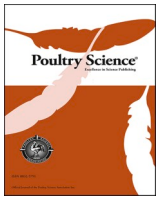
104 (2025) 104606

Contents lists available at ScienceDirect

Poultry Science

journal homepage: www.elsevier.com/locate/psj



Transcriptomic and proteomic analysis of the jejunum revealed the effects and mechanism of protocatechuic acid on alleviating *Salmonella* typhimurium infection in chickens

Sheng Zhang [a,1], Qin Wang [a,1], Xiangtian Yao [a,b], Jingjing Dong [a], Guanhuo Li [a,b], Yingan Zang [b], Shouqun Jiang [a,*], Yibing Wang [a,*]

[a] *Institute of Animal Science, Guangdong Academy of Agricultural Sciences, State Key Laboratory of Swine and Poultry Breeding Industry, Key Laboratory of Animal Nutrition and Feed Science in South China, Ministry of Agriculture and Rural Affairs, Guangdong Provincial Key Laboratory of Animal Breeding and Nutrition, Guangzhou, 510640, Guangdong, China*

[b] *Zhongkai University of Agriculture and Engineering, 510225, Guangdong, China*

A R T I C L E I N F O

Keywords:
Salmonella typhimurium
Protocatechuic acid
Immunity
Chickens
Omics analysis

A B S T R A C T

Salmonella typhimurium is a common cause of gastroenteritis, which infects animals and human. Protocatechuic acid has anti-inflammatory, immunoregulatory and anti-pathogenic effects, and is expected to be an effective choice for alleviating *Salmonella* infection and intestinal injury. A total of 180 1-d female, yellow-feathered chickens were randomly allocated into 3 treatment groups, the controls (Ctr), the *Salmonella*-challenged treatment (Sal) and the protocatechuic acid treatment (PA). Birds were fed a basal diet for 18 d, with birds in PA supplemented with 600 mg/kg protocatechuic acid. On 14 and 16 d, birds in Sal and PA were orally challenged with 10^9 CFU *S.* typhimurium, while birds in Ctr received an equal amount of PBS. The results showed that protocatechuic acid improved growth performance and jejunal structure in *Salmonella*-infected chickens ($P < 0.05$). In addition, protocatechuic acid suppressed ($P < 0.05$) the secretion of plasmal IgG, IL-1β, and IFN-β, and jejunal mucosal IL-6, IFN-β, complement protein 3 and 4. Mechanistically, the transcriptomic results showed that dietary protocatechuic acid inhibited acute inflammatory response and activated the signaling pathways of cytokine-cytokine receptor interaction, complement and coagulation cascades. Results of proteomic showed that protocatechuic acid inhibited the positive regulation of dendritic cell cytokine production and I-κB kinase/NF-κB signaling, as well as cytokine-cytokine receptor interaction and MAPK signaling pathways, and activated the mTOR signaling pathways. These results were critical to both suppressing the secretion of inflammatory cytokines and restoring the intestinal function. Dietary protocatechuic acid (600 mg/kg) was effective to alleviate *Salmonella* infection, as it suppressed the levels of cytokines and complement protein by inhibiting MAPK, I-κB kinase/NF-κB and enhancing the mTOR signaling pathway, thus offset the decline in weight loss and intestinal injury caused by infection.

Introduction

Salmonella typhimurium is considered to be one of the most serious food-borne pathogens worldwide, and it is a common cause of gastroenteritis, infecting a broad range of hosts, including mice, poultry, livestock, and humans (Zambry et al., 2022). *Salmonella* infection reduces the growth performance of chickens, and infects chicks through vertical transmission of eggs, causing huge economic losses (Hofer 2021). *Salmonella* uses the invasion-associated type 3 secretion system (T3SS) to invade intestinal epithelial cells, induce intestinal inflammatory damage, destroy intestinal epithelial integrity, and thus disseminate through the bloodstream causing systemic infection and reducing the growth performance of animals (Galán 2021; Jennings et al., 2017). Therefore, there is an urgent need to control *Salmonella* infection, effectively reducing the harm to poultry and livestock.

Antibiotic use leads to antibiotic resistance, antibiotic residues in food and other serious problems. Due to its importance for the nutrition and health of chicks, there is a continuously growing demand for

* Corresponding authors.
E-mail addresses: jiangshouqun@gdaas.cn (S. Jiang), wangyibing77@163.com (Y. Wang).
[1] These two authors contributed equally to this work.

https://doi.org/10.1016/j.psj.2024.104606
Received 8 September 2024; Accepted 26 November 2024
Available online 27 November 2024

antibiotic substitutes to alleviate the *Salmonella*-infection. The supplementation of feeds with plant extracts, probiotics, and antimicrobial peptides, etc. is a safer and more effective way of nutritional regulation in animals. Among these additives, the exploitation of protocatechuic acid may offer an effective alternative in alleviating *Salmonella*-infection and intestinal injury in view of its polyphenol structure, which exerts anti-inflammatory, antioxidant and pathogen-inhibiting activities (Tan et al., 2023; Varì et al., 2015; Wu et al., 2022).

Protocatechuic acid directly destroyed the cell membrane structure of pathogenic bacteria and inhibited the production of metabolism-related enzymes and toxins of bacteria (Zhao et al., 2021) and also inhibited pathogens, *such as Escherichia coli, Staphylococcus aureus* and *Helicobacter pylori* (Jalali et al., 2020; Song et al., 2020). Research in this laboratory (Cui et al., 2023) also showed that protocatechuic acid affected the intestinal inflammatory response and microbiota composition in yellow feather chickens, and relieved *Salmonella*-induced weight loss and injury of the intestinal barrier. However, the mechanism of its alleviating *Salmonella* infection has not been clearly explored, nor the specific pathway through which it impacts immunity. In the present study, transcriptomic and proteomic analyses were used to reveal the effects of protocatechuic acid treatment on suppressing the inflammatory response and modulating immune function through the mTOR and MAPK signaling pathways on *Salmonella*-infected chicks. These results provide new insight for the application of plant extracts in alleviating intestinal bacterial infection.

Materials and methods

Experimental design

A total of 180 1-d female, yellow-feathered chickens were randomly allocated into 3 treatment groups, the control group (**Ctr**), the *Salmonella*-challenged group (**Sal**) and the protocatechuic acid treatment group (**PA**), each with 6 replicates, and each replicate with 10 birds. Birds were fed a basal diet for 18 d, with birds in PA being supplemented with 600 mg/kg protocatechuic acid (> 97%, Sigma-Aldrich, MO). On 14 and 16 d of age of the trial, birds in Sal and PA were orally challenged with 10^9 CFU *S.* typhimurium, while birds in Ctr received an equal amount of PBS.

The basal diet was formulated to meet the nutritional requirements of birds recommended by Chinese Nutrient Requirements of Yellow Chickens (Ministry of Agriculture, PRC 2020), and details of ingredient composition and calculated nutrient contents are shown in Table 1. The crude protein of diets was determined according to the Association of Official Analytical Chemists (AOAC International, 2007). The calcium and total phosphorus concentrations of diets was measured by the methods (SAC, 2018a) of potassium permanganate and vanadomolybdate colorimetry, respectively. Protocatechuic acid was added to the premix. Birds were raised in cages (100 cm × 50 cm × 50 cm) and allowed *ad libitum* access to fresh water and mashed diets.

Table 1
Composition and nutrient levels of the basal diet (%, dry matter basis).

Ingredients	%	Nutrient levels[2)]	%
Corn	59.20	Metabolic energy (MJ/kg)	11.91
Soybean meal	31.00	Crude protein	21.17
Corn gluten	3.00	Calcium	0.88
Soybean oil	2.30	Total phosphorus	0.69
$CaHPO_4$	1.85	Nonphytate phosphorus	0.40
Limestone	0.85	Lysine	1.16
DL-Met (99%)	0.10	Methionine	0.43
L-Lys HCL (78%)	0.20		
NaCl	0.30		
Zeolite powder	0.20		
Premix[1)]	1.00		
Total	100.00		

[1)] Premix provided the following per kilogram of the diet: VA 15 000 IU, VD_3 3 300 IU, VE 20 IU, VK_3 4.0 mg, VB_1 1.8 mg, VB_2 9.0 mg, VB_6 3.5 mg, VB_{12} 0.01 mg, choline chloride 500 mg, niacin 60 mg, pantothenic acid 16 mg, folic acid 0.55 mg, biotin 0.15 mg, Fe 80 mg, Cu 8 mg, Mn 80 mg, Zn 60 mg, I 0.35 mg, Se 0.30 mg.

[2)] Crude protein, calcium and phosphorus were determined by analysis AOAC International, 2007; SAC, 2018a, 2018b) Other values were calculated from data provided by Nutrient Requirements of Yellow Chickens Ministry of Agriculture, PRC 2020.

Culture of Salmonella typhimurium

Salmonella was cultured in liquid Luria-Bertani medium on a shaker for 12 h (180 rpm, 37°C), and then streaked on *Salmonella-Shigella* agar medium, and cultured at constant temperature (37°C) for 24 h. A single colony of typical *Salmonella* was selected and cultured in liquid Luria-Bertani medium for 8 h (180 rpm, 37°C) to logarithmic stage. The inoculum concentration was adjusted to match the standard growth curve, which corresponded to an optical density of 0.6 at OD_{600}, as measured by a spectrophotometer. The bacterial suspension was resuspended in PBS after centrifugation to achieve a final bacterial concentration of approximately 10^9 CFU/mL.

Calculation of growth performance

Birds were weighed per replicate at birth (0 d of age), and each phase (14, 16, and 18 d of age). Feed take was recorded daily on a per replicate basis. Average body weight (BW), average daily gain (ADG), average daily feed intake (ADFI) and the ratio of feed to gain (F/G) were calculated to assess growth performance.

Sample collection and the relative weights of immune organs calculation

After feed withdrawal overnight, 12 birds per group (2 birds/replicate) close to average BW per replicate were weighed, electrically stunned and exsanguinated. Blood samples were obtained into tubes containing sodium heparin, and then centrifuged (3,000 rpm, 10 min, 4°C) to collect plasma. The spleen, liver, thymus and bursa of Fabricius were dissected, blotted and then weighed. The relative weights of immune organs were calculated as follows: relative weight = weights of organ/BW × 100%.

After gently washing with physiological saline, segments of jejunum (1 cm) were fixed in 4% paraformaldehyde. Jejunal mucosal samples were also collected in Eppendorf tubes by gentle scraping after opening lengthwise and stored at -80°C for future analysis.

Morphological observation of jejunum

Serial sections of Paraffin-embedded sections (5 μm) of jejunum were cut using a microtome (Microm-HM340E, Thermo Fisher Scientific, USA), mounted then dewaxed in xylene, rehydrated with an ethanolic series and routinely stained with H&E to observe the morphology of villi. The height of villi, and depth of adjacent crypts were examined microscopically with a Panoramic Scanner (P-MIDI P250, 3D Histech, HUN), and the ratio of villus height to crypt depth was calculated with Image-Pro Plus software.

The TUNEL (TdT-mediated dUTP nick end labeling) assay was provided by Wuhan Servicebio Technology Co., Ltd (Wuhan, China). Briefly, the paraffin-embedded liver sections were deparaffinized with xylene, hydrated with gradient concentrations of alcohol, and covered with proteinase K. Slides were incubated with terminal deoxynucleotidyl transferase and biotinylated nucleotides and then treated with saline-sodium citrate buffer, 6% hydrogen peroxide, streptavidin–HRP conjugate, and DAB substrate solution. Finally, the slides were counterstained in hematoxylin solution. Images were captured using an

Upright fluorescence microscope (Eclipse C1, Nikon, Japan) and imaging system (DS-U3, Nikon, Japan).

Determination of immunoglobulin and immune cytokines in plasma and jejunal mucosa

The mucosal samples were homogenized with 10 volumes of ice-cold physiologic saline and centrifuged at 3,500 rpm for 10 min to obtain clarified supernatants. The concentrations of complement protein 3 (C3), C4, IgG, IgM, IgA, IL-1β, IL-6, IL-8, TNF-α, IFN-β and IFN-γ in plasma and jejunal mucosa were determined by commercial kits (MM-1610O1, MM-1611O1 MM-0505O1, MM-0912O1, MM-0913O1, MM-0521O1, MM-0768O1, MM-0938O1, MM-0520O1, MM-36910O1 and MM-34122O1, Jiangsu Meimian industrial Co., Ltd, Suzhou, China) and a spectrophotometer (Spectra Max M-5, Molecular Devices, San Jose, CA).

Determination of mRNA relative expression in jejunal mucosa

Total RNA was extracted from the jejunal mucosa using RNAiso Plus (9109, Takara, Japan) and reverse transcribed using PrimeScript II cDNA synthesis Kit (D6210A, Takara, Japan). Real-time PCR used the CFX96 RT-PCR Detection System (Bio-Rad, Hercules, CA) with SYBR PremixExTaq II (RR820A, Takara, Japan), specific experimental operation as previously described (Wang et al., 2021). The primers were designed (Table 2) and synthesised by Shenggong Bioengineering Co., Ltd (Guangzhou, China). The relative RNA expression level was calculated using $2^{-\Delta\Delta CT}$ analyses.

Analysis of transcriptome and proteome

After the quality and integrity of the above RNA were determined, the RNA sequencing library was constructed using the KC-Digital™ stranded mRNA library Prep Kit for Illumina®, and the library was filtered and quantified.

The total protein of jejunal mucosa was extracted from jejunal mucosal with urea lysis buffer (7 M urea, 2 M thiourea, and 1% SDS) with protease inhibitor, and quantified using the Pierce BCA kit (Thermo Scientific Pierce, Rockford, IL). Following reduction, cysteine alkylation, and digestion, samples were labeled with iTRAQ Reagents (4390812, Applied Biosystems, Waltham, MA) according to the manufacturer's instructions. After desalting by C18 solid-phase extraction, peptides were used for Nano Liquid Chromatography–Mass Spectrometry/Mass Spectrometry (LC-MS/MS) analysis (Zhu et al., 2020).

Table 2
Primers sequences for real-time PCR.

Gene	GenBank ID	Primers sequence (5′ to 3′)
β-actin	NM_001101.5	F: GAGAAATTGTGCGTGACATCA
		R: CCTGAACCTCTCATTGCCA
CXCR2	XM_015289858	F: GCAGACCAAGAACGTCCAGA
		R: TGATGTTGTAGGGCAGCCAG
CXCR3	XM_046921656.1	F: AAAGTGCCCTGCTCTACACC
		R: CAACCCAAACAGGAAGCGTG
CXCR4	NM_204617.3	F: CTGACAATGGCTCGGAGGAG
		R: AGCCAACAGGCAAAGAAGGT
MAPK13	XM_040691288.2	F: TGAAATGACAGGCTATGTGGTC
		R: CAAAGTATGGGTGGGCTAATG
TNF-α	XM_046927262.1	F: AATTTGCAGGCTGTTTCTGC
		R: TATGAAGGTGGTGCAGATGG
IFN-γ	NM_205427.1	F: GGACATGGCTCCCACACTAC
		R: TGAAGAGGTGCTGAAGGATG
NF-kB	XM_046915553.1	F: TGAGGGTGATGTAGACGCGG
		R: ACACGTGCTTTCAGGGTCTT

CXCR = CXC chemokine receptors, *MAPK*13 = mitogen-activated protein kinase 13, *TNF-α* = tumor necrosis factor α, *IFN-γ* = interferon γ, *NF-κB* = nuclear factor κB.

Statistical analysis

Effects of treatment were analyzed by the one-way analysis of variance (ANOVA) procedure in SPSS 25.0 (SPSS Inc., Chicago, IL). When effects of treatment were significant ($P < 0.05$), means were separated by Duncan's multiple range test. Tabulated results are shown as means with standard error of the mean (SEM).

Differentially expressed genes (DEG) were identified using the DESeq2 method with the criteria of |fold change| ≥ 2.0 and *P*-value < 0.05. Differentially expressed proteins (DEP) were identified by Proteome Discoverer™ Software 2.2 and R Language. The threshold for up-regulated proteins was $P < 0.05$ and FC ≥ 2, and the threshold for down-regulated proteins was $P < 0.05$ and FC ≤ 0.5. Finally, KEGG (Kyoto Encyclopedia of Genes and Genomes, http://www.genome.jp/kegg//) and GO (gene ontology, http://www.blast2go.com/b2ghome; http://geneontology.org/) were used for metabolic pathway and function cluster analysis of DEG and DEP.

Results

Effects of protocatechuic acid on growth performance of chickens infected with Salmonella

As shown in Table 3, compared with Ctr, BW of chicks in Sal was significantly ($P < 0.05$) reduced at 16 and 18 d of age. The ADG and ADFI were also significantly ($P < 0.05$) decreased during 0 to 18 d and 14 to 18 d of age. These suppressions were completely relieved by supplementation with protocatechuic acid ($P < 0.05$). In addition, the F/G was also decreased ($P < 0.05$) by protocatechuic acid, compared with birds in Sal.

Effects of protocatechuic acid on relative weights of immune organs of chickens infected with Salmonella

As shown in Table 4, compared with birds in Ctr, infection with *Salmonella* increased the relative weight of liver ($P < 0.05$) and

Table 3
Effects of protocatechuic acid on growth performance of chickens infected with *Salmonella*.

Items	Treatments			SEM	*P*-value
	Ctr	Sal	PA		
BW, g					
0 d of age	33.58	33.57	33.57	0.042	0.982
7 d of age	78.28	76.17	79.60	1.014	0.398
14 d of age	150.66	152.91	155.14	1.841	0.188
16 d of age	167.04[b]	161.32[c]	172.12[a]	1.323	< 0.001
18 d of age	189.89[a]	177.84[b]	196.51[a]	2.356	< 0.001
0 to 7 d of age					
ADG, g	6.39	6.08	6.58	0.141	0.397
ADFI, g	9.26	9.17	9.71	0.202	0.528
F/G	1.45	1.52	1.48	0.021	0.488
7 to 14 d of age					
ADG, g	10.34	10.94	10.79	0.152	0.234
ADFI, g	18.17	18.38	18.54	0.174	0.683
F/G	1.76	1.69	1.72	0.028	0.588
14 to 18 d of age					
ADG, g	9.81[a]	6.27[b]	10.35[a]	0.561	0.001
ADFI, g	17.18[b]	16.24[b]	18.90[a]	0.351	0.001
F/G	1.83[b]	2.62[a]	1.85[b]	0.104	0.001
0 to 18 d of age					
ADG, g	8.68[b]	8.01[c]	9.05[a]	0.120	< 0.001
ADFI, g	14.48[b]	14.32[b]	15.19[a]	0.144	0.015
F/G	1.67[b]	1.79[a]	1.68[b]	0.017	0.038

BW = body weight; ADG = average daily gain; ADFI = average daily feed intake; F/G = the ratio of feed to gain (g/g); Ctr = Control group; Sal = *Salmonella*-challenged group; PA = protocatechuic acid treatment group.

[ab] Within a row, values with different superscripts mean significant difference ($P < 0.05$). Results are presented as mean ± SEM (n=6).

Table 4
Effects of protocatechuic acid on relative weights of immune organs of chickens infected with *Salmonella*.

Items	Treatments			SEM	*P*-value
	Ctr	Sal	PA		
Relative weight of liver, %	0.13	0.15	0.12	0.011	0.119
Relative weight of spleen, %	0.54	0.53	0.55	0.024	0.955
Relative weight of thymus, %	0.43[a]	0.34[b]	0.42[a]	0.027	0.023
Relative weight of bursa of Fabricius, %	2.60[b]	3.19[a]	2.74[b]	0.121	< 0.001

Ctr = Control group; Sal = *Salmonella*-challenged group; PA = protocatechuic acid treatment group.
[ab] Within a row, values with different superscripts mean significant difference ($P < 0.05$). Results are presented as mean ± SEM (n=6).

decreased the relative weight of bursa of Fabricius ($P < 0.05$). Compared with birds in Sal, supplementation with protocatechuic acid significantly decreased the relative weight of liver and increased the relative weight of bursa of Fabricius of infected birds ($P < 0.05$).

Effects of protocatechuic acid supplementation on jejunal morphology of chickens challenged with Salmonella

The jejunal villi of bird in Ctr were intact, as shown by H&E staining (Fig. 1A), whereas those from birds infected with *Salmonella* were sparse and deranged. Infection with *Salmonella* induced apoptosis, as detected by TUNEL staining (Fig. 1B). In *Salmonella*-infected birds supplemented with protocatechuic acid, a protective effect on the derangement of villi was apparent.

Compared with birds in Ctr, infection with *Salmonella* decreased ($P < 0.05$) the villus height and villus height/crypt depth in jejunum (Fig. 1C and 1E). Compared with birds in Sal, supplementation with protocatechuic acid increased ($P < 0.05$) the villus height and villus height/crypt depth, and decreased ($P < 0.05$) the crypt depth in jejunum (Fig. 1D).

Effects of protocatechuic acid supplementation on immunoglobulins and cytokines of chickens challenged with Salmonella

Compared with birds in Ctr, infection with *Salmonella* increased ($P < 0.05$) the concentrations of IgG, IL-1β, IL-8, IFN-β and IFN-γ in plasma (Table 5), as well as the concentrations of complement C3, complement C4, IL-6, TNF-α and IFN-β in jejunal mucosa following the infection. Compared with birds in Sal, supplementation with protocatechuic acid decreased ($P < 0.05$) the plasma contents of IgG, IL-1β and IFN-β, and also decreased ($P < 0.05$) jejunal mucosal IL-6 and IFN-β.

Effects of protocatechuic acid supplementation on gene expression in jejunum of chickens challenged with Salmonella

As shown in Fig. 2A, *Salmonella* infection significantly ($P < 0.05$) up-regulated 163 DEG and down-regulated 125 DEG compared with Ctr, and compared with Sal treatment with protocatechuic acid significantly ($P < 0.05$) up-regulated 181 DEG and down-regulated 215 DEG. Venn diagram (Fig. 2B) showed that protocatechuic acid had significantly contrary influence on the expression of 77 genes which were affected by *Salmonella*. In detail, compared with birds in Sal, protocatechuic acid up-

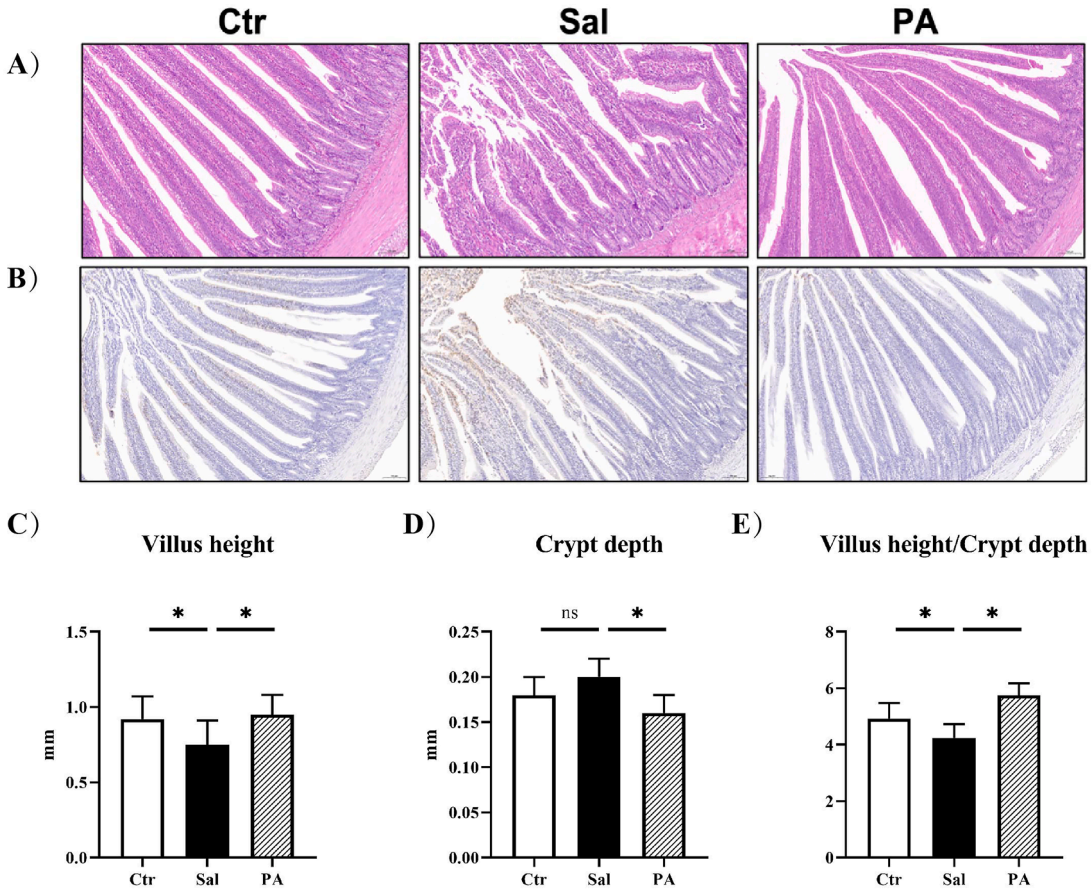


Fig. 1. Effects of protocatechuic acid supplementation on jejunal morphology of chickens challenged with *Salmonella*. Images of the jejunum stained with A) H&E and B) TUNEL assay, scale bar at 100 μm. C) Villus height, D) crypt depth and E) villus height/crypt depth of chickens. Ctr = Control group; Sal = *Salmonella*-challenged group; PA = protocatechuic acid treatment group. Results are presented as mean ± SEM (n=6); * $P < 0.05$; ns = not significant.

Table 5
Effects of protocatechuic acid on concentration of immunoglobulins and cytokines of chickens infected with *Salmonella*.

Items	Treatments			SEM	*P*-value
	Ctr	Sal	PA		
Plasma					
IgG, μg/mL	1088.69[b]	1221.94[a]	1123.66[b]	20.426	0.016
IgM, μg/mL	1036.23	1145.74	1149.89	24.084	0.088
IgA, ng/mL	327.54	356.21	336.31	6.654	0.195
IL-1β, pg/mL	327.78[b]	345.99[a]	307.38[b]	4.719	0.001
IL-6, pg/mL	17.15	16.87	17.53	0.288	0.670
IL-8, pg/mL	52.45[b]	60.18[a]	58.55[ab]	1.217	0.015
TNF-α, pg/mL	35.04	37.80	37.18	0.827	0.372
IFN-β, pg/mL	28.77[b]	33.72[a]	30.76[b]	0.671	0.006
IFN-γ, pg/mL	30.51[b]	34.42[a]	32.13[ab]	0.657	0.046
C3, μg/mL	181.07	182.18	176.18	1.887	0.398
C4, μg/mL	128.63	134.43	130.59	1.758	0.400
Jejunum					
IL-1β, pg/mg prot	113.25	114.32	107.66	2.717	0.563
IL-6, pg/mg prot	6.22[b]	6.91[a]	5.88[b]	0.151	0.017
IL-8, pg/mg prot	24.63	28.34	25.26	1.158	0.405
TNF-α, pg/mg prot	15.61[b]	19.98[a]	17.93[ab]	0.618	0.011
IFN-β, pg/mg prot	13.17[b]	15.96[a]	12.78[b]	0.496	0.013
IFN-γ, pg/mg prot	22.46	22.86	22.52	0.657	0.852
C3, μg/mg prot	157.84[b]	210.50[a]	163.57[b]	7.668	0.006
C4, μg/mg prot	119.10[b]	153.38[a]	122.34[b]	5.671	0.022

IgG = immunoglobulin G, IgM = immunoglobulin M, IgA = immunoglobulin A, IL-1β = interleukin-1β, IL-6 = interleukin 6, IL-8 = interleukin 8, TNF-α = tumor necrosis factor α, IFN-β = interferon β, IFN-γ = interferon γ; C3 = complement protein 3; C4 = complement protein 4. Ctr = Control group; Sal = *Salmonella*-challenged group; PA = protocatechuic acid treatment group.

[ab] Within a row, values with different superscripts mean significant difference ($P < 0.05$). Results are presented as mean ± SEM (n=6).

regulated 41 genes which were down-regulated by *Salmonella* compared with Ctr (**Supplemental Table S1**) and down-regulation 36 genes which were up-regulated by *Salmonella* compared with Ctr (**Supplemental Table S2**).

GO (Fig. 2C) analysis showed that 41 up-regulated DEG were enriched mainly in the function of IL-8 receptor binding, CXCR chemokine receptor binding, leukocyte chemotaxis, leukocyte migration, and neutrophil activation. KEGG (Fig. 2D) analysis showed that these up-regulated DEG were enriched in the signaling pathways of cytokine-cytokine receptor interaction, viral protein interaction with cytokine and cytokine receptor, chemokine and B cell receptor. Furthermore, 36 down-regulated DEG were enriched in the function of acute inflammatory response, blood coagulation, fibrin clot formation, and positive regulation of ceramide biosynthetic process (Fig. 2E), and signaling pathways of complement and coagulation cascades, vitamin B_6 metabolism, estrogen, systemic lupus erythematosus, vitamin digestion and absorption, and prion disease (Fig. 2F).

Effects of protocatechuic acid supplementation on protein expression in jejunum of chickens challenged with Salmonella

As shown in Fig. 3A, *Salmonella* infection significantly up-regulated 35 DEP and down-regulated 25 DEP compared with Ctr, and compared with Sal, treatment with protocatechuic acid significantly up-regulated 16 DEP and down-regulated 35 DEP. The Venn diagram (Fig. 3B) shown that dietary protocatechuic acid had significantly contrary influence on 11 common DEP which were affected by *Salmonella*, including XP_025004075.2, NP_990340.2, NP_001244132.1, NP_001183985.1, XP_040553571.1, XP_015147310.1, NP_001074360.1, XP_001234703.3, XP_015135083.2, XP_040562974.1, and XP_040503704.1 (Fig. 3C-M).

GO analysis showed that 3 up-regulated DEP were enriched in the functions of cytolysis (Fig. 4A). KEGG analysis showed that these up-regulated DEP were enriched in the signaling pathways of fanconi anemia and mTOR (Fig. 4B). In addition, 8 down-regulated DEP were enriched in the function of positive regulation of I-κB kinase/NF-κB signaling, positive regulation of dendritic cell cytokine production, regulation of interferon-alpha secretion, negative regulation of translation, and negative regulation of cellular amide metabolic process (Fig. 4C), as well as the signaling pathways of RNA degradation, herpes simplex virus 1 infection, MAPK, and cytokine-cytokine receptor interaction (Fig. 4D).

The verification of the expression levels of inflammation-related genes in the omics results is shown in Fig. 5. Compared with Ctr, the transcript abundances of *CXCR2*, *MAPK13*, *TNF-α*, *IFN-γ*, and *NF-κB* were significantly up-regulated ($P < 0.05$) in *Salmonella*-infected birds, which was consistent with the omics results. In addition, dietary protocatechuic acid supplementation significantly down-regulated ($P < 0.05$) the relative mRNA expression of CXC receptors, including *CXCR2/3/4*, as well as *MAPK13*, *TNF-α* and *IFN-γ*.

Discussion

Protocatechuic acid improved growth performance and regulated the relative weight of immune organs

Salmonella, the main food-borne bacteria in chicken production, seriously threatens animal production and human health. After colonizing the intestines, *Salmonella* causes intestinal inflammation, impairs intestinal function and reduces growth performance of chickens (Liu et al., 2023a). The study of Huang et al. (2022) showed that *Salmonella* infection could lead to weight loss in chicks. In addition, *Salmonella* infection significantly decreased feed intake, BW and feed conversion efficiency in chicks (Abudabos et al., 2019). These reports are consistent with the reduction of ADG and ADFI in *Salmonella*-infected chickens at 16 and 18 d of age in the present study. Protocatechuic acid is a phenolic compound that has been shown to influence animal immunity, antioxidant, anti-inflammatory status, improve intestinal health, and promote growth performance (Mahfuz et al., 2022). In previous studies, our laboratory has found that protocatechuic acid significantly increased BW and ADG, and decreased the F/G of broilers over 1 to 21 d of age (Jiang et al., 2023; Wang et al., 2019). Under the conditions of the present study, supplementation with 600 mg/kg protocatechuic acid reduced the F/G and alleviated the BW loss caused by *Salmonella*.

The development of immune organs is the basis of the formation of the immune system of chicks, in which the thymus is mainly responsible for the development and differentiation of T lymphocytes, and the bursa of Fabricius is an important place for the growth and development of B lymphocytes. *Salmonella* infection and LPS challenge shrink the thymus and reduce the weight of bursa of Fabricius (Ahiwe et al., 2019; Wang et al., 2022), resulting in a dysfunctional immune system. In the present study, the relative weight of thymus was increased and that of the bursa of Fabricius was decreased in *Salmonella*-infected chickens. Dietary protocatechuic acid alleviated the negative effects of *Salmonella*, possibly by suppressing the increase of inflammation caused by DNA methylation and inhibiting fibrosis (Li et al., 2023; Lyu et al., 2023), as well as inhibiting infection by reducing TLR4 expression and promoting the release of eosinophils and mast cells (Nam and Lee, 2018). During infection *in vivo*, changes in the weight of immune organs caused by supplementation with protocatechuic acid might affect the immune milieu, thus redering Salmonella less effective.

Protocatechuic acid alleviated the damage of jejunal morphology and inflammatory response in chickens challenged with Salmonella

Intestinal tissue acts as an effective barrier against microbial habitats from the external environment, maintaining its homeostasis through a delicate network of interactions between the gut-associated immune system and microbiota (Dolowschiak et al., 2016). The main symptoms of *Salmonella* occur in the early stages of infection, and *Salmonella* destroyed and invaded the intestinal epithelium, including severe rupture of intestinal villi, decreased villi height and VH/CD, and damage

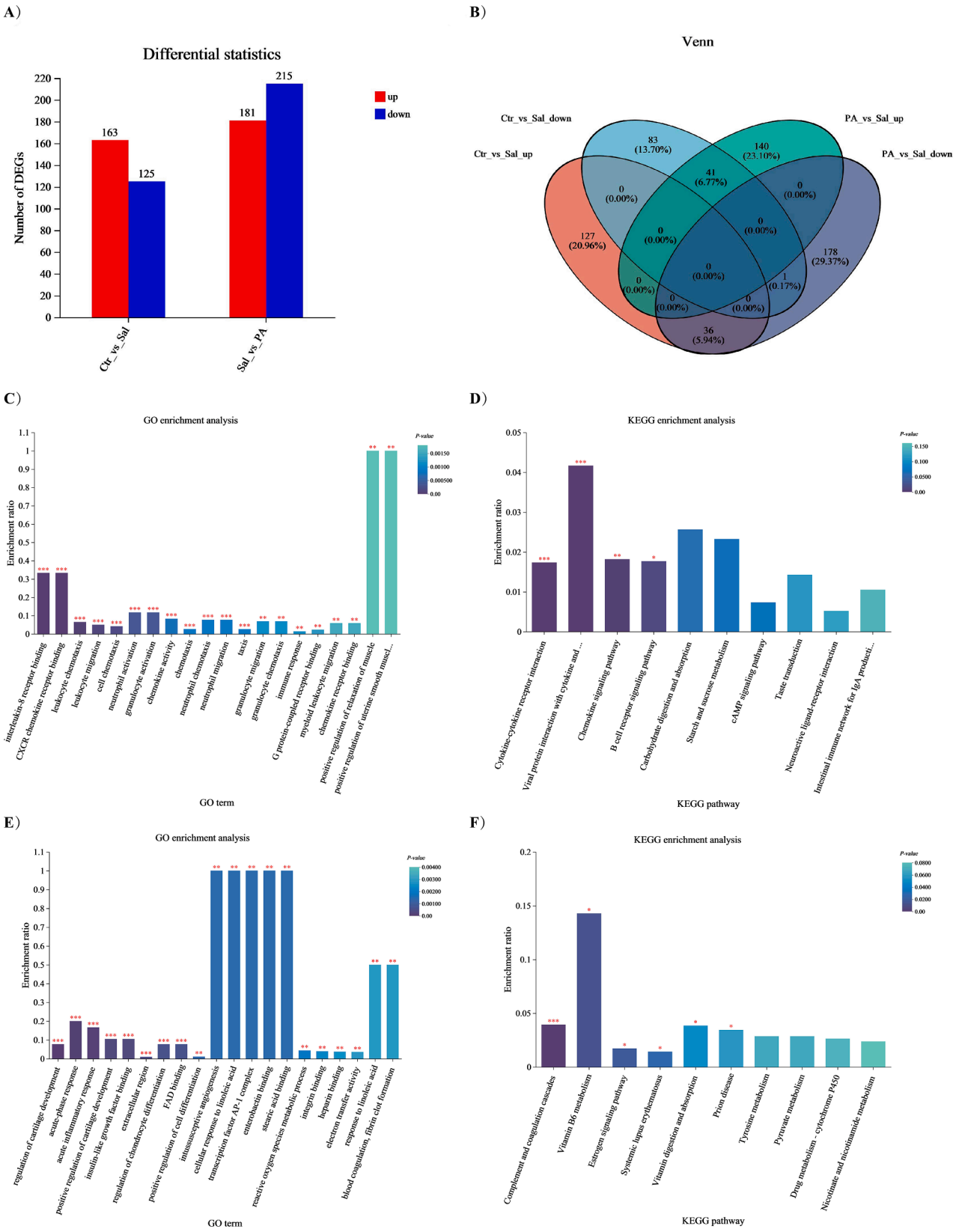


Fig. 2. Effects of protocatechuic acid on the jejunal mucosal transcriptome of chickens infected with *Salmonella* (n=3). A) Differentially expressed genes statistics and B) Venn diagram analysis. C) Gene ontology enrichment analysis of function and D) Kyoto Encyclopedia of Genes and Genomes enrichment analysis of signaling pathways were performed for the up-regulated genes by dietary protocatechuic acid. E) Gene ontology enrichment analysis of function and F) Kyoto Encyclopedia of Genes and Genomes enrichment analysis of signaling pathways were performed for the down-regulated genes by dietary protocatechuic acid. Ctr = Control group; Sal = *Salmonella*-challenged group; PA = protocatechuic acid treatment group.

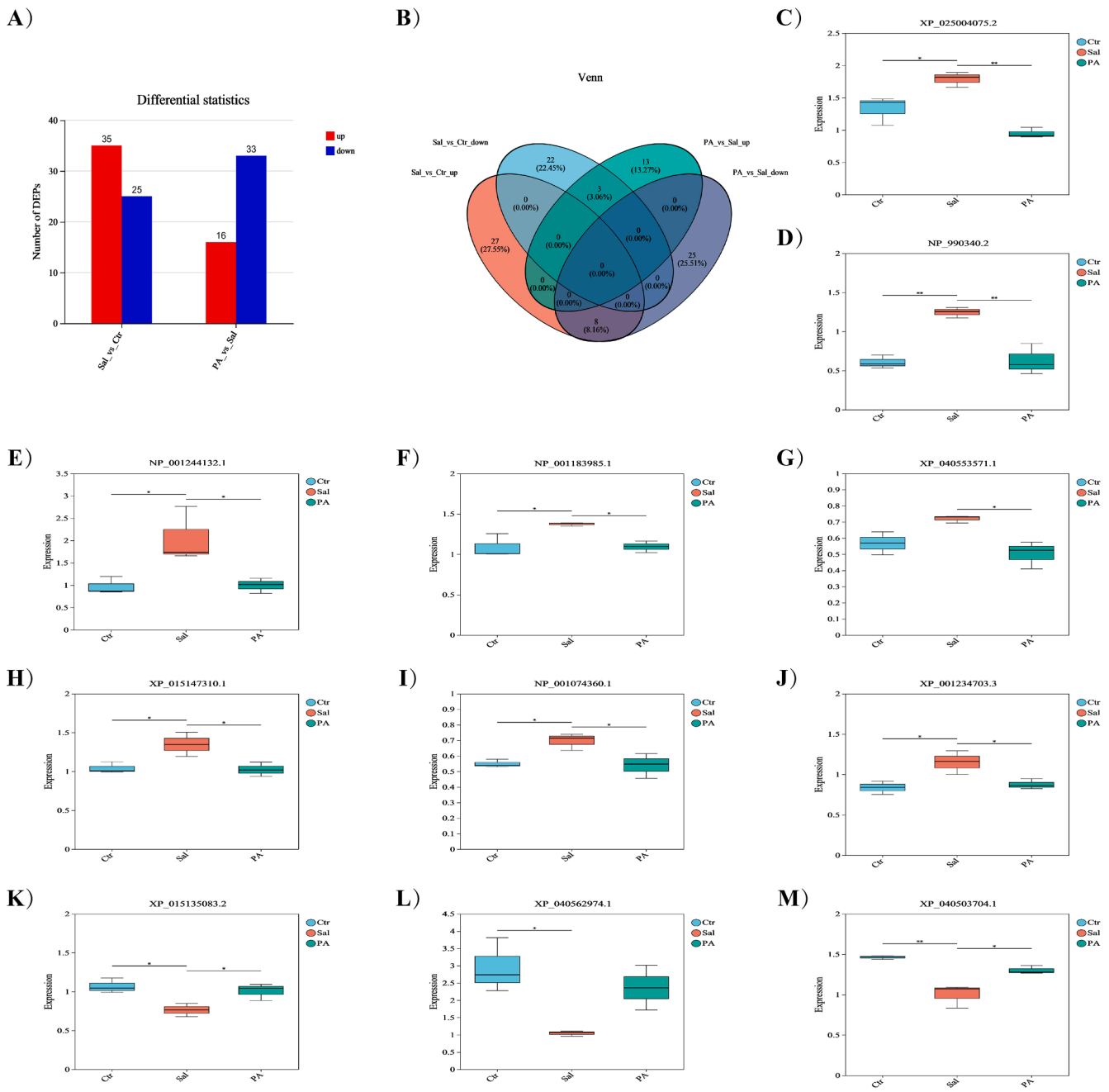


Fig. 3. Effects of protocatechuic acid on the jejunal mucosal transcriptome of chickens infected with *Salmonella* (n=3). A) Differentially expressed proteins statistics and B) Venn diagram analysis. C)-M) The expression levels of differentially expressed proteins in jejunal mucosa. Ctr = Control group; Sal = *Salmonella*-challenged group; PA = protocatechuic acid treatment group. Results are presented as mean ± SEM (n=3); * $P < 0.05$, ** $P < 0.01$.

to the tight connections of the intestine, leading to the translocation of pathogens from the gut to the blood, liver, and other organs (Felix et al., 2023; Hu et al., 2023). Besides the absorption and transport of nutrients, the complete intestinal epithelial structure also has the role of preventing pathogenic bacteria and toxic substances from invading the host, which is crucial in the process of resisting the invasion of *Salmonella*. In the present study, dietary protocatechuic acid alleviated the damage of *Salmonella* to the jejunal structure of chickens, and inhibited apoptosis. Strikingly, dietary protocatechuic acid effectively increased the jejunal VH and VH/CD, and decreased CD in chickens challenged with *Salmonella*. These findings were consistent with protocatechuic acid suppressing lipopolysaccharide and TNF-α-induced increase in intestinal permeability to reduce intestinal barrier structure damage in mice (So et al., 2023). In addition, studies also showed that protocatechuic acid alleviated intestinal inflammation through inhibiting necrosis and pyrodeath signaling pathways (Xiao et al., 2023), and exerted its apoptotic effect to reducing intestinal injury, such as up-regulating *DJ-1* (*PARK7*) mediated Nrf2 translocation (Cheng et al., 2019).

As a result of invasion of the gut mucosa and further replication, *Salmonella* induces a strong inflammatory response in the gastrointestinal tract, thereby damaging the intestine and facilitating colonization in animals (Gillis et al., 2018). After the invasion of the mucosal tissue, *Salmonella* promotes the secretion of pro-inflammatory cytokines. In the current research with chickens, *Salmonella* infection was found to increase the concentrations in plasma of IgG, IL-1β, IL-8, IFN-β, and IFN-γ. At the end of infection, IFN-γ plays an important role in pathogen clearance and alleviating inflammation (Thiemann et al., 2017).

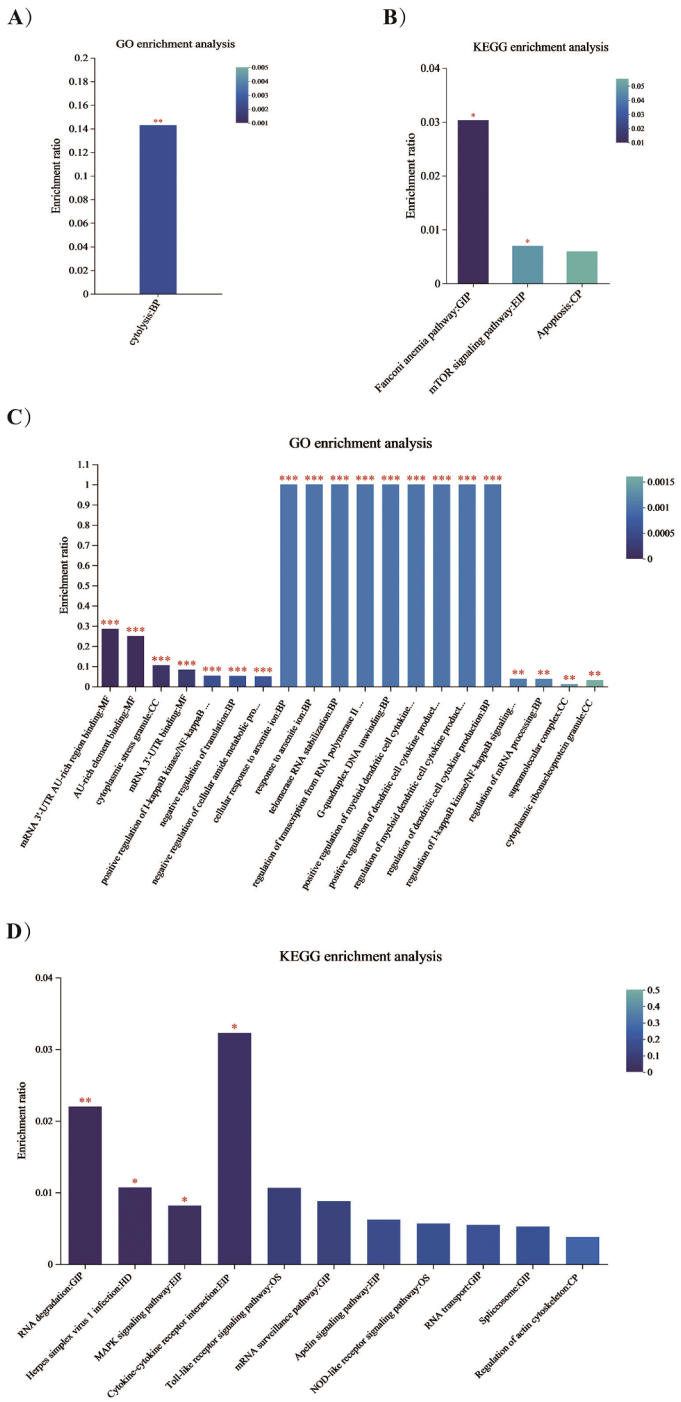


Fig. 4. A) Gene ontology enrichment analysis of function and B) Kyoto Encyclopedia of Genes and Genomes enrichment analysis of signaling pathways were performed for the up-regulated proteins by dietary protocatechuic acid. C) Gene ontology enrichment analysis of function and D) Kyoto Encyclopedia of Genes and Genomes enrichment analysis of signaling pathways were performed for the down-regulated proteins by dietary protocatechuic acid. Ctr = Control group; Sal = *Salmonella*-challenged group; PA = protocatechuic acid treatment group.

Dolowschiak et al. (2016) found that IFN-γ production by mucosal T and NK cells retarded disease by maintaining signaling through the transcriptional regulator *STAT1* and boosting expression of inflammatory mediators like *IL-1β, TNF*, and *iNOS*. Meanwhile, mucosal defense against *Salmonella* infection was reflected in the increased secretion of C3, C4, IL-6, TNF-α and IFN-β in jejunum. Indirect mechanisms of resistance against pathogen colonization include engagement and activation of immune pathways in the host, altering function of epithelial and immune cells (Jacobson et al., 2018).

The present study suggested that protocatechuic acid decreased C3 and C4 complement and IFN-β in plasma and jejunal mucosa, and also had a certain inhibitory effect on inflammatory cytokines, such as plasma IL-1β and jejunal mucosal IL-6. The effective anti-inflammatory activity of protocatechuic acid was demonstrated in LPS-induced and cadmium-induced inflammation by regulating various inflammatory cytokines and related signaling pathways in mice (Al Olayan et al., 2020; Salama et al., 2023). Kassab et al. (2022) found that protocatechuic acid modulated inflammation and apoptosis in liver and kidney in mice by reducing gene expression levels of *IL-1β, TNF-α*, and *NF-κB* and activating the Nrf-2 signaling pathway, thereby counteracting inflammatory damage. To conclude, protocatechuic acid alleviated intestinal damage by reducing the concentrations of inflammatory cytokines, thereby protecting the intestinal mucosa from inflammation caused by *Salmonella* infection.

Protocatechuic acid improved the expression of immune-related response genes in the chickens challenged with Salmonella

After reaching the intestinal cavity, *Salmonella* competes with the intestinal microbiota of the host and promote the occurrence of acute intestinal inflammation, resulting in intestinal bleeding and damage (Liu et al., 2023b; Wang et al., 2023). The complement system is a non-specific defense mechanism against pathogens. Complement C3 maintains T cell homeostasis and induces the production of autocrine pro-inflammatory cytokines *in vivo* (Li et al., 2023). Complement C4 has been shown to stimulate the activation of protease receptors, which leads to increased vascular endothelial permeability and promotes inflammation (Copenhaver et al., 2020). Activation of the complement causes a series of inflammatory responses and recruitment of immune response cells, directly killing the pathogens (Hakroush et al., 2021). This is consistent with the transcriptomic results that *Salmonella*-infection activated the function of acute inflammatory response, blood coagulation, and fibrin clot formation. *Salmonella* invasion led to the activation of signaling pathways associated with complement and coagulation cascades, as well as increased concentrations of complement C3 and C4 in the jejunal mucosa and plasma.

Enterocyte immune activation emerges as an important pathway for epithelial cells to defend against *Salmonella* infection. Interestingly, in the current study, immune-related responses such as leukocyte chemotaxis, leukocyte migration neutrophil activation, chemokine activity, and cytokine activity were all suppressed after infection. These may be related to the fact that *Salmonella* can manipulate the host's immune system for its own benefit during persistent infection, and alter its metabolism to inhibit apoptosis, cytolysis to resist antimicrobial treatment. Such changes retain nutrients for replication and the formation and maintenance of *Salmonella*-containing vacuoles as a conservative strategy (Ehrhardt et al., 2023; Jones et al., 2008).

Similarly, dietary protocatechuic acid supplementation up-regulated the functional genes of signaling pathway associated with vitamin B_6 (VB_6) metabolism in *Salmonella*-infected chickens. VB_6 regulates the synthesis and methylation of DNA associated with single carbon metabolism, as well as the ability to reduce oxidative stress, inflammation, and cell growth (Munteanu and Schwartz, 2023). Activation of DNA methylation induces differentiation, maturation and functional expression of the immune cell types and the occurrence of immune responses. During infection, pathogens induce epigenetic modifications in host cells by regulating the expression of genes involved in the immune response. These allow the bacteria to evade immune responses, survive and replicate inside the host cell (Barbachowska and Arimondo, 2023). Previous studies have shown that dietary protocatechuic acid supplementation regulated the development of immune organs of yellow-feathered chickens (Wang et al., 2019) and alleviated the

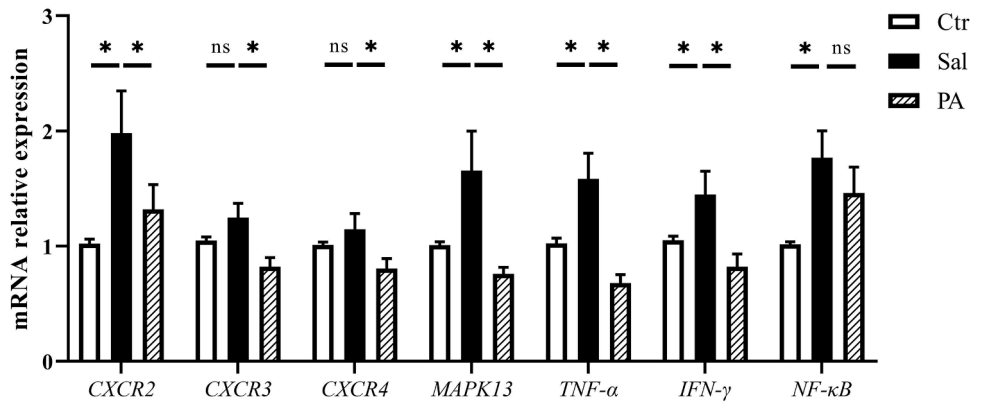


Fig. 5. Effects of protocatechuic acid on the jejunal mucosal inflammatory mRNA expression of chickens infected with *Salmonella*. Ctr = Control group; Sal = *Salmonella*-challenged group; PA = protocatechuic acid treatment group. *CXCR* = CXC chemokine receptors, *MAPK*13 = mitogen-activated protein kinase 13, *TNF-α* = tumor necrosis factor α, *IFN-γ* = interferon γ, *NF-κB* = nuclear factor κB. Results are presented as mean ± SEM (n=6); * $P < 0.05$; ns = not significant.

infection of avian influenza virus (AIV) and infectious bursal disease (IBD) in SPF chickens by enhancing humoral and cellular immune responses (Guo et al., 2018). The up-regulation of immune-related functional genes may be an important strategy to alleviate *Salmonella* infection.

Protocatechuic acid regulated the expression of mTOR and MAPK signaling pathways related to alleviate jejunal inflammatory damage

Proteome profiling showed that *Salmonella* infection significantly inhibited the mTOR signaling pathway, while protocatechuic acid activated this pathway in infected chickens. As a conventional autophagy pathway, the mTOR signaling pathway is widely involved in eukaryotes impacting autophagy and protein anabolism. *Salmonella* down-regulates autophagy by the AKT/mTOR signaling pathway (Wu et al., 2022), and promotes downstream NLRP3 and IL-1β induction of autophagy. The AKT/mTOR activation may improve intestinal epithelial function by increasing synthesis of tight junction proteins (He et al., 2021). Activation of the mTOR signaling pathway may potentially be the mechanistic role of protocatechuic acid in improving intestinal function, resisting *Salmonella* infection, inhibiting inflammation, and regulating apoptosis. In addition, I-κB kinase/NF-κB and immune-related signaling pathways (MAPK and cytokine-cytokine receptor interaction) were up-regulated in *Salmonella*-infected chickens. *Salmonella* effector proteins were likely secreted into host cells to initiate transformation by activating the host MAPK and AKT pathways, promoting bacterial uptake and intracellular survival (Scanu et al., 2015). Luis et al. (2022) showed that *Salmonella* activated PI3K/AKT pathway through SopB during B-cell infection. Furthermore, LPS and endotoxins contained in the cytoderm of *Salmonella* activated TLR4 and MyD88 in intestinal macrophages, and I-κB/NF-κB and MAPK signaling pathways downstream, ultimately caused inflammation (He et al., 2021; Zhang et al., 2021). In the present study, the increased concentrations of IL-6, TNF-α and IFN-β in jejunal mucosa of *Salmonella*-infected chickens were correlated with activation of the signaling pathway for cytokine-cytokine receptor interaction. Supplementation with protocatechuic acid effectively inhibited the secretions of inflammatory cytokines in jejunal mucosa and the enrichment of inflammatory signaling pathways.

Conclusion

The present study provided the basis for the speculation that *Salmonella* infection provoked an acute intestinal inflammatory response and activation of the MAPK signaling pathway, and these changes might cause the lower BW and intestinal damage in infected chickens. Dietary supplementation with 600 mg/kg protocatechuic acid alleviated the decrease of growth performance, intestinal structure damage and increased levels of inflammation cytokines in chickens challenged with *Salmonella*, and improved intestinal epithelial function through inhibiting the MAPK inflammatory signaling pathway and activating the mTOR signaling pathway. The current findings suggested that protocatechuic acid had potential as a promising non-antibiotic alternative in alleviating *Salmonella* infection.

Authors contributions

Sheng Zhang: Manuscript writing, Methodology, Formal analysis; **Qin Wang:** Designed the experiments, Data curation; **Xiangtian Yao, Jingjing Dong,** and **Guanhuo Li:** Performed animal husbandry, Data curation; **Yingan Zang:** Provided experimental guidance; **Shouqun Jiang:** Conceptualization, Funding acquisition; **Yibing Wang:** Writing-review&editing; Supervision, Funding acquisition. All authors read and approved the final version of the manuscript.

Animal ethics statement

Experimental procedures followed to guidelines established by the Institutional Animal Care and Use Committee, Guangdong Academy of Agricultural Sciences in China (Number: GAASISA-2022-003).

Declaration of competing interest

None.

Acknowledgments

We are thankful to W. Bruce Currie (Emeritus Professor) from Cornell University for his suggestions on presentation. This research was supported by the China Agriculture Research System of MOF and MARA (CARS-41-G06), the National Key R&D Project (2021YFD1300404, 2022YFD1300502), the Guangzhou Municipal Applied Basic Research Program (SL2022A04J00940), and Special Fund for Scientific Innovation Strategy-Construction of High-Level Academy of Agriculture Science (R2023PY-QY012)

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.psj.2024.104606.

References

Abudabos, A.M., Ali, M.H., Nassan, M.A., Saleh, A.A., 2019. Ameliorative effect of Bacillus subtilis on growth performance and intestinal architecture in broiler infected with *Salmonella*. Animals-Basel 9 (4), 190.

Ahiwe, E.U., Abdallh, M.E., Chang'a, E.P., Al-Qahtani, M., Omede, A.A., Graham, H., Iji, P.A., 2019. Influence of autolyzed whole yeast and yeast components on broiler chickens challenged with *Salmonella* lipopolysaccharide. Poultry Sci. 98 (12), 7129–7138.

Al Olayan, E.M., Aloufi, A.S., AlAmri, O.D., Ola, H., Moneim, A.E.A., 2020. Protocatechuic acid mitigates cadmium-induced neurotoxicity in rats: role of oxidative stress, inflammation and apoptosis. Sci. Total. Environ. 723, 137969.

AOAC International, 2007. International Association of Official Analytical Chemists. Official Methods of Analysis. Chemists, Gaithersburg.

Barbachowska, M., Arimondo, P.B., 2023. To target or not to target? The role of DNA and histone methylation in bacterial infections. Epigenetics-US 18 (1), 2242689.

Cheng, Y.T., Lin, J.A., Jhang, J.J., Yen, G.C., 2019. Protocatechuic acid-mediated DJ-1/PARK7 activation followed by PI3K/mTOR signaling pathway activation as a novel mechanism for protection against ketoprofen-induced oxidative damage in the gastrointestinal mucosa. Free Radical Bio. Med. 130, 35–47.

Copenhaver, M.M., Yu, C.Y., Zhou, D., Hoffman, R.P., 2020. Relationships of complement components C3 and C4 and their genetics to cardiometabolic risk in healthy, non-Hispanic white adolescents. Pediatr. Res. 87 (1), 88–94.

Cui, X.Y., Zhang, S., Jiang, S.Q., Gou, Z.Y., Wang, Y.B., 2023. Dietary protocatechuic acid ameliorates ileal mucosal barrier injury and inflammatory response and improves intestinal microbiota composition in Yellow chickens challenged with *Salmonella* typhimurium. Poultry Sci. 102 (4), 102496.

Dolowschiak, T., Mueller, A.A., Pisan, L.J., Namineni, S., Nguyen, B.D., Wotzka, S.Y., Heikenwalder, M., von Mering, C., Mueller, C., Hardt, W.D., 2016. IFN-γ hinders recovery from mucosal inflammation during antibiotic therapy for *Salmonella* gut infection. Cell Host Microbe 20 (2), 238–249.

Ehrhardt, K., Becker, A.L., Grassl, G.A., 2023. Determinants of persistent Salmonella infections. Curr. Opin. Immunol. 82, 102306.

Felix, L., Moreira Filho, A., Santos, M.R., Saraiva, M., Freitas Neto, O., Givisie, P., 2023. Intestinal morphometric changes associated with the use of non-antibiotic feed additives in broiler chicks challenged with *Salmonella* Enteritidis. J. Anim. Sci. Technol. 2023.

Galán, J.E., 2021. *Salmonella* typhimurium and inflammation: a pathogen-centric affair. Nat. Rev. Microbiol. 19 (11), 716–725.

Gillis, C.C., Hughes, E.R., Spiga, L., Winter, M.G., Zhu, W., Furtado de Carvalho, T., Chanin, R.B., Behrendt, C.L., Hooper, L.V., Santos, R.L., Winter, S.E., 2018. Dysbiosis-associated change in host metabolism generates lactate to support *Salmonella* growth. Cell Host Microbe 23 (1), 54–64.e6.

Guo, Y.X., Zhang, Q., Zuo, Z.H., Chu, J., Xiao, H.Z., Javed, M.T., He, C., 2018. Protocatechuic acid (PCA) induced a better antiviral effect by immune enhancement in SPF chickens. Microb. Pathogenesis 114, 233–238.

Hakroush, S., Tampe, D., Korsten, P., Ströbel, P., Tampe, B., 2021. Complement components C3 and C4 indicate vasculitis manifestations to distinct renal compartments in ANCA-associated glomerulonephritis. Int. J. Mol. Sci. 22 (12), 6588.

He, Y., Ayansola, H., Hou, Q.H., Liao, C.Y., Lei, J.Q., Lai, Y.J., Jiang, Q.Y., Masatoshi, H., Zhang, B.K., 2021. Genistein inhibits colonic goblet cell loss and colorectal inflammation induced by *Salmonella* typhimurium infection. Mol. Nutr. Food. Res. 65 (16), 2100209.

Hofer, U., 2021. *Salmonella* Enteritidis: chicken or egg? Nat. Rev. Microbiol. 19 (11), 682. -682.

Hu, Z.Q., Liu, L., Guo, F.S., Huang, J., Qiao, J.N., Bi, R.C., Huang, J.Y., Zhang, K.C., Guo, Y.M., Wang, Z., 2023. Dietary supplemental coated essential oils and organic acids mixture improves growth performance and gut health along with reduces Salmonella load of broiler chickens infected with *Salmonella* Enteritidis. J. Anim. Sci. Biotechno. 14 (1), 95.

Huang, J.Q., Liang, L., Cui, K.T., Li, P.Y., Hao, G.J., Sun, S.H., 2022. *Salmonella* phage CKT1 significantly relieves the body weight loss of chicks by normalizing the abnormal intestinal microbiome caused by hypervirulent *Salmonella* Pullorum. Poultry Sci. 101 (3), 101668.

Jacobson, A., Lam, L., Rajendram, M., Tamburini, F., Honeycutt, J., Phamet, T., Van Treuren, W., Pruss, K., Stabler, S.R., Lugo, K., Bouley, D.M., Vilches-Moure, J.G., Smith, M., Sonnenburg, J.L., Bhatt, A.S., Huang, K.C., Monack, D.A., 2018. A gut commensal-produced metabolite mediates colonization resistance to *Salmonella* infection. Cell Host Microbe 24 (2), 296–307.

Jalali, O., Best, M., Wong, A., Schaeffer, B., Johnson, L., 2020. Protocatechuic acid as a topical antimicrobial for surgical skin antisepsis: preclinical investigations. JBJS Open Access 5 (3), e19.00079.

Jennings, E., Thurston, T.L.M., Holden, D.W., 2017. *Salmonella* SPI-2 type III secretion system effectors: molecular mechanisms and physiological consequences. Cell Host Microbe 22 (2), 217–231.

Jiang, S.Q., Chen, Z.L., Zhang, S., Ye, J.L., Wang, Y.B., 2023. Protective effects of protocatechuic acid on growth performance, intestinal barrier and antioxidant capacity in broilers challenged with lipopolysaccharide. Animal 17 (1), 100693.

Jones, R.M., Wu, H.X., Wentworth, C., Luo, L., Collier-Hyams, L., Neish, A.S., 2008. *Salmonella* AvrA coordinates suppression of host immune and apoptotic defenses via JNK pathway blockade. Cell Host Microbe 3 (4), 233–244.

Kassab, R.B., Theyab, A., Al-Ghamdy, A.O., Algahtani, M., Mufti, A.H., Alsharif, K.F., Abdella, E.M., Habotta, O.A., Omran, M.M., Lokman, M.S., Bauomy, A.A., Albrakati, A., Baty, R.S., Hassan, K.E., Alshiekheid, M.A., Abdel Moneim, A.E., Elmasry, H.A., 2022. Protocatechuic acid abrogates oxidative insults, inflammation, and apoptosis in liver and kidney associated with monosodium glutamate intoxication in rats. Environ. Sci. Pollut. R. 29 (8), 12208–12221.

Li, H.G., Zheng, J., Xu, Q., Yang, Y.J., Zhou, J., Guo, X.L., Cai, Y.F., Cai, J.J., Xie, L.L., Awika, J., Han, X.L., Li, Q.S., Kennedy, L., Francis, H., Glaser, S., Huo, Y.Q., Alpini, G., Wu, C.D., 2023. Hepatocyte adenosine kinase promotes excessive fat deposition and liver inflammation. Gastroenterology 164 (1), 134–146.

Li, Z.Q., Qi, Z., Wang, X.R., Lu, L.T., Wang, H.Y., He, Z.J., Chen, Z., Shao, Y., Tu, J., Song, X.J., 2023. Avian pathogenic Escherichia coli infection causes infiltration of heterophilic granulocytes of chick tracheal by the complement and coagulation cascades pathway. BMC Vet. Res. 19 (1), 262.

Liu, J.X., Liu, H.H., Teng, Y., Qin, N.B., Ren, X.M., Xia, X.D., 2023a. A high-sucrose diet causes microbiota composition shift and promotes the susceptibility of mice to *Salmonella* Typhimurium infection. Food Funct. 14 (6), 2836–2846.

Liu, J.D., Shanmugasundaram, R., Doupovec, B., Schatzmayr, D., Murugesan, G.R., Applegate, T.J., 2023b. Short-term exposure to fumonisins and deoxynivalenol, on broiler growth performance and cecal *Salmonella* load during experimental *Salmonella* Enteritidis infection. Poultry Sci. 102 (6), 102677.

Luis, L.B., Ana, G.T., Carlos, G.E., Abraham, G.G., Iris, E.G., Martha, M.L., Vianney, O.N., 2022. *Salmonella* promotes its own survival in B cells by inhibiting autophagy. Cells-Basel 11 (13), 2061.

Lyu, S.Y., Xiao, W., Cui, G.Z., Yu, C., Liu, H., Lyu, M., Kuang, Q.Y., Xiao, E.H., Luo, H.Y., 2023. Role and mechanism of DNA methylation and its inhibitors in hepatic fibrosis. Frontiers Genet. 14, 1124330.

Mahfuz, S., Mun, H.S., Dilawar, M.A., Ampode, K.M.B., Yang, C.J., 2022. Potential role of protocatechuic acid as natural feed additives in farm animal production. Animals-Basel 12 (6), 741.

Ministry of Agriculture of the People's Republic of China (PRC), 2020. Nutrient Requirements of Yellow Chicken. China Agricultural Press, Beijing, China.

Munteanu, C., Schwartz, B., 2023. B Vitamins, Glucoronolactone and the immune system: bioavailability, doses and efficiency. Nutrients 16 (1), 24.

Nam, Y.J., Lee, C.S., 2018. Protocatechuic acid inhibits Toll-like receptor-4-dependent activation of NF-κB by suppressing activation of the Akt, mTOR, JNK and p38-MAPK. Int. Immunopharmacol. 55, 272–281.

Salama, A.A.A., Elgohary, R., Fahmy, M.I., 2023. Protocatechuic acid ameliorates lipopolysaccharide-induced kidney damage in mice via downregulation of TLR-4-mediated IKBKB/NF-κB and MAPK/Erk signaling pathways. J. App. Toxicol. 43 (8), 1119–1129.

Scanu, T., Spaapen, R.M., Bakker, J.M., Pratap, C.B., Wu, L., Hofland, I., Broeks, A., Shukla, V.K., Kumar, M., Janssen, H., Song, J.Y., Neefjes-Borst, E.A., te Riele, H., Holden, D.W., Nath, G., Neefjes, J., 2015. *Salmonella* manipulation of host signaling pathways provokes cellular transformation associated with gallbladder carcinoma. Cell Host Microbe 17 (6), 763–774.

So, B.R., Kim, S., Jang, S.H., Kim, M.J., Lee, J.J., Kim, S.R., Jung, S.K., 2023. Dietary protocatechuic acid redistributes tight junction proteins by targeting Rho-associated protein kinase to improve intestinal barrier function. Food Funct. 14 (10), 4777–4791.

Song, J., He, Y.N., Luo, C.H., Feng, B., Ran, F., Xu, H., Ci, Z.M., Xu, R.C., Han, L., Zhang, D.K., 2020. New progress in the pharmacology of protocatechuic acid: a compound ingested in daily foods and herbs frequently and heavily. Pharmacol. Res. 161, 105109.

Standardization Administration of China (SAC), 2018a. Determination of Calcium in Feeds (GB/T 6436-2018). China Agricultural Press, Beijing, China.

Standardization Administration of China (SAC), 2018b. Determination ofPhosphorus in Feeds (GB/T 6437-2018). China Agricultural Press, Beijing, China.

Tan, J.J., Hu, R.Z., Gong, J.T., Fang, C.K., Li, Y.L., Liu, M., He, Z., Hou, D.X., Zhang, H., He, J., Wu, S., 2023. Protection against metabolic associated fatty liver disease by protocatechuic acid. Gut Microbes 15 (1), 2238959.

Thiemann, S., Smit, N., Roy, U., Lesker, T.R., Galvez, E.J.C., Helmecke, J., Basic, M., Bleich, A., Goodman, A.L., Kalinke, U., Flavell, R.A., Erhardt, M., Strowig, T., 2017. Enhancement of IFN-γ production by distinct commensals ameliorates *Salmonella*-induced disease. Cell Host Microbe 21 (6), 682–694.

Varì, R., Scazzocchio, B., Santangelo, C., Filesi, C., Galvano, F., D'Archivio, M., Masella, R., Giovannini, C., 2015. Research article protocatechuic acid prevents oxLDL-induced apoptosis by activating JNK/Nrf2 survival signals in macrophages. Cell 1, 0–2.

Wu, L.H., Pangilinan, C.R., Lee, C.H., 2022. Downregulation of AKT/mTOR signaling pathway for Salmonella-mediated autophagy in human anaplastic thyroid cancer. J. Cancer 13 (11), 3268.

Wang, D., Zheng, Y.C., Fan, Y.Y., He, Y.J., Liu, K.X., Deng, S.X., Liu, Y., 2023. Sodium humate-derived gut microbiota ameliorates intestinal dysfunction induced by *Salmonella* typhimurium in mice. Microbiol. Spectr. 11 (3), e05348. -22.

Wang, Y.B., Wang, Y., Lin, X.J., Gou, Z.Y., Fan, Q.L., Jiang, S.Q., 2021. Effects of *Clostridium butyricum*, sodium butyrate, and butyric acid glycerides on the reproductive performance, egg quality, intestinal health, and offspring performance of yellow-feathered breeder hens. Front. Microbiol. 12, 657542.

Wang, Y.B., Wang, Y.Y., Wang, B.K., Mei, X.Q., Jiang, S.Q., Li, W.F., 2019. Protocatechuic acid improved growth performance, meat quality, and intestinal health of Chinese yellow-feathered broilers. Poultry Sci. 98 (8), 3138–3149.

Wang, Z., Li, X., Du, S.H., Sun, X.S., Huang, J.G., Shao, Y.X., 2022. Protective effects of Zinc on *Salmonella* invasion, intestinal morphology and immune response of young pigeons infected with *Salmonella* enterica serovar Typhimurium. Biol. Trace. Elem. Res. 200 (11), 4817–4827.

Wu, M., Tian, L., Fu, J., Liao, S.C., Li, H., Gai, Z.C., Gong, G.L., 2022. Antibacterial mechanism of Protocatechuic acid against *Yersinia enterocolitica* and its application in pork. Food Control 133, 108573.

Xiao, K., Zhou, M.H., Lv, Q.Q., He, P.W., Qin, X., Wang, D., Zhao, J.C., Liu, Y.L., 2023. Protocatechuic acid and quercetin attenuate ETEC-caused IPEC-1 cell inflammation and injury associated with inhibition of necroptosis and pyroptosis signaling pathways. J. Anim. Sci. Biotechno. 14 (1), 1–18.

Zambry, N.S., Ahmad, N.M., Awang, M.S., Selvam, K., Khalid, M.F., Bustami, Y., Hamzah, H.H., Ozsoz, M., Abd, M.A., Aziah, I., 2022. Aptamer-based electrochemical biosensors for the detection of *Salmonella*: A scoping review. Diagnostics 12 (12), 3186.

Zhang, L., Sun, Y., Xu, W., Geng, Y., Su, Y.H., Wang, Q.N., Wang, J.L., 2021. Baicalin inhibits *Salmonella* typhimurium-induced inflammation and mediates autophagy through TLR4/MAPK/NF-κB signaling pathway. Basic Clin. Pharmacol. 128 (2), 241–255.

Zhao, Y.M., He, Z.Y., Hao, W.J., Zhu, H.Y., Liu, J.H., Ma, K.Y., He, W.S., Chen, Z.Y., 2021. Cholesterol-lowering activity of protocatechuic acid is mediated by increasing the excretion of bile acids and modulating gut microbiota and producing short-chain fatty acids. Food Funct. 12 (22), 11557–11567.

Zhu, D.F., Fang, X.X., Chen, Y.H., Shan, M.Y., Jiang, R.Z., Qiu, Z.D., Luo, H.M., 2020. Structure-activity relationship analysis of Panax ginseng glycoproteins with cytoprotective effects using LC-MS/MS and bioinformatics. Int. J. Biol. Macromol. 158, 1352–1361.