony forming units. *Means significant difference from the CON group ($P < 0.05$). #Means significant difference from the *E. coli* challenge group ($P < 0.05$).

Relative organ weight

Compared with the control group (Table 4), the APEC challenge caused an increase in the relative weight of the liver, kidney, gizzard, proventriculus, jejunum, and ileum ($P < 0.05$). Additionally, there was a trend towards increased relative weight of the heart ($P = 0.068$) and duodenum ($P = 0.054$) in birds exposed to APEC. In contrast, incorporation of CGA reduced the relative weight of the heart, liver, kidney, gizzard, proventriculus, duodenum, jejunum, and ileum in APEC-challenged broiler chickens ($P < 0.05$). Furthermore, CGA-treated birds exhibited an increase in the relative weight of the thymus compared with their challenged counterparts receiving a basal diet ($P < 0.05$). There were no statistically significant differences in the relative weight of spleen, bursa of Fabricius, or lung among the experimental treatments ($P > 0.05$).

Serum biochemical indices

Compared with the control group (Table 5), APEC infection decreased the levels of total protein and glucose, but increased triglyceride concentration and aspartate aminotransferase activity in serum ($P < 0.05$). There was also a trend towards increased serum alanine aminotransferase activity in *E. coli*-challenged broilers ($P = 0.052$). However, dietary supplementation with CGA reduced serum triglyceride level and alanine aminotransferase activity in comparison with APEC challenge group ($P < 0.05$). In addition to this result, CGA showed a tendency to increase serum glucose level in broiler chickens exposed to an oral *E. coli* challenge ($P = 0.097$). Neither albumin nor total cholesterol levels in serum were affected by the treatments ($P > 0.05$).

Cytokine levels

The exposure to APEC infection increased the concentrations of IFN-γ, TNF-α, IL-1β, and IL-6 in both serum and duodenal mucosa, as well as elevated concentrations of IFN-γ, TNF-α, and IL-6 in jejunal mucosa, and IL-6 concentration in ileal mucosa (Table 6, $P < 0.05$). Additionally, it also led to decreased levels of IL-10 in both serum and intestinal mucosa (duodenum, jejunum, and ileum) when compared with the control group ($P < 0.05$). In contrast, the administration of CGA reduced the concentrations of IFN-γ, TNF-α, IL-1β, and IL-6 in serum, concentrations of IFN-γ, TNF-α, and IL-6 in duodenal mucosa, concentrations of IFN-γ and TNF-α in jejunal mucosa, and IL-6 concentration in ileal mucosa ($P < 0.05$), when compared with the APEC group. Moreover, CGA supplementation increased the levels of IL-10 in all three segments of intestinal mucosa ($P < 0.05$) in comparison with the APEC infection group. A decreased tendency towards decreased jejunal IL-6 level was also found in APEC-challenged birds receiving a CGA-supplemented diet. No significant differences were found for jejunal and ileal IL-1β, ileal IFN-γ, or ileal TNF-α concentrations between the treatments ($P > 0.05$).

Table 4
Effects of dietary chlorogenic acid supplementation on relative organ weight of avian pathogenic *Escherichia coli*-challenged broiler chickens (g/kg).

Items	Treatments[1]			SEM[2]	*P*-values	
	CON	APEC	CGA		CON vs *E. coli*	CGA vs *E. coli*
Heart	5.35	5.92	5.39	0.12	0.068	0.041
Liver	24.31	30.54	24.98	0.88	0.002	0.003
Thymus	4.10	3.46	4.65	0.21	0.265	0.002
Spleen	0.83	0.90	0.96	0.04	0.445	0.536
Bursa of Fabricius	2.35	2.57	2.31	0.13	0.600	0.295
Lung	5.64	6.37	6.60	0.23	0.131	0.721
Kidney	6.03	7.78	6.12	0.28	0.009	0.009
Gizzard	18.43	21.00	17.20	0.42	<0.001	<0.001
Proventriculus	5.45	6.81	5.25	0.21	0.003	0.002
Duodenum	7.45	8.09	6.59	0.20	0.054	0.002
Jejunum	12.93	15.55	12.53	0.40	0.003	0.001
Ileum	7.46	8.74	7.74	0.19	0.003	0.022

[1] CON, normal broilers fed a basal diet; APEC, avian pathogenic *Escherichia coli*-challenged broilers fed a basal diet; CGA, avian pathogenic *Escherichia coli*-challenged broilers fed a basal diet supplemented with 1.0 g/kg of chlorogenic acid.
[2] SEM, standard error of the mean ($n = 6$).

Table 5
Effects of dietary chlorogenic acid supplementation on serum biochemical indices of avian pathogenic *Escherichia coli*-challenged broiler chickens.

Items	Treatments[1]			SEM[2]	*P*-values	
	CON	APEC	CGA		CON vs *E. coli*	CGA vs *E. coli*
Total protein (g/L)	21.94	20.06	20.99	0.33	0.026	0.299
Albumin (g/L)	13.61	13.73	12.92	0.25	0.816	0.254
Glucose (mmol/L)	13.24	12.23	13.07	0.21	0.049	0.097
Total cholesterol (mmol/L)	4.67	5.14	4.58	0.15	0.285	0.201
Triglyceride (mmol/L)	0.81	1.11	0.78	0.06	0.024	0.007
Alanine aminotransferase (U/L)	15.47	17.71	15.20	0.44	0.052	0.024
Aspartate aminotransferase (U/L)	66.99	82.03	77.29	3.25	0.043	0.579

[1] CON, normal broilers fed a basal diet; APEC, avian pathogenic *Escherichia coli*-challenged broilers fed a basal diet; CGA, avian pathogenic *Escherichia coli*-challenged broilers fed a basal diet supplemented with 1.0 g/kg of chlorogenic acid.
[2] SEM, standard error of the mean ($n = 6$).

Expression of intestinal genes related to inflammation

Compared with the control group (Fig. 2), the APEC challenge resulted in an up-regulation of *TLR4* and *MyD88* expression in three segments of the intestinal mucosa of broiler chickens ($P < 0.05$). This was accompanied by a higher abundance of *IFN-γ* and *IL-6* in the duodenum and jejunum, *TNF-α* in the ileum, and *IL-1β* in the duodenum and ileum, as well as a lower abundance of *IL-10* in the ileum ($P < 0.05$). In contrast, CGA administration down-regulated the expression of *TLR4* and *MyD88* in all three segments of the intestine in chickens subjected to APEC challenge ($P < 0.05$). In addition, CGA supplementation also reduced the expression of duodenal *IFN-γ*, duodenal *IL-1β*, duodenal and jejunal *IL-6*, and ileal *TNF-α*, and increased the expression of ileal *IL-10* ($P < 0.05$). However, no statistically significant differences were found for the abundance of *TNF-α* and *IL-10* in the duodenum and jejunum, *IL-1β* in the jejunum, or *IFN-γ* and *IL-6* in the ileum ($P > 0.05$).

Intestinal permeability and morphology

The APEC challenge elevated circulating diamine oxidase activity and d-lactate and endotoxin concentrations in broiler chickens compared with their normal counterparts (Table 7, $P < 0.05$), and the values of these three parameters in the APEC-challenged birds were reduced when feeding a CGA-supplemented diet ($P < 0.05$). Compared with the control group, APEC infection decreased jejunal VH and the ratio between the VH and CD in the intestine (duodenum, jejunum, and ileum), while increased duodenal CD ($P < 0.05$). There was also a trend towards increased jejunal CD in APEC-challenged chickens ($P = 0.091$). The incorporation of CGA increased VH/CD in both the duodenum and jejunum ($P < 0.05$) and exhibited a tendency to increase VH/CD in ileum ($P = 0.091$) compared with APEC challenge group. No significant differences were found in duodenal VH, ileal VH, or ileal CD between the treatments ($P > 0.05$).

Table 6
Effects of dietary chlorogenic acid supplementation on cytokine concentrations in serum and intestinal mucosa of avian pathogenic *Escherichia coli*-challenged broiler chickens.

Items[1]	Treatments[2]			SEM[3]	*P*-values	
	CON	APEC	CGA		CON vs *E. coli*	CGA vs *E. coli*
Serum						
INF-γ (pg/mL)	46.31	57.25	45.25	1.76	<0.001	0.006
TNF-α(pg/mL)	47.69	56.99	49.91	1.20	0.002	0.008
IL-1β (pg/mL)	378.58	486.23	381.94	14.10	<0.001	<0.001
IL-6 (pg/mL)	17.78	23.37	19.82	0.68	<0.001	0.003
IL-10 (pg/mL)	60.33	49.13	49.75	1.81	0.017	0.767
Duodenum						
IFN-γ (pg/mg protein)	5.52	7.42	6.33	0.24	0.001	0.002
TNF-α (pg/mg protein)	5.14	6.86	6.19	0.22	0.001	0.046
IL-1β (pg/mg protein)	46.30	53.95	54.27	1.34	0.032	0.893
IL-6 (pg/mg protein)	2.45	2.82	2.47	0.06	0.018	0.014
IL-10 (pg/mg protein)	7.43	5.86	7.06	0.21	0.003	0.001
Jejunum						
IFN-γ (pg/mg protein)	6.53	7.91	6.26	0.27	0.033	0.023
TNF-α (pg/mg protein)	6.11	7.35	6.01	0.18	0.004	0.003
IL-1β (pg/mg protein)	54.19	58.38	56.48	1.89	0.479	0.716
IL-6 (pg/mg protein)	2.46	3.06	2.74	0.08	0.001	0.060
IL-10 (pg/mg protein)	8.29	6.37	7.86	0.29	0.014	0.006
Ileum						
IFN-γ (pg/mg protein)	6.03	6.40	6.28	0.13	0.248	0.726
TNF-α (pg/mg protein)	5.89	6.14	6.21	0.18	0.444	0.908
IL-1β (pg/mg protein)	48.39	55.25	50.66	2.20	0.139	0.447
IL-6 (pg/mg protein)	2.40	2.94	2.39	0.09	0.017	0.003
IL-10 (pg/mg protein)	8.32	6.82	7.87	0.25	0.031	0.021

[1] IFN-γ, interferon-γ; TNF-α, tumor necrosis factor-α; IL-1β, interleukin-1β; IL-6, interferon-6; IL-10, interferon-10.
[2] CON, normal broilers fed a basal diet; APEC, avian pathogenic *Escherichia coli*-challenged broilers fed a basal diet; CGA, avian pathogenic *Escherichia coli*-challenged broilers fed a basal diet supplemented with 1.0 g/kg of chlorogenic acid.
[3] SEM, standard error of the mean ($n = 6$).

Expression of intestinal tight junction proteins and apoptosis-related genes

As shown in Fig. 3, the expression of *CLDN1* and *CLDN2* in both the duodenal and jejunal mucosa was up-regulated in the APEC-challenged group compared to the control group ($P < 0.05$). Additionally, the expression of *CLDN2* in the ileal mucosa was also up-regulated, while the expression of *ZO-1* in both the duodenal and jejunal mucosa and *OCLN* in the ileal mucosa was down-regulated ($P < 0.05$). Furthermore, *E.coli* infection resulted in an up-regulation of *Caspase 9* in the duodenal and ileal mucosa, and *Bax* in the jejunal and ileal mucosa, while down-regulated the expression of *Bcl-2* in the jejunal mucosa ($P < 0.05$). In contrast to these results, supplementation with CGA normalized the expression of *CLDN1* and *CLDN2* in both the duodenal and jejunal mucosa, *ZO-1* in the jejunal mucosa, *OCLN* in the ileal mucosa, *Bax* and *Bcl-2* in the jejunal mucosa, and *Caspase 9* in the duodenal and ileal mucosa ($P < 0.05$) of APEC-challenged birds. However, the treatments did not alter the expression of duodenal and jejunal *OCLN*, ileal *CLDN1*, ileal *ZO-1,* duodenal *Bax*, duodenal and ileal *Bcl-2*, jejunal *Caspase 9*, or intestinal *Caspase 3* in broiler chickens ($P > 0.05$).

Antioxidant capacity

Compared with the control group (Table 8), the APEC challenge decreased T-AOC activity in the serum, duodenum, and jejunum, SOD activity in the serum and jejunum, GSH-Px activity in the serum, duodenum, and ileum, CAT activity in the serum and intestinal mucosa (duodenum, jejunum, and ileum), and GSH level in all three segments of the intestine, but increased MDA level in the duodenum ($P < 0.05$). Additionally, APEC challenge also tended to reduce jejunal GSH-Px ($P = 0.091$) and ileal SOD ($P = 0.081$) activities in broiler chickens. In contrast, dietary supplementation with CGA increased T-AOC activity in the serum, duodenum, and jejunum, SOD activity in the serum, GSH-Px activity in the serum, CAT activity in the jejunum and ileum, and GSH level in the duodenum and jejunum, but decreased MDA accumulation in the duodenum and ileum of the APEC-challenged broiler chickens ($P < 0.05$). Furthermore, an increased tendency was also found for duodenal GSH-Px ($P = 0.091$) and CAT ($P = 0.070$) activities, as well as ileal GSH concentration ($P = 0.066$). No significant differences were found for serum MDA and GSH levels, duodenal SOD activity, jejunal MDA level, or ileal T-AOC activity between the treatments ($P > 0.05$).

Expression of intestinal antioxidant-related genes

The APEC challenge down-regulated the expression of *Nrf2* in the jejunum and ileum, as well as *HO-1* in the duodenum and jejunum (Fig. 4, $P < 0.05$), when compared with normal counterparts. This was accompanied by a lower level of *SOD1* expression in the jejunum and ileum ($P < 0.05$). Dietary CGA supplementation increased the expression of *Nrf2* in the jejunum and ileum and *SOD1* in the ileum in comparison with *E. coli*-challenged birds receiving a basal diet only ($P < 0.05$). The treatments did not alter the expression of *GPX1* in the intestinal mucosa of broiler chickens ($P > 0.05$).

Discussion

The negative effects of APEC infection on the growth performance and mortality rate of poultry have been frequently reported in a variety of species (Christensen et al., 2021; Hu et al., 2022; Joseph et al., 2023; Tilli et al., 2024; Waliaula et al., 2024). In broiler chickens, an oral APEC exposure has been shown to result in apparently inferior weight gain, feed intake, and feed efficiency, as well as a higher mortality during the infection period compared with their healthy counterparts without challenge (Jahanian et al., 2021; Wu et al., 2021; Pham et al., 2022; Tan et al., 2024; Xu et al., 2024). The current research also found that APEC challenge had detrimental effects on the growth performance of broiler chickens, as evidenced by the lower BW, ADG, and ADFI, as well as a high mortality rate. These negative consequences can be attributed to local and systemic bacterial infection and inflammatory processes, digestive and metabolic disorders, organ dysfunction, oxidative stress, intestinal barrier injury damage and subsequent bacterial translocation, and disrupted intestinal microflora (Guabiraba and Schouler, 2015; Dubreuil et al., 2016; Alber et al., 2021; Christensen et al., 2021; Joseph et al., 2023). Until recently, there has been limited research on the protective effects of CGA on the growth performance of broiler chickens subjected to the APEC challenge. Although our study did not find any improvements in ADFI, ADG or BW with CGA supplementation in APEC-challenged birds, its supplementation increased FCR. This was partially consistent with the finding of Tan et al. (2023), who reported no changes in BW or weight gain in broiler chickens challenged with lipopolysaccharide, a key virulence factor of APEC. The harmful consequences of APEC on growth performance of broiler chickens can vary

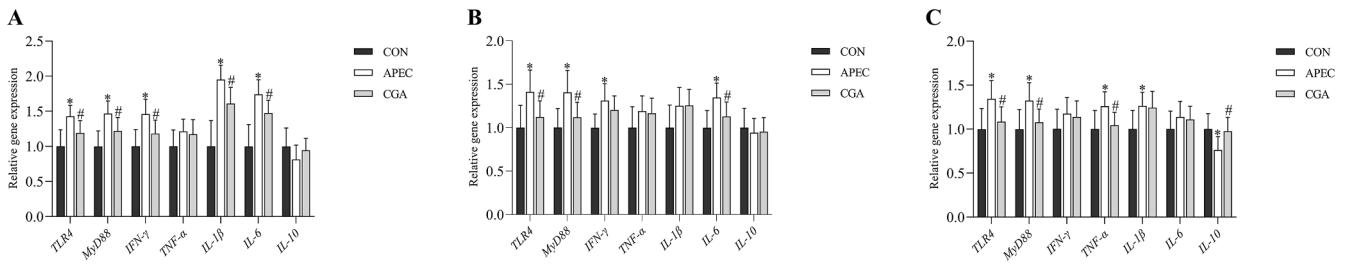

Fig. 2. Effects of dietary chlorogenic acid supplementation on expression of genes related to inflammation in the duodenal (A), jejunal (B), and ileal (C) mucosa of avian pathogenic *Escherichia coli*-challenged broiler chickens The columns with error bars represent the mean ± standard error of measurement for each experimental group ($n = 6$). CON, normal broilers fed a basal diet; APEC, avian pathogenic *Escherichia coli*-challenged broilers fed a basal diet; CGA, avian pathogenic *Escherichia coli*-challenged broilers fed a basal diet supplemented with 1.0 g/kg of chlorogenic acid; *TLR4*, toll-like receptor 4; *MyD88*, myeloid differentiation factor; *IFN-γ*, interferon-γ; *TNF-α*, tumor necrosis factor-α; *IL-1β*, interleukin-1β; *IL-6*, interleukin-6; *IL-10*, interleukin-10. *Means significant difference from the CON group ($P < 0.05$). #Means significant difference from the *E. coli* challenge group ($P < 0.05$).

Table 7
Effects of dietary chlorogenic acid supplementation on the intestine permeability and morphology of avian pathogenic *Escherichia coli*-challenged broiler chickens.

Items	Treatments[1]			SEM[2]	*P*-values	
	CON	APEC	CGA		CON vs *E. coli*	CGA vs *E. coli*
Serum						
Diamine oxidase (U/L)	10.42	25.55	12.57	1.79	<0.001	<0.001
Endotoxin (EU/mL)	0.205	0.301	0.278	0.011	0.001	0.011
D-Lactate (mmol/L)	0.527	0.853	0.556	0.049	<0.001	0.015
Duodenum						
Villus height (μm)	1071.59	1087.53	1095.67	12.12	0.640	0.830
Crypt depth (μm)	181.49	200.50	191.16	2.91	0.017	0.202
Villus height/ Crypt depth	5.91	5.44	5.74	0.06	<0.001	0.002
Jejunum						
Villus height (μm)	1021.34	955.60	951.80	13.05	0.028	0.900
Crypt depth (μm)	187.16	195.95	188.99	2.51	0.091	0.350
Villus height/ Crypt depth	5.47	4.89	5.05	0.06	<0.001	0.017
Ileum						
Villus height (μm)	831.05	764.42	791.71	15.54	0.167	0.524
Crypt depth (μm)	156.49	154.77	156.54	2.62	0.839	0.819
Villus height/ Crypt depth	5.32	4.94	5.06	0.04	<0.001	0.091

[1] CON, normal broilers fed a basal diet; APEC, avian pathogenic *Escherichia coli*-challenged broilers fed a basal diet; CGA, avian pathogenic *Escherichia coli*-challenged broilers fed a basal diet supplemented with 1.0 g/kg of chlorogenic acid.
[2] SEM, standard error of the mean ($n = 6$).

depending on the time after infection (Xu et al., 2024). In a previous study, the beneficial effects of resveratrol on the growth performance of APEC-challenged birds at an early age were time dependent, and the improvement in growth performance was only observed after two weeks rather than in the first week after bacterial infection (Mohebodini et al., 2019). Therefore, the lack of significant changes in feed intake, weight gain, and BW in the CGA-supplemented group in our study may be due to the dosage of APEC, age of the birds, and the short duration of the experiment. Consequently, it would be necessary to implement an extended experimental duration in future feeding trials to thoroughly assess the protective effects of CGA on the growth performance of APEC-challenged broiler chickens. In this study, however, CGA supplementation did significantly reduce mortality rate of broiler chickens after oral APEC challenge. This is in agreement with previous research showing that CGA can reduce mortality rate in broiler chickens infected with *Eimeria* (Liu et al., 2022a). The inhibited colonization of intestinal *E. coli*, alleviated inflammation and oxidative stress, and improved intestinal barrier function would together provide an explanation for this decrease in the morality of CGA-supplemented group. Additionally, the significant difference in the FCR between the CGA and APEC challenge groups may be closely correlated with the difference in morality rather than changes in ADG or ADFI.

The APEC challenge resulted in an increase in the relative weight of multiple organs (liver, kidney, gizzard, proventriculus, jejunum, and ileum) in this experiment, which is a typical and common characteristic of colibacillosis. This was accompanied by alternations in serum biochemical indices (total protein, glucose, and triglyceride levels, as well as aminotransferase activity), indicating that APEC infection impaired the structure and functionality of these organs. These results were in parallel with the simultaneously increased presence of *E. coli* colonies in the cecal content. The impaired functionality of these weighed organs and the altered serum biochemical indices can be directly linked to the increased colonization of APEC in the intestine and their potential translocation from the lumen to the bloodstream due to a compromised intestinal barrier.

This assumption is supported by the observed increase in intestinal permeability and damage to the intestinal barrier in our study. Similar results have been reported in previous studies on broiler chickens that were orally challenged with *E. coli* (Manafi et al., 2017; Mohebodini et al., 2019; Tang et al., 2021). The negative effects of APEC on the organ weight and serum biochemical indices were mitigated when the chickens were fed a basal diet supplemented with CGA. Specifically, CGA supplementation restored the relative weight of the measured organs (heart, liver, kidney, gizzard, proventriculus, and intestine (duodenum, jejunum, and ileum)) and improved the circulating triglyceride level and alanine aminotransferase activity in the APEC-challenged chickens. These results can be explained by the antibacterial, organ-protective, and metabolic regulatory properties of CGA (Tajik et al., 2017; Lu et al., 2020; Nguyen et al., 2024). On one hand, CGA can effectively inhibit the colonization of APEC in the intestine by disrupting the bacterial cell membrane and inducing cell death (Lou et al., 2011; Lee and Lee, 2018), thereby preventing the translocation of pathogenic bacteria. On the other hand, once absorbed, CGA can protect multiple organs from APEC challenge and improve their functionality and metabolic status, owing to its multiple biological activities, such as anti-bacterial, anti-inflammatory, anti-oxidant, and metabolic-regulatory effects, as previously summarized (Lu et al., 2020).

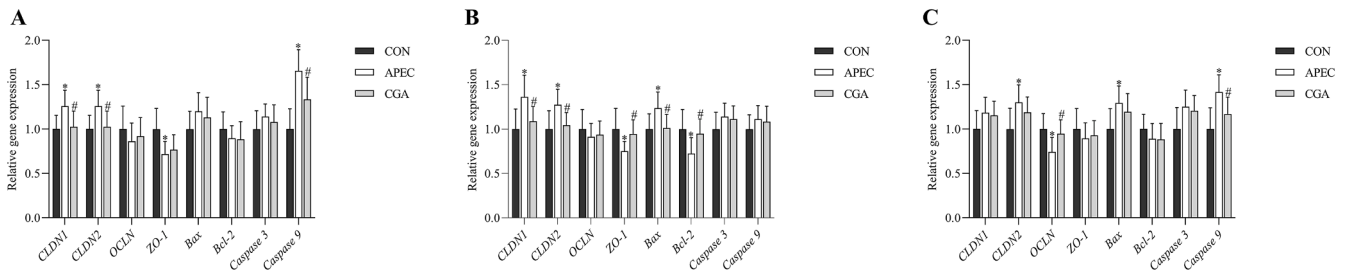

Fig. 3. Effects of dietary chlorogenic acid supplementation on expression of tight junction proteins and apoptosis-related genes in the duodenal (A), jejunal (B), and ileal (C) mucosa of avian pathogenic *Escherichia coli*-challenged broiler chickens The columns with error bars represent the mean ± standard error of measurement for each experimental group ($n = 6$). CON, normal broilers fed a basal diet; APEC, avian pathogenic *Escherichia coli*-challenged broilers fed a basal diet; CGA, avian pathogenic *Escherichia coli*-challenged broilers fed a basal diet supplemented with 1.0 g/kg of chlorogenic acid; *CLDN1*, claudin-1; *CLDN2*, claudin-2; *OCLN*, occludin; *ZO-1*, zonula occludens-1; *Bax*, B cell lymphoma-2 associated X protein; *Bcl-2*, B cell lymphoma-2; *Caspase 3*, cysteine-requiring aspartate protease 3; *Caspase 9*, cysteine-requiring aspartate protease 9. *Means significant difference from the CON group ($P < 0.05$). #Means significant difference from the *E. coli* challenge group ($P < 0.05$).

The APEC-induced inflammatory response is a key underlying mechanism of avian colibacillosis pathogenesis and is responsible for the detrimental consequences of APEC on the growth and health of poultry (Dziva and Stevens, 2008; Lutful Kabir et al., 2010; Guabiraba and Schouler, 2015). A recent study has shown that an oral APEC challenge elevated the levels of pro-inflammatory cytokines (IL-1β, IL-6, and IFN-γ) and TNF-α in the jejunum, and TNF-α in the ileum, while decreasing the levels of IL-10 in the jejunum and ileum. This may be due to the activation of the TLR4-mediated signaling pathway (Tan et al., 2024). Similarly, the *E. coli* infection has been shown to increase the protein expression of jejunal TNF-α and ileal TNF-α and IL-6, which is also thought to be regulated by the TLR4 signaling pathway (Tang et al., 2021). Consistent with these findings, an oral APEC challenge resulted in increased levels of pro-inflammatory cytokines (IFN-γ, TNF-α, IL-1β, or IL-6) and decreased IL-10 levels of (an anti-inflammatory cytokine) in the serum and/or intestinal mucosa (duodenum, jejunum, and/or ileum). These changes in APEC-challenged birds were accompanied by a higher abundance of intestinal pro-inflammatory cytokines (*IFN-γ* and *IL-6* in the duodenum and jejunum, *IL-1β* in the duodenum and ileum, and *TNF-α* in the ileum) and a lower abundance of *IL-10* in the ileum. This transcriptional response and the subsequent imbalance between pro-inflammatory and anti-inflammatory cytokine production are detrimental to intestinal barrier integrity and function (Vancamelbeke and Vermeire, 2017). The APEC-induced inflammation in this study is likely to be mediated by the TLR4/MyD88 signaling pathway, as evidenced by the up-regulation of their gene expression in the intestine. The underlying mechanism can be attributed to bacterial lipopolysaccharide, a critical virulence factor of APEC, which specifically interacts with TLR4 on intestinal epithelial cells. This interaction triggers nuclear translocation of nuclear factor kappa B (**NF-κB**) in a MyD88-dependent manner and subsequent production of diverse inflammatory mediators (Lu et al., 2008; Kuzmich et al., 2017; Fitzgerald and Kagan, 2020). In models of lipopolysaccharide-induced inflammation, dietary supplementation with CGA has been recently demonstrated to effectively decrease the levels of pro-inflammatory cytokines (IFN-γ, TNF-α, IL-1β, and/or IL-6) in both serum and intestinal mucosa, down-regulate their gene expression in the intestinal mucosa, and increased the levels of intestinal mucosal IL-10 at both protein and mRNA grades in broiler chickens (Tan et al., 2023; Liu et al., 2024; Zha et al., 2024). In addition to these findings, CGA has been shown to counteract the inflammatory response induced by pathogenic bacteria-induced (*Clostridium perfringens*) inflammatory response in broiler chickens by inhibiting the expression of pro-inflammatory mediators and increasing the concentrations and gene expressions of anti-inflammatory factors (Lv et al., 2024; Zhang et al., 2020c). The anti-inflammatory effects of CGA have also been demonstrated in this research. The addition of CGA to a basal diet significantly decreased the levels of pro-inflammatory cytokines in the serum (IFN-γ, TNF-α, IL-1β, and IL-6), duodenum (IFN-γ, TNF-α, and IL-6), jejunum (IFN-γ and TNF-α), and ileum (IL-6), while increasing the level of the anti-inflammatory cytokine IL-10 in all three segments of the intestinal mucosa. This was accompanied by a decrease in the gene expression of pro-inflammatory cytokines (*IFN-γ, IL-1β, IL-6*, and *TNF-α*) and an increase in the expression of the anti-inflammatory cytokine IL-10 in the intestinal mucosa of broiler chickens. The reduced colonization of *E. coli*, likely due to the antibacterial effect of CGA, may have contributed to the alleviated inflammatory response observed in APEC-challenged birds fed a CGA-supplemented diet. Additionally, the anti-inflammatory effect of CGA may also be attributed to the inactivation of TLR4-mediated NF-κB signaling. Previous studies have shown that CGA can inhibit the nuclear translocation of NF-κB in the intestinal mucosa by down-regulating *TLR4* expression (Liu et al., 2024; Zha et al., 2024). In this study, CGA also significantly decreased the expression of *TLR4* and *MyD88* in the intestinal mucosa of broiler chickens exposed to the APEC infection. It has been shown that interleukin 1 receptor-associated kinase-4 is an important signal transducer in the TLR4/MyD88-mediated innate immunity and inflammatory response, promoting transcriptional activity of NF-κB or activator protein-1 (Bai et al., 2023). This key molecular is a molecular target of CGA in the treatment of inflammation following the insult of various toll-like receptor pathogens (Park et al., 2015; Bai et al., 2023), which, in turn, provides a deeper explanation for the observed anti-inflammatory effect of CGA in this study.

In this study, the APEC challenge caused an increase in the intestinal permeability, as indicated by the elevated d-lactate and endotoxin levels, as well as an increase in diamine oxidase activity in the blood. This increase in intestinal permeability was accompanied by impaired intestinal morphology in broiler chickens, including a lower jejunal VH, a higher duodenal CD, and a lower VH/CD in the small intestine. These findings can be explained by the alternations in the expression of tight junction proteins (*CLDN1, CLDN2, OCLN*, and *ZO-1*) and genes responsible for the apoptosis of intestinal epithelial cells (*Caspase 9, Bax*, and *Bcl-2*). A similar study by Tan et al. (2024) also reported an increase in serum diamine oxidase activity and d-lactate levels, as well as a down-regulation of tight junction proteins (*ZO-1, OCLN*, and *CLDN-1*) in the intestinal mucosa of broiler chickens following an *E. coli* challenge. Additionally, Mao et al. (2025) found that APEC infection caused pathological damage, disrupted morphology, and reduced the expression of tight junction proteins in the small intestine of broiler chickens. Similar results have been reported in other studies involving broiler chickens exposed to *E. coli* challenge (Wang et al., 2016; Daneshmand et al., 2019; Mohebodini et al., 2019; Zhang et al., 2020a; Wu et al., 2021). The APEC-induced intestinal inflammatory response is assumed to be an important reason contributing to the observed intestinal barrier damage in this study. Pro-inflammatory cytokines have been shown to disrupt intestinal tight junction proteins and subsequently increase intestinal permeability by activating myosin light chain kinase, protein

Table 8
Effects of dietary chlorogenic acid supplementation on antioxidant capacity in serum and intestinal mucosa of avian pathogenic *Escherichia coli*-challenged broiler chickens.

Items[1]	Treatments[2]			SEM[3]	*P*-values	
	CON	APEC	CGA		CON vs *E. coli*	CGA vs *E. coli*
Serum						
T-AOC (U/mL)	8.70	5.89	7.18	0.34	0.001	0.012
SOD (U/mL)	259.06	211.43	243.95	7.20	0.005	0.030
GSH-Px (U/mL)	1104.38	974.01	1045.12	20.80	0.030	0.031
CAT (U/mL)	7.45	6.10	6.99	0.27	0.032	0.203
GSH (mg/L)	7.12	7.27	5.27	0.56	0.906	0.170
MDA (nmol/mL)	1.97	2.04	2.07	0.06	0.515	0.829
Duodenum						
T-AOC (U/mg protein)	0.92	0.74	0.87	0.03	0.017	0.040
SOD (U/mg protein)	156.74	169.87	166.17	3.59	0.184	0.578
GSH-Px (U/mg protein)	18.88	14.56	16.66	0.79	0.049	0.091
CAT (U/mg protein)	1.83	1.56	1.76	0.04	0.011	0.070
GSH (mg/g protein)	27.53	17.22	23.79	1.43	0.003	0.017
MDA (nmol/mg protein)	0.30	0.56	0.40	0.03	<0.001	0.003
Jejunum						
T-AOC (U/mg protein)	0.81	0.64	0.79	0.03	0.039	0.040
SOD (U/mg protein)	155.67	128.62	145.12	4.61	0.008	0.110
GSH-Px (U/mg protein)	16.63	12.94	14.95	0.79	0.091	0.193
CAT (U/mg protein)	2.04	1.46	1.95	0.09	0.010	0.024
GSH (mg/g protein)	31.64	24.74	31.42	1.22	0.030	0.006
MDA (nmol/mg protein)	0.36	0.37	0.33	0.03	0.930	0.638
Ileum						
T-AOC (U/mg protein)	0.20	0.15	0.17	0.01	0.132	0.420
SOD (U/mg protein)	157.83	131.73	151.06	5.45	0.081	0.139
GSH-Px (U/mg protein)	18.95	14.99	16.55	0.58	0.001	0.225
CAT (U/mg protein)	2.45	1.81	2.39	0.10	0.001	0.010
GSH (mg/g protein)	15.76	11.37	14.78	0.80	0.020	0.066
MDA (nmol/mg protein)	0.37	0.42	0.29	0.02	0.411	0.047

[1] T-AOC, total antioxidant capacity; SOD, superoxide dismutase; GSH-Px, glutathione peroxidase; CAT, catalase; GSH, glutathione; MDA, malondialdehyde.

[2] CON, normal broilers fed a basal diet; APEC, avian pathogenic *Escherichia coli*-challenged broilers fed a basal diet; CGA, avian pathogenic *Escherichia coli*-challenged broilers fed a basal diet supplemented with 1.0 g/kg of chlorogenic acid.

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

kinase C, and mitogen-activated protein kinase (Al-Sadi et al., 2009; Neurath et al., 2014; Kaminsky et al., 2021). Furthermore, these pro-inflammatory cytokines have been linked to excessive apoptosis of intestinal epithelial cells through activating apoptosis signaling and inactivating and interfering with intestinal stem cell renewal and differentiation, ultimately leading to intestinal mechanical barrier damage (Ramachandran et al., 2000; Günther et al., 2013; Andrews et al., 2018; Biton et al., 2018). The disrupted intestinal microbiota in the APEC-challenged broilers may also provide an explanation for the increased permeability and intestinal barrier damage, as previously reported in the broiler chickens (Zhang et al., 2014; Manafi et al., 2017; Jahanian et al., 2021; Xu et al., 2024). Aside from regulating inflammation, the activation of TLR4 has been shown to directly and indirectly mediate intestinal tight junction permeability, cell proliferation, and apoptosis in the intestine (Fukata et al., 2006; Guo et al., 2013; Zhan et al., 2022). The up-regulated expression of *TLR4* in the intestinal mucosa of the APEC-challenged chickens may also be therefore partially account for the damaged intestinal barrier in this study. Previous research has demonstrated that CGA effectively inhibited small intestine structural injury and prevented damage to the intestinal mucosal layer construction and tight junctions in broilers challenged with *Clostridium perfringens* type A by alleviating inflammatory response and oxidative stress (Zhang et al., 2020c). This finding is supported by other studies which have reported that CGA decreased serum diamine oxidase activity and levels of d-lactate and endotoxin, improved intestinal morphology, and normalized the intestinal expression of tight junction proteins and genes involved in cell apoptosis and renewal in broilers subjected to an intraperitoneal lipopolysaccharide-induced immunological stress (Tan et al., 2023; Liu et al., 2024; Zha et al., 2024). In this study, we also observed the protective effects of CGA on the intestinal health in APEC-challenged chickens. Briefly, CGA incorporation significantly decreased intestinal permeability and improved intestinal morphology, as evidenced by decreased diamine oxidase activity and d-lactate and endotoxin levels, as well as a higher duodenal and jejunal VH/CD ratio. Apart from these results, CGA also restored the gene expression of tight junction proteins (duodenal and jejunal *CLDN1* and *CLDN2*, jejunal *ZO-1*, and ileal *OCLN*) and genes involved in apoptosis (jejunal *Bax* and *Bcl-2*, and duodenal and ileal *Caspase 9*). Tight junctions, the most apical component of epithelial intercellular junctions, play essential roles in establishing epithelial barrier function and maintaining cellular polarity. Among the key structural components, CLDN1 and CLDN2 constitute the fundamental units of tight junction strands and are critically involved in modulating paracellular permeability (Otani and Furuse, 2020). Although the exact molecular mechanisms of OCLN remain to be fully elucidated, emerging evidence suggests that its phosphorylation status contributes to the dynamic regulation of tight junction functionality (Cummins, 2012). Furthermore, ZO-1, as a cytoplasmic scaffolding protein, orchestrates the assembly and organization of these cellular junctions (Fanning and Anderson, 2009). The restoration of these gene expressions following CGA administration in APEC-challenged chickens may indicate an improvement in intestinal health status. The amelioration of intestinal barrier damage in CGA-treated chickens can be explained by the simultaneous alleviation of the inflammatory response, as CGA inhibited the production of pro-inflammatory cytokines at both the mRNA and protein levels by inactivating the TLR4 signaling pathway. This explanation is further supported by the findings in broiler chickens and weaned piglets, where dietary CGA supplementation effectively prevented intestinal barrier injury by inhibiting the TLR4/NF-κB pathway and subsequent production of intestinal inflammatory mediators (Chen et al., 2018a; Tan et al., 2023; Liu et al., 2024; Zha et al., 2024). Aside from this possible explanation, CGA has been found to alleviate intestinal barrier damage by reducing endoplasmic reticulum stress and regulating autophagy-mediated Nrf2 pathway in broiler chickens under different stressful conditions (Liu et al., 2022b, 2024). Additionally, CGA has been shown to improve intestinal barrier function by inhibiting myosin light chain kinase and promoting the expression and dynamic distribution of tight junction proteins (Ruan et al., 2016; Song et al., 2022). In this study, we also observed that CGA exhibited an anti-apoptotic effect on the intestinal epithelial cells of APEC-challenged birds. This finding was evidenced by a high expression of *Bcl-2* and a lower expression of *Bax* and *Caspase 9* in the intestine, which may have contributed to the improved intestinal barrier function in CGA-supplemented birds. The anti-apoptotic effect of CGA has been

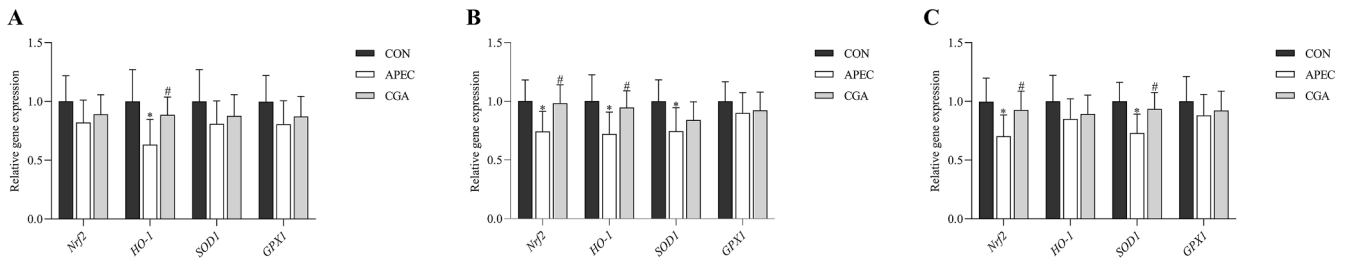

Fig. 4. Effects of dietary chlorogenic acid supplementation on expression of antioxidant-related genes in the duodenal (A), jejunal (B), and ileal (C) mucosa of avian pathogenic *Escherichia coli*-challenged broiler chickens The columns with error bars represent the mean ± standard error of measurement for each experimental group ($n = 6$). CON, normal broilers fed a basal diet; APEC, avian pathogenic *Escherichia coli*-challenged broilers fed a basal diet; CGA, avian pathogenic *Escherichia coli*-challenged broilers fed a basal diet supplemented with 1.0 g/kg of chlorogenic acid; *Nrf2*, nuclear factor-erythroid 2-related factor-2; *HO-1*, heme oxygenase-1; *SOD1*, superoxide dismutase 1; *GPX1*, glutathione peroxidase 1. *Means significant difference from the CON group ($P < 0.05$). #means significant difference from the *E. coli* challenge group ($P < 0.05$).

previously reported in the intestine of weaned piglets and broiler chickens (Chen et al., 2018b; Zha et al., 2024). Its anti-apoptotic effect may be associated with the promotion of intestinal stem cell activity, leading to improved intestinal cell proliferation and differentiation (Qin et al., 2023; Lv et al., 2024; Zha et al., 2024). In addition to these explanations, CGA has been reported to regulate the composition and metabolism of the intestinal microbiota and promote the production of short-chain fatty acids, which have been correlated with improved intestinal barrier function (Chen et al., 2021; Zhu et al., 2022; Liu et al., 2023a; Bi et al., 2024; Hu et al., 2024).

The accompanying oxidative damage is a typical cellular process implicated in APEC infection (Christensen et al., 2021). The interrelations between inflammation and oxidative stress are complicated and bidirectional. The generation of a variety of indispensable free radicals during the inflammatory processes can disturb the redox balance and lead to the exaggerated oxidative stress, while the excessive production of free radicals, in turn, can exacerbate local and systematic inflammation (Gill et al., 2010; Lugrin et al., 2014; Lauridsen, 2019). In broiler chickens, APEC infection has been shown to cause excessive generation of reactive oxygen species in the blood, brain, and liver (da Rosa et al., 2020), indicating the presence of oxidative stress. The APEC challenge has also been found to decrease serum SOD and CAT activities in broilers (Mohebodini et al., 2019). In laying hens, APEC exposure has resulted in an increase in plasma MDA levels, an end product of lipoperoxidation (Abbas et al., 2020). In accordance with existing literature, this study also found compromised antioxidant capacity in APEC-challenged broiler chicken in this study. Oral APEC gavage decreased the activities of antioxidant enzymes (SOD, GSH-Px, and/or CAT) and the level of the antioxidant molecule (GSH), while increasing the accumulation of MDA in the blood and/or intestinal mucosa. The inflammatory response in the APEC-challenged birds may provide an explanation for this oxidative stress. Additionally, the down-regulated expression of *Nrf2* and *HO-1* and their downstream gene (*SOD1*) in the intestinal mucosa suggests that the Nrf2 signaling pathway may play an important role in APEC-induced oxidative stress in this study. A previous study have also reported that *E.coli*-induced inactivation of Nrf2 signaling pathway and subsequent antioxidant enzyme expression in the intestine of piglets and (Jin et al., 2024). The unique phenolic structure of CGA endows it with a strong free-radical scavenging ability and a prominent antioxidant activity *in vitro* (Liang and Kitts, 2015; Lu et al., 2020). *In vivo* studies on poultry and livestock have demonstrated the protective effects of CGA against oxidative stress. In broiler chickens challenged with *Clostridium perfringens*, CGA has been shown to increase the activities of SOD and GSH-Px and decrease MDA level (Zhang et al., 2020c). In a lipopolysaccharide-induced immunological stress model, CGA effectively improved the antioxidant capacity of broiler chickens by increasing SOD, GSH-Px, and CAT activities, and decreasing MDA and protein carbonyl levels in both the systemic and jejunal levels (Liu et al., 2024). Similarly, in a dextran sodium sulfate-induced intestinal inflammation model, CGA was found to elevate GSH levels in serum and intestinal mucosa and inhibit the production of intestinal MDA in broiler chickens (Chen et al., 2023). The beneficial effects of CGA on the antioxidant system have also been observed in broilers and laying hens exposed to various oxidative challenges (Zhao et al., 2019; Chen et al., 2021; Liu et al., 2022a; Zha et al., 2023). Consistent with these findings, the incorporation of CGA in the diet of broiler chickens exposed to APEC challenge resulted in increased antioxidant enzyme activities (SOD, GSH-Px, and CAT), elevated GSH concentrations, and reduced MDA accumulations in both serum and/or intestinal mucosa of broiler chickens exposed to the APEC challenge. This effect is believed to be regulated by the Nrf2 signaling, as evidenced by the simultaneously increased gene expression of *Nrf2* and *SOD1* in the intestinal mucosa. The modulatory effect of CGA on the Nrf2 pathway has also been previously reported in broiler chickens (Liu et al., 2022b; Zhang et al., 2022; Song et al., 2024). The explanations for the improved redox status in APEC-challenged chickens fed a CGA-supplemented diet are complex and multi-faceted. The antioxidant activity of CGA may directly contribute to the maintenance of the antioxidant system directly through its free radical scavenging abilities and its regulatory effect on a variety of antioxidant signaling pathways (Liang and Kitts, 2015). Additionally, the reduced inflammation in the CGA-treated birds may also play a role in enhancing the antioxidant capacity, as there is a bidirectional relationship between inflammation and oxidative stress (Lugrin et al., 2014). Recently, it has been reported that CGA decreased levels of reactive oxygen species in the mitochondria, increased mitochondrial membrane potential, and enhanced the activities of mitochondrial complexes in the intestines of broilers challenged with necrotic enteritis (Lv et al., 2024). This finding suggests that the incorporation of CGA may improve the function of the mitochondria in the intestine, which could explain the improved redox status observed in APEC-infected birds. The function of mitochondria is crucial for maintaining cellular redox balance, and any dysfunction can lead to a series of events including energy deficiency, oxidative stress, and inflammatory and apoptotic cascades (Tiku et al., 2020; Faas and de Vos, 2020). The alleviated oxidative damage has been considered as an important reason accounting for the improved intestinal health in CGA-treated chickens exposed to different challenges, such as bacterial infections, lipopolysaccharide injections, and heat stress (Zhang et al., 2020c; Chen et al., 2021, 2023; Bi et al., 2024; Liu et al., 2024; Song et al., 2024), which, in turn, provides another explanation for the improved intestinal barrier function and overall health status observed in the current research.

Conclusions

This study has demonstrated that an incorporation of CGA effectively reduced mortality, improved organ function and metabolism, inhibited the colonization of cecal *E. coli*, decreased intestinal permeability,

improved intestinal morphology, and restored the altered expression of tight junction proteins and genes involved in apoptosis in APEC-challenged broiler chickens at an early age. The protective and beneficial effects of CGA on the intestinal health of broiler chickens were correlated with the alleviated inflammatory response and oxidative damage, possibly through the inactivation of TLR4 signaling and activation of Nrf2 pathway.

Declaration of competing interest

All authors declare no conflict of interest.

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References

Abbas, A.O., Alaqil, A.A., El-Beltagi, H.S., Abd El-Atty, H.K., Kamel, N.N., 2020. Modulating laying hens productivity and immune performance in response to oxidative stress induced by *E. coli* challenge using dietary propolis supplementation. Antioxidants 9, 893.

Abdel-Moneim, A.E., Shehata, A.M., Alzahrani, S.O., Shafi, M.E., Mesalam, N.M., Taha, A.E., Swelum, A.A., Arif, M., Fayyaz, M., Abd El-Hack, M.E., 2020. The role of polyphenols in poultry nutrition. J. Anim. Physiol. Anim. Nutr. 104, 1851–1866.

Alber, A., Stevens, M.P., Vervelde, L., 2021. The bird's immune response to avian pathogenic *Escherichia coli*. Avian Pathol 50, 382–391.

Al-Sadi, R., Boivin, M., Ma, T., 2009. Mechanism of cytokine modulation of epithelial tight junction barrier. Front. Biosci. 14, 2765–2778.

Ameer, K., Shahbaz, H.M., Kwon, J.H., 2017. Green extraction methods for polyphenols from plant matrices and their byproducts: a review. Compr. Rev. Food Sci. Food Saf. 16, 295–315.

Andrews, C., McLean, M.H., Durum, S.K., 2018. Cytokine tuning of intestinal epithelial function. Front. Immunol. 9, 1270.

Bai, Y.R., Yang, W.G., Hou, X.H., Shen, D.D., Zhang, S.N., Li, Y., Qiao, Y.Y., Wang, S.Q., Yuan, S., Liu, H.M., 2023. The recent advance of interleukin-1 receptor associated kinase 4 inhibitors for the treatment of inflammation and related diseases. Eur. J. Med. Chem. 258, 115606.

Bi, R., Yang, M., Liu, X., Guo, F., Hu, Z., Huang, J., Abbas, W., Xu, T., Liu, W., Wang, Z., 2024. Effects of chlorogenic acid on productive and reproductive performances, egg quality, antioxidant functions, and intestinal microenvironment in aged breeder laying hens. Poult. Sci. 103, 104060.

Biton, M., Haber, A.L., Rogel, N., Burgin, G., Beyaz, S., Schnell, A., Ashenberg, O., Su, C. W., Smillie, C., Shekhar, K., Chen, Z., Wu, C., Ordovas-Montanes, J., Alvarez, D., Herbst, R.H., Zhang, M., Tirosh, I., Dionne, D., Nguyen, L.T., Xifaras, M.E., Shalek, A. K., von Andrian, U.H., Graham, D.B., Rozenblatt-Rosen, O., Shi, H.N., Kuchroo, V., Yilmaz, O.H., Regev, A., Xavier, R.J., 2018. T helper cell cytokines modulate intestinal stem cell renewal and differentiation. Cell 175, 1307–1320 e22.

Chen, F., Zhang, H., Zhao, N., Yang, X., Du, E., Huang, S., Guo, W., Zhang, W., Wei, J., 2021. Effect of chlorogenic acid on intestinal inflammation, antioxidant status, and microbial community of young hens challenged with acute heat stress. Anim. Sci. J. 92, e13619.

Chen, J., Li, Y., Yu, B., Chen, D., Mao, X., Zheng, P., Luo, J., He, J., 2018c. Dietary chlorogenic acid improves growth performance of weaned pigs through maintaining antioxidant capacity and intestinal digestion and absorption function. J. Anim. Sci. 96, 1108–1118.

Chen, J., Xie, H., Chen, D., Yu, B., Mao, X., Zheng, P., Yu, J., Luo, Y., Luo, J., He, J., 2018b. Chlorogenic acid improves intestinal development via suppressing mucosa inflammation and cell apoptosis in weaned pigs. ACS Omega 3, 2211–2219.

Chen, J., Yu, B., Chen, D., Huang, Z., Mao, X., Zheng, P., Yu, J., Luo, J., He, J., 2018a. Chlorogenic acid improves intestinal barrier functions by suppressing mucosa inflammation and improving antioxidant capacity in weaned pigs. J. Nutr. Biochem. 59, 84–92.

Chen, J., Yu, B., Chen, D., Zheng, P., Luo, Y., Huang, Z., Luo, J., Mao, X., Yu, J., He, J., 2019. Changes of porcine gut microbiota in response to dietary chlorogenic acid supplementation. Appl. Microbiol. Biotechnol. 103, 8157–8168.

Chen, Y., Zha, P., Xu, H., Zhou, Y., 2023. An evaluation of the protective effects of chlorogenic acid on broiler chickens in a dextran sodium sulfate model: a preliminary investigation. Poult. Sci. 102, 102257.

Christensen, H., Bachmeier, J., Bisgaard, M., 2021. New strategies to prevent and control avian pathogenic *Escherichia coli* (APEC). Avian Pathol 50, 370–381.

Cummins, P.M., 2012. Occludin: one protein, many forms. Mol. Cell Biol. 32, 242–250.

Dai, C., Li, H., Zhao, W., Fu, Y., Cheng, J., 2024. Bioactive functions of chlorogenic acid and its research progress in pig industry. J. Anim. Physiol. Anim. Nutr. 108, 439–450.

Daneshmand, A., Kermanshahi, H., Sekhavati, M.H., Javadmanesh, A., Ahmadian, M., 2019. Antimicrobial peptide, cLF36, affects performance and intestinal morphology, microflora, junctional proteins, and immune cells in broilers challenged with *E. coli*. Sci. Rep 9, 14176.

da Rosa, G., Alba, D.F., Silva, A.D., Gris, A., Mendes, R.E., Mostardeiro, V.B., Lopes, T.F., Schetinger, M.R.C., Stefani, L.M., Lopes, M.T., Boiago, M.M., da Silva, A.S., 2020. Impact of *Escherichia coli* infection in broiler breeder chicks: the effect of oxidative stress on weight gain. Microb. Pathog. 139, 103861.

Dubreuil, J.D., Isaacson, R.E., Schifferli, D.M., 2016. Animal enterotoxigenic *Escherichia coli*. EcoSal Plus 7. https://doi.org/10.1128/ecosalplus.ESP-0006-2016.

Dziva, F., Stevens, M.P., 2008. Colibacillosis in poultry: unravelling the molecular basis of virulence of avian pathogenic *Escherichia coli* in their natural hosts. Avian Pathol 37, 355–366.

Faas, M.M., de Vos, P., 2020. Mitochondrial function in immune cells in health and disease. Biochim. Biophys Acta Mol. Basis Dis. 1866, 165845.

Fancher, C.A., Zhang, L., Kiess, A.S., Adhikari, P.A., Dinh, T.T.N., Sukumaran, A.T., 2020. Avian pathogenic *Escherichia coli* and *Clostridium perfringens*: challenges in no antibiotics ever broiler production and potential solutions. Microorganisms 8, 1533.

Fanning, A.S., Anderson, J.M., 2009. Zonula occludens-1 and -2 are cytosolic scaffolds that regulate the assembly of cellular junctions. Ann. N. Y. Acad. Sci. 1165, 113–120.

Farman, A.A., Hadwan, M.H., 2021. Simple kinetic method for assessing catalase activity in biological samples. Methods X 8, 101434.

Feng, S., Zhang, Y., Fu, S., Li, Z., Zhang, J., Xu, Y., Han, X., Miao, J., 2023. Application of chlorogenic acid as a substitute for antibiotics in multidrug-resistant *Escherichia coli*-induced mastitis. Int. Immunopharmacol. 114, 109536.

Fitzgerald, K.A., Kagan, J.C., 2020. Toll-like receptors and the control of immunity. Cell 180, 1044–1066.

Fleckenstein, J.M., Kuhlmann, F.M., 2019. Enterotoxigenic *Escherichia coli* infections. Curr. Infect. Dis. Rep. 21, 9.

Fukata, M., Chen, A., Klepper, A., Krishnareddy, S., Vamadevan, A.S., Thomas, L.S., Xu, R., Inoue, H., Arditi, M., Dannenberg, A.J., Abreu, M.T., 2006. Cox-2 is regulated by toll-like receptor-4 (TLR4) signaling: role in proliferation and apoptosis in the intestine. Gastroenterology 131, 862–877, 2006.

Ghani, M.A., Barril, C., Bedgood, D.R., Prenzler, P.D., 2017. Measurement of antioxidant activity with the thiobarbituric acid reactive substances assay. Food Chem 230, 195–207.

Gilani, S., Chrystal, P.V., Barekatain, R., 2021. Current experimental models, assessment and dietary modulations of intestinal permeability in broiler chickens. Anim. Nutr. 7, 801–811.

Gill, R., Tsung, A., Billiar, T., 2010. Linking oxidative stress to inflammation: toll-like receptors. Free radic. Biol. Med. 48, 1121–1132.

Giustarini, D., Fanti, P., Matteucci, E., Rossi, R., 2014. Micro-method for the determination of glutathione in human blood. J. Chromatogr. B 964, 191–194.

Guabiraba, R., Schouler, C., 2015. Avian colibacillosis: still many black holes. FEMS Microbiol. Lett. 362, fnv118.

Günther, C., Neumann, H., Neurath, M.F., Becker, C., 2013. Apoptosis, necrosis and necroptosis: cell death regulation in the intestinal epithelium. Gut 62, 1062–1071.

Guo, S., Al-Sadi, R., Said, H.M., Ma, T.Y., 2013. Lipopolysaccharide causes an increase in intestinal tight junction permeability *in vitro* and *in vivo* by inducing enterocyte membrane expression and localization of TLR-4 and CD14. Am. J. Pathol. 182, 375–387.

Haida, Z., Hakiman, M., 2019. A comprehensive review on the determination of enzymatic assay and nonenzymatic antioxidant activities. Food Sci. Nutr. 7, 1555–1563.

Hu, J., Afayibo, D.J.A., Zhang, B., Zhu, H., Yao, L., Guo, W., Wang, X., Wang, Z., Wang, D., Peng, H., Tian, M., Qi, J., Wang, S., 2022. Characteristics, pathogenic mechanism, zoonotic potential, drug resistance, and prevention of avian pathogenic *Escherichia coli* (APEC). Front. Microbiol. 13, 1049391.

Hu, X., Zhen, W., Bai, D., Zhong, J., Zhang, R., Zhang, H., Zhang, Y., Ito, K., Zhang, B., Ma, Y., 2024. Effects of dietary chlorogenic acid on cecal microbiota and metabolites in broilers during lipopolysaccharide-induced immune stress. Front. Microbiol. 15, 1347053.

Jahanian, E., Mahdavi, A.H., Jahanian, R., 2021. Silymarin improved the growth performance via modulating the microbiota and mucosal immunity in *Escherichia coli*-challenged broiler chicks. Livest. Sci. 249, 104529.

Jin, S., Xu, H., Yang, C., Karmin, O., 2024. Regulation of oxidative stress in the intestine of piglets after enterotoxigenic *Escherichia coli* (ETEC) infection. Biochim. Biophys. Acta Mol. Cell Res. 1871, 119711.

Joseph, J., Zhang, L., Adhikari, P., Evans, J.D., Ramachandran, R., 2023. Avian pathogenic *Escherichia coli* (APEC) in broiler breeders: an overview. Pathogens 12, 1280.

Kaminsky, L.W., Al-Sadi, R., Ma, T.Y., 2021. IL-1β and the intestinal epithelial tight junction barrier. Front. Immunol. 12, 767456.

Kathayat, D., Lokesh, D., Ranjit, S., Rajashekara, G., 2021. Avian pathogenic *Escherichia coli* (APEC): an overview of virulence and pathogenesis factors, zoonotic potential, and control strategies. Pathogens 10, 467.

Kono, Y., 2022. Reprint of: generation of superoxide radical during autoxidation of hydroxylamine and an assay for superoxide dismutase. Arch. Biochem. Biophys. 726, 109247.

Kuralkar, P., Kuralkar, S.V., 2021. Role of herbal products in animal production– An updated review. J. Ethnopharmacol. 278, 114246.
Kuzmich, N.N., Sivak, K.V., Chubarev, V.N., Porozov, Y.B., Savateeva-Lyubimova, T.N., Peri, F., 2017. TLR4 signaling pathway modulators as potential therapeutics in inflammation and sepsis. Vaccines 5, 34.
Lauridsen, C., 2019. From oxidative stress to inflammation: redox balance and immune system. Poult. Sci. 98, 4240–4246.
Le, Y.J., He, L.Y., Li, S., Xiong, C.J., Lu, C.H., Yang, X.Y., 2022. Chlorogenic acid exerts antibacterial effects by affecting lipid metabolism and scavenging ROS in *Streptococcus pyogenes*. FEMS Microbiol. Lett. 369, fnac061.
Lee, B., Lee, D.G., 2018. Depletion of reactive oxygen species induced by chlorogenic acid triggers apoptosis-like death in *Escherichia coli*. Free Radic. Res. 52, 605–615.
Li, G., Wang, X., Xu, Y., Zhang, B., Xia, X., 2014. Antimicrobial effect and mode of action of chlorogenic acid on *Staphylococcus aureus*. Eur. Food Res. Technol. 238, 589–596.
Li, Y., Li, W., Fu, C., Song, Y., Fu, Q., 2020. *Lonicerae japonicae flos* and *Lonicerae flos*: a systematic review of ethnopharmacology, phytochemistry and pharmacology. Phytochem. Rev. 19, 1–61.
Liang, N., Kitts, D.D., 2015. Role of chlorogenic acids in controlling oxidative and inflammatory stress conditions. Nutrients 8, 16.
Liu, H., Chen, P., Lv, X., Zhou, Y., Li, X., Ma, S., Zhao, J., 2022a. Effects of chlorogenic acid on performance, anticoccidial indicators, immunity, antioxidant status, and intestinal barrier function in coccidia-infected broilers. Animals 12, 963.
Liu, H., Li, X., Shi, S., Zhou, Y., Zhang, K., Wang, Y., Zhao, J., 2022b. Chlorogenic acid improves growth performance and intestinal health through autophagy-mediated nuclear factor erythroid 2-related factor 2 pathway in oxidatively stressed broilers induced by dexamethasone. Poult. Sci. 101, 102036.
Liu, H., Li, X., Zhang, K., Lv, X., Zhang, Q., Chen, P., Wang, Y., Zhao, J., 2023a. Integrated multi-omics reveals the beneficial role of chlorogenic acid in improving the growth performance and immune function of immunologically stressed broilers. Anim. Nutr. 14, 383–402.
Liu, H., Meng, H., Du, M., Lv, H., Wang, Y., Zhang, K., 2024. Chlorogenic acid ameliorates intestinal inflammation by inhibiting NF-κb and endoplasmic reticulum stress in lipopolysaccharide-challenged broilers. Poult. Sci. 103, 103586.
Liu, W., Zha, P., Guo, L., Chen, Y., Zhou, Y., 2023b. Effects of different levels of dietary chlorogenic acid supplementation on growth performance, intestinal integrity, and antioxidant status of broiler chickens at an early age. Anim. Feed Sci. Technol. 297, 115570.
Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. Methods 25, 402–408.
Lou, Z., Wang, H., Zhu, S., Ma, C., Wang, Z., 2011. Antibacterial activity and mechanism of action of chlorogenic acid. J. Food Sci. 76, M398–M403.
Lu, H., Tian, Z., Cui, Y., Liu, Z., Ma, X., 2020. Chlorogenic acid: a comprehensive review of the dietary sources, processing effects, bioavailability, beneficial properties, mechanisms of action, and future directions. Compr. Rev. Food Sci. Food Saf. 19, 3130–3158.
Lu, Y.C., Yeh, W.C., Ohashi, P.S., 2008. LPS/TLR4 signal transduction pathway. Cytokine 42, 145–151.
Lugrin, J., Rosenblatt-Velin, N., Parapanov, R., Liaudet, L., 2014. The role of oxidative stress during inflammatory processes. Biol. Chem. 395, 203–230.
Lutful Kabir, S.M., 2010. Avian colibacillosis and salmonellosis: a closer look at epidemiology, pathogenesis, diagnosis, control and public health concerns. Int. J. Environ. Res. Public Health. 7, 89–114.
Lv, H., Chen, P., Wang, Y., Xu, L., Zhang, K., Zhao, J., Liu, H., 2024. Chlorogenic acid protects against intestinal inflammation and injury by inactivating the mtDNA-cGAS-STING signaling pathway in broilers under necrotic enteritis challenge. Poult. Sci. 103, 103274.
Manafi, M., Khalaji, S., Hedayati, M., Pirany, N., 2017. Efficacy of *Bacillus subtilis* and bacitracin methylene disalicylate on growth performance, digestibility, blood metabolites, immunity, and intestinal microbiota after intramuscular inoculation with *Escherichia coli* in broilers. Poult. Sci. 96, 1174–1183.
Mao, N., Yu, Y., Cui, J., He, J., Yang, Y., Wang, D., 2025. Effect of Matrine on growth performance, gut health, and gut microbiota in chickens infected with avian pathogenic *Escherichia coli*. Poult. Sci. 104, 104520.
Maria Cardinal, K., Kipper, M., Andretta, I., Machado Leal Ribeiro, A., 2019. Withdrawal of antibiotic growth promoters from broiler diets: performance indexes and economic impact. Poult. Sci. 98, 6659–6667.
Mirsepasi-Lauridsen, H.C., Vallance, B.A., Krogfelt, K.A., Petersen, A.M., 2019. *Escherichia coli* pathobionts associated with inflammatory bowel disease. Clin. Microbiol. Rev. 32 e00060–e00018.
Mohebodini, H., Jazi, V., Bakhshalinejad, R., Shabani, A., Ashayerizadeh, A., 2019. Effect of dietary resveratrol supplementation on growth performance, immune response, serum biochemical indices, cecal microflora, and intestinal morphology of broiler chickens challenged with *Escherichia coli*. Livest. Sci. 229, 13–21.
Nawaz, S., Wang, Z., Zhang, Y., Jia, Y., Jiang, W., Chen, Z., Yin, H., Huang, C., Han, X., 2024. Avian pathogenic *Escherichia coli* (APEC): current insights and future challenges. Poult. Sci. 103, 104359.
Neurath, M.F., 2014. Cytokines in inflammatory bowel disease. Nat. Rev. Immunol. 14, 329–342.
Nguyen, V., Taine, E.G., Meng, D., Cui, T., Tan, W., 2024. Chlorogenic acid: a systematic review on the biological functions, mechanistic actions, and therapeutic potentials. Nutrients 16, 924.
Otani, T., Furuse, M., 2020. Tight junction structure and function revisited. Trends Cell Biol 30, 805–817.
Park, S.H., Baek, S.I., Yun, J., Lee, S., Yoon, D.Y., Jung, J.K., Jung, S.H., Hwang, B.Y., Hong, J.T., Han, S.B., Kim, Y., 2015. IRAK4 as a molecular target in the amelioration of innate immunity-related endotoxic shock and acute liver injury by chlorogenic acid. J. Immunol. 194, 1122–1130.
Pham, V.H., Abbas, W., Huang, J., Guo, F., Zhang, K., Kong, L., Zhen, W., Guo, Y., Wang, Z., 2022. Dietary coated essential oil and organic acid mixture supplementation improves health of broilers infected with avian pathogenic *Escherichia coli*. Anim. Nutr. 12, 245–262.
Pliego, A.B., Tavakoli, M., Khusro, A., Seidavi, A., Elghandour, M.M.M.Y., Salem, A.Z.M., Márquez-Molina, O., Rene Rivas-Caceres, R., 2022. Beneficial and adverse effects of medicinal plants as feed supplements in poultry nutrition: a review. Anim. Biotechnol. 33, 369–391.
Qin, Y., Wang, S., Huang, W., Li, K., Wu, M., Liu, W., Han, J., 2023. Chlorogenic acid improves intestinal morphology by enhancing intestinal stem-cell activity. J. Sci. Food Agric. 103, 3287–3294.
Rafeeq, M., Bilal, R.M., Batool, F., Yameen, K., Farag, M.R., Madkour, M., Shaaban, S.E., El-Shall, A.N., Kuldeep, D., Alagawany, M., 2023. Application of herbs and their derivatives in broiler chickens: a review. World's Poult. Sci. J. 79, 95–117.
Ramachandran, A., Madesh, M., Balasubramanian, K.A., 2000. Apoptosis in the intestinal epithelium: its relevance in normal and pathophysiological conditions. J. Gastroenterol. Hepatol. 15, 109–120.
Riley, L.W., 2020. Distinguishing pathovars from nonpathovars: *escherichia coli*. Microbiol. Spectr. 8, 10.
Roth, N., Käsbohrer, A., Mayrhofer, S., Zitz, U., Hofacre, C., Domig, K.J., 2019. The application of antibiotics in broiler production and the resulting antibiotic resistance in *Escherichia coli*: a global overview. Poult. Sci. 98, 1791–1804.
Ruan, Z., Mi, S., Zhou, L., Zhou, Y., Li, J., Liu, W., Deng, Z., Yin, Y., 2016. Chlorogenic acid enhances intestinal barrier by decreasing MLCK expression and promoting dynamic distribution of tight junction proteins in colitic rats. J. Funct. Foods 26, 698–708.
Sargeant, J.M., Bergevin, M.D., Churchill, K., Dawkins, K., Deb, B., Dunn, J., Logue, C.M., Novy, A., O'Connor, A.M., Reist, M., Winder, C.B., 2019. The efficacy of antibiotics to control colibacillosis in broiler poultry: a systematic review. Anim. Health Res. Rev. 20, 263–273.
Schumm, W.R., Pratt, K..K., Hartenstein, J.L., Jenkins, B.A., Johnson, G.A., 2013. Determining statistical significance (alpha) and reporting statistical trends: controversies, issues, and facts. Compr. Psychol. 2, 10. Article.
Singh, A.K., Singla, R.K., Pandey, A.K., 2023. Chlorogenic acid: a dietary phenolic acid with promising pharmacotherapeutic potential. Curr. Med. Chem. 30, 3905–3926.
Song, D., Zhang, S., Chen, A., Song, Z., Shi, S., 2024. Comparison of the effects of chlorogenic acid isomers and their compounds on alleviating oxidative stress injury in broilers. Poult. Sci. 103, 103649.
Song, L., Wu, T., Zhang, L., Wan, J., Ruan, Z., 2022. Chlorogenic acid improves the intestinal barrier by relieving endoplasmic reticulum stress and inhibiting ROCK/MLCK signaling pathways. Food Funct 13, 4562–4575.
Su, M., Liu, F., Luo, Z., Wu, H., Zhang, X., Wang, D., Zhu, Y., Sun, Z., Xu, W., Miao, Y., 2019. The antibacterial activity and mechanism of chlorogenic acid against foodborne pathogen *Pseudomonas aeruginosa*. Foodborne Pathog. Dis. 16, 823–830.
Swelum, A.A., Elbestawy, A.R., El-Saadony, M.T., Hussein, E.O.S., Alhotan, R., Suliman, G.M., Taha, A.E., Ba-Awadh, H., El-Tarabily, K.A., Abd El-Hack, M.E., 2021. Ways to minimize bacterial infections, with special reference to *Escherichia coli*, to cope with the first-week mortality in chicks: an updated overview. Poult. Sci. 100, 101039.
Tajik, N., Tajik, M., Mack, I., Enck, P., 2017. The potential effects of chlorogenic acid, the main phenolic components in coffee, on health: a comprehensive review of the literature. Eur. J. Nutr. 56, 2215–2244.
Tan, H., Zhen, W., Bai, D., Liu, K., He, X., Ito, K., Liu, Y., Li, Y., Zhang, Y., Zhang, B., Ma, Y., 2023. Effects of dietary chlorogenic acid on intestinal barrier function and the inflammatory response in broilers during lipopolysaccharide-induced immune stress. Poult. Sci. 102, 102623.
Tan, Z., Chen, Y., Wen, C., Zhou, Y., 2024. Dietary supplementation with a silicate clay mineral (palygorskite) alleviates inflammatory responses and intestinal barrier damage in broiler chickens challenged with *Escherichia coli*. Poult. Sci. 103, 104017.
Tang, L.P., Li, W.H., Liu, Y.L., Lun, J.C., He, Y.M., 2021. Heat stress aggravates intestinal inflammation through TLR4-NF-κb signaling pathway in Ma chickens infected with *Escherichia coli O157:H7*. Poult. Sci. 100, 101030.
Tiku, V., Tan, M.W., Dikic, I., 2020. Mitochondrial functions in infection and immunity. Trends Cell Biol 30, 263–275.
Tilli, G., Vougat Ngom, R., Cardoso de Carvalho Ferreira, H., Apostolakos, I., Paudel, S., Piccirillo, A., 2024. A systematic review on the role of biosecurity to prevent or control colibacillosis in broiler production. Poult. Sci. 103, 103955.
Vancamelbeke, M., Vermeire, S., 2017. The intestinal barrier: a fundamental role in health and disease. Expert Rev. Gastroenterol. Hepatol. 11, 821–834.
Veiga, M., Costa, E.M., Silva, S., Pintado, M., 2020. Impact of plant extracts upon human health: a review. Crit. Rev. Food Sci. Nutr. 60, 873–886.
Waliaula, P.K., Kiarie, E.G., M.S., 2024. Diarra. Predisposition factors and control strategies of avian pathogenic *Escherichia coli* in laying hens. Front. Vet. Sci. 11, 1474549.
Wang, C.Y., Tang, L., He, J.W., Li, J., Wang, Y.Z., 2019. Ethnobotany, phytochemistry and pharmacological properties of *Eucommia ulmoides*: a review. Am. J. Chin. Med. 47, 259–300.
Wang, W., Li, Z., Han, Q., Guo, Y., Zhang, B., D'inca, R., 2016. Dietary live yeast and mannan-oligosaccharide supplementation attenuate intestinal inflammation and barrier dysfunction induced by *Escherichia coli* in broilers. Br. J. Nutr. 116, 1878–1888.
Watts, A., Wigley, P., 2024. Avian pathogenic *Escherichia coli*: an overview of infection biology, antimicrobial resistance and vaccination. Antibiotics 13, 809.

Wood, J., Freemantle, N., King, M., Nazareth, I., 2014. Trap of trends to statistical significance: likelihood of near significant P value becoming more significant with extra data. BMJ 348, g2215.

Wu, Z., Yang, K., Zhang, A., Chang, W., Zheng, A., Chen, Z., Cai, H., Liu, G., 2021. Effects of *Lactobacillus acidophilus* on the growth performance, immune response, and intestinal barrier function of broiler chickens challenged with *Escherichia coli* O157. Poult. Sci. 100, 101323.

Xie, K., Sun, Y., Deng, L., Yu, B., Luo, Y., Huang, Z., Mao, X., Yu, J., Zheng, P., Yan, H., Li, Y., Li, H., He, J., 2023. Effects of dietary chlorogenic acid supplementation on growth performance, meat quality, and muscle flavor substances in finishing pigs. Foods 12, 3047.

Xu, P., Wang, J., Chen, P., Ding, H., Wang, X., Li, S., Fan, X., Zhou, Z., Shi, D., Li, Z., Cao, S., Xiao, Y., 2024. Effects of pomegranate (*Punica granatum* L.) peel on the growth performance and intestinal microbiota of broilers challenged with *Escherichia coli*. Poult. Sci. 103, 103304.

Xue, H., Wei, M., Ji, L., 2023. Chlorogenic acids: a pharmacological systematic review on their hepatoprotective effects. Phytomedicine 118, 154961.

Yehia, N., Salem, H.M., Mahmmod, Y., Said, D., Samir, M., Mawgod, S.A., Sorour, H.K., AbdelRahman, M.A.A., Selim, S., Saad, A.M., El-Saadony, M.T., El-Meihy, R.M., Abd El-Hack, M.E., El-Tarabily, K.A., Zanaty, A.M., 2023. Common viral and bacterial avian respiratory infections: an updated review. Poult. Sci. 102, 102553.

Zha, P., Liu, W., Zhou, Y., Chen, Y., 2024. Protective effects of chlorogenic acid on the intestinal barrier of broiler chickens: an immunological stress model study. Poult. Sci. 103, 103949.

Zha, P., Wei, L., Liu, W., Chen, Y., Zhou, Y., 2023. Effects of dietary supplementation with chlorogenic acid on growth performance, antioxidant capacity, and hepatic inflammation in broiler chickens subjected to diquat-induced oxidative stress. Poult. Sci. 102, 102479.

Zhan, L., Zheng, J., Meng, J., Fu, D., Pang, L., Ji, C., 2022. Toll-like receptor 4 deficiency alleviates lipopolysaccharide-induced intestinal barrier dysfunction. Biomed. Pharmacother. 155, 113778.

Zhang, K., Li, X., Zhao, J., Wang, Y., Hao, X., Liu, K., Liu, H., 2022. Protective effects of chlorogenic acid on the meat quality of oxidatively stressed broilers revealed by integrated metabolomics and antioxidant analysis. Food Funct 13, 2238–2252.

Zhang, L., Cao, G.T., Zeng, X.F., Zhou, L., Ferket, P.R., Xiao, Y.P., Chen, A.G., Yang, C.M., 2014. Effects of *Clostridium butyricum* on growth performance, immune function, and cecal microflora in broiler chickens challenged with *Escherichia coli* K88. Poult. Sci. 93, 46–53.

Zhang, M., Kou, J., Wu, Y., Wang, M., Zhou, X., Yang, Y., Wu, Z., 2020a. Dietary genistein supplementation improves intestinal mucosal barrier function in *Escherichia coli* O78-challenged broilers. J. Nutr. Biochem. 77, 108267.

Zhang, S., Abbas, M., Rehman, M.U., Huang, Y., Zhou, R., Gong, S., Yang, H., Chen, S., Wang, M., Cheng, A., 2020b. Dissemination of antibiotic resistance genes (ARGs) via integrons in *Escherichia coli*: a risk to human health. Environ. Pollut. 266, 115260.

Zhang, X., Zhao, Q., Ci, X., Chen, S., Xie, Z., Li, H., Zhang, H., Chen, F., Xie, Q., 2020c. Evaluation of the efficacy of chlorogenic acid in reducing small intestine injury, oxidative stress, and inflammation in chickens challenged with *Clostridium perfringens* type A. Poult. Sci. 99, 6606–6618.

Zhang, Y., Wang, Y., Chen, D., Yu, B., Zheng, P., Mao, X., Luo, Y., Li, Y., He, J., 2018. Dietary chlorogenic acid supplementation affects gut morphology, antioxidant capacity and intestinal selected bacterial populations in weaned piglets. Food Funct 9, 4968–4978.

Zhao, J.S., Deng, W., Liu, H.W., 2019. Effects of chlorogenic acid-enriched extract from *Eucommia ulmoides* leaf on performance, meat quality, oxidative stability, and fatty acid profile of meat in heat-stressed broilers. Poult. Sci. 98, 3040–3049.

Zhu, H., Jiang, W., Liu, C., Wang, C., Hu, B., Guo, Y., Cheng, Y., Qian, H., 2022. Ameliorative effects of chlorogenic acid on alcoholic liver injury in mice via gut microbiota informatics. Eur. J. Pharmacol. 928, 175096.