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Influence of paeoniflorin dietary supplementation on growth performance, antioxidant status, blood parameters, carcass characteristics and meat quality in broiler chickens

Yefei Zhou [a,*], Cunyi Qiu [b], Zhiding Zhou [c], Dunlin Zhang [a], Yao Cai [a], Jun Yuan [a], Shanguo Mao [a]

[a] Department of Food Science, Nanjing Xiaozhuang University, Nanjing, 211171, China
[b] Gansu Polytechnic College of Animal Husbandry & Engineering, Wuwei, 733006, China
[c] Key Laboratory of Marine Animal Disease Prevention and Control, Dalian Ocean University, Dalian, 116023, China

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ABSTRACT

Paeonia lactiflora Pall, known for its antioxidative and anti-apoptotic properties, is a traditional Chinese medicine. To address the growing demand for animal protein, large-scale commercial broiler production systems often induce excessive stress responses in chickens, impacting their performance and immune function. This study examined the effects of adding paeoniflorin at doses of 150, 300, and 450 mg/kg to broiler diets on antioxidant activities, blood biochemical parameters, carcass characteristics, and meat quality. The results showed that different levels of paeoniflorin significantly enhanced the activity of antioxidant enzyme in serum and liver, and decreased in malondialdehyde level both in serum and meat tissue compared with basal diet broilers ($P < 0.05$). Paeoniflorin supplementation markedly decreased levels of creatinine, uric acid, aspartate aminotransferase, alanine aminotransferase, total cholesterol, and triglycerides ($P < 0.05$). Diets containing different levels of paeoniflorin significantly increased the eviscerated yield percentage of birds and reduced abdominal fat ($P < 0.05$). Furthermore, paeoniflorin supplementation notably enhanced the redness and reduced the yellowness of pectoral and thigh muscles, while also significantly decreasing drip and cooking loss in the pectoral muscle ($P < 0.05$). Although the levels of crude protein, ether extract, and crude ash in the pectoral and thigh muscles did not significantly vary between treatments ($P > 0.05$), paeoniflorin significantly increased the nucleotide 5′-monophosphate content in meat muscles ($P < 0.05$). Therefore, the data suggest that paeoniflorin can be an effective natural feed additive for broiler diets, with an optimal dosage of 150–300 mg/kg.

1. Introduction

To address the increasing demand for animal protein, commercial poultry production systems are frequently utilized for large-scale broiler production (Chowdhury et al., 2014). However, due to nutrition, metabolism, temperature, stocking density, vaccination, disease, transportation and feed quality, broilers can develop excessive stress responses in this system (Gasparino et al., 2018). A large amount of reactive oxygen species (ROS) is produced in some severe pathological conditions, which disturbs the balance between the oxidation and antioxidant defense systems in the bird's body, and has a large negative impact on the performance and immune function of the broiler (Kebreab et al., 2016). At the same time, oxidation is the result of the body's natural metabolic process, the production of high levels of ROS in body can accelerate the induction of lipid peroxidation and affect the meat quality (Pirgozliev et al., 2019). Therefore, dietary supplementation of synthetic antioxidants, such as butylated hydroxytoluene (BHT), butylated hydroxyanisole and ethoxyquin in poultry feed, has been implemented to achieve optimal reproduction and meat quality. However, their use is debated due to their side-effects (Farahat et al., 2017). In order to find effective substitutes to synthetic ones and meet consumer's demands, many herbs or their extracts have been evaluated in poultry industry (Habibi et al., 2014).

Paeonia lactiflora Pall, commonly known as baishao in China, is a member of the Ranunculaceae family. Its root is often used in traditional Chinese medicine. The primary active component of this root is

* Corresponding author.
E-mail address: yfzhou@njxzc.edu.cn (Y. Zhou).

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paeoniflorin, a natural monoterpene glycoside (He & Dai, 2011). Paeoniflorin is recognized for its diverse biological activities, including antioxidative, antihepatic injury, anti-inflammatory, immunoregulatory, and neuroprotective effects (Wang et al., 2018). Research has demonstrated that paeoniflorin can effectively reduce serum lipid and cholesterol levels in rats (Hu et al., 2017). Given its broad pharmacological potential, paeoniflorin has been the subject of extensive studies across various therapeutic areas. However, research exploring the use of paeoniflorin in broilers remains scarce. This study investigates the potential of paeoniflorin as a natural antioxidant, examining its impact on the antioxidant activity, blood profiles, carcass characteristics, and meat quality of broilers, thereby providing theoretical and experimental data to support its inclusion in broiler diet formulations.

2. Materials and methods

The experiment was conducted at the facility of Nanjing Xiaozhuang University farm. The birds were raised in cages measuring 0.90 m × 1.54 m each. All cages were equipped with automatic feeding and nipple drinkers, and the chickens were free to feed and drink. All experimental protocols were approved by the Institutional Animal Care and Ethics Committee of Nanjing Xiaozhuang University (permit number IACECNXU20220326), and all protocols also meet the International Guiding Principles for Biomedical Research Involving Animals.

2.1. Experimental birds and management

Two hundred and forty one-day-old Arbor Acres (AA) female broilers were obtained from the hatchery of the Nanjing Poultry Institute (Nanjing, China). Upon arrival at the animal facility, all chicks were fed with commercial anti-stress vitamins and starter meal. At 7 days of age, the chicks were individually weighed and identified with wing tags, with an average body weight of 172.16 ± 1.45 g per chick. Subsequently, all chicks were randomly assigned to five dietary treatment groups, each comprising six replicate cages (8 chicks per cage).The brooder temperature was 33± 1 °C in the first week, and then decreased by 2 °C per week until it reached 22 °C ± 1 °C.The lighting was implemented with a "23-hour-on-1-hour-off" lighting regimen with a relative humidity of 60 %. The experimental period was extended from 7 to 42 days old.

The chickens were immunized as follows: Newcastle disease virus LaSota strain and infectious bronchitis virus H_{120} strain were immunized by eye-drops on the 7th and 14th day, respectively; the infectious bursal disease virus B_{87} strain was vaccinated through drinking water on the 10th and 17th days; avian influenza virus (reassortant inactivated H_5N_1 subtype, Re-4/Re-5 strain) were inoculated 0.3 ml/bird by intramuscular injection on day 21.

2.2. Experimental diets

The basal diet consisted of corn-soybean meal pellet feed, and the feeding program was divided into two phases: the chick-rearing period (from day 7 to day 21) and the finishing period (from day 22 to day 42). The diet is provided in the mash form of isocaloric and isonitrogenous, and synthetic antibiotics are not included in the diet. In the light of the National Research Council (National Research Council & Subcommittee on Poultry Nutrition, 1994) and Chinese feeding standards of chickens (Feed Standard of Chicken, NY/T 33–2004), the composition and nutritional level of the basal diet are shown in Table 1 and Table 2. The composition of the five treatments diets was as follows: treatment 1 basal diet (negative control, CON); Treatment 2, 3, and 4 were fed basal diets plus 150 (PF_{150}), 300 (PF_{300}), and 450 (PF_{450}) mg/kg paeoniflorin (PF), respectively; treatment 5, basal diet plus 200 mg/Kg butylated hydroxytoluene (BHT, positive control, BHT dosage according to the recommendations of the production company). Paeoniflorin (99 % purity) was purchased from Sigma-Aldrich (St. Louis, MO, USA), and BHT (99 % purity) was obtained from Nanjing Tongying Biological Science and Technology Company (Nanjing, China).

Table 1
The ingredients of the basal-diet.

Ingredient	Formulation of Premixes at Different Stages (g/kg)	
	Starter (1–21 Days)	Finisher (22–42 Days)
Corn grain	570	619
Soybean meal (44 % Crude protein)	313	256
Corn-gluten meal (50 % Crude protein)	39	43
Soybean oil	31	38
Dicalcium Phosphate	18	16
Limestone	13	12
L-Lysine HCl	1.6	2
DL-methionine	1.4	1
Salt	3	3
Vitamin premix [1]	5	5
Mineral premix [2]	5	5

[1] vitamin A, 3100,000 IU; D3, 620,000 IU; vitamin E, 10,000 IU; vitamin k3, 1400 mg; vitamin B1, 100 mg; vitamin B2, 1600 mg; vitamin B6, 200 mg; vitamin B12, 6 mg; vitamin Niacin, 7500 mg; pantothenic acid, 4100 mg; vitamin B9, 150 mg; choline chloride, 100,000 mg and vitamin Biotin, 17 mg.
[2] Fe, 30,000 mg; Cu, 10,000 mg; Mn, 30,000 mg; Zn, 50,000 mg; I, 170 mg and Se, 80 mg.

Table 2
The nutritive contents of the basal-diet.

nutritive content	Nutrient Levels[1] of Premixes at Various Stages	
	Starter (1–21 Days)	Finisher (22–42 Days)
ME (MJ/kg)	12.13	13.75
Crude protein (%)	20.24	18.33
Lysine (%)	1.01	0.99
Methionine (%)	0.41	0.35
Methionine + Cysteine (%)	0.71	0.61
Ca (%)	0.95	0.87
Total phosphorus (%)	0.68	0.65
Available phosphorus (%)	0.47	0.43

[1] The value of crude protein was analyzed and others are calculated values.

2.3. Bird performance

Every Monday morning, the body weight (BW, g/bird) of broiler chickens in each treatment group were individually weighed after feed deprivation for 12 h with free drinking water, and the residual feed of each group was also recorded. The average daily feed intake (AFDI, g/bird/day), average daily gain (ADG, g/bird/day), and feed conversion ratio (F/G, g feed/g bird weight gain) were calculated during the entire experimental period.

2.4. blood collections and parameters

At 42 days of age, twelve chickens (2 chickens from each replicate cage) were randomly selected from each experimental group to collect blood samples for serum biochemical indices. The 2 mL blood samples were extracted from each chicken in the brachial wing veins and collected in a non-heparinized blood collection tube, centrifuged at 3500 × *g* for 20 min at 4 °C, and serum samples were harvested for determination of serum creatinine, uric acid, total protein (TP), albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), total cholesterol (TC) and triglycerides (TG).

Creatinine and uric acid were evaluated by the sarcosine oxidase method and the automatic colorimetric method, respectively. AST and ALT were determined by UV method according to the IFCC modified method without pyridoxal phosphate. The TP was measured by the Biuret method, and the albumin was assayed by bromocresol green dye

binding procedure. The TC and TG were measured by enzymatic photometry (CHOD-PAP method) and colorimetric enzyme method, respectively. The samples were determined using a fully automated biochemistry analyzer (WHYA6, Shanghai, China), and concentrations of blood biochemical parameters were measured using commercial kits with prepared reagents according to manufacturer instruction (Nanjing Jiancheng Bioengineering Institute. Nanjing, China).

2.5. Carcass characteristics

After collecting blood from 12 chickens in each of the above test groups, the broiler chickens were slaughtered by rupture of the jugular vein according to the standard slaughtering procedure of broilers. After bleeding and deplumation, the chilled carcass (15 min at 12 °C, air cooling) was weighed to calculate the dressing percentage. Next, the sacrificed chicks were dissected immediately. The eviscerated carcass weight (without carcass rinsing or washing) was determined after the carcasses were hand eviscerated and removed the lungs and fat pad. The dressing percentage and eviscerated percentage were measured as percentages of live body weight. In order to evaluate the production yield after slaughter, the abdominal fat (including leaf fat around the cloaca and the abdominal fat surrounding the gizzard), the whole breast muscle (pectoralis major and pectoralis minor without sternums) and leg muscles (drumsticks and thighs) were removed about 30 min after slaughter, and then weighed. The breast muscle yield, leg muscles yield and abdominal fat percentage were measured as percentages of live body weight, respectively.

2.6. Antioxidant capacity determinations in serum and tissues

The blood samples of 42-day-old broiler chickens were collected in the same manner as above, and serum samples were isolated for determination of antioxidant enzyme activities, including total superoxide dismutase (T-SOD), glutathione peroxidase (GSH-Px), catalase (CAT) activity and malondialdehyde (MDA) concentration. Similarly, the liver, breast muscles and thigh muscles were quickly removed 45 min after slaughter to analyze tissue antioxidant capacity. The 9 ml of pre-cooled phosphate buffer (pH = 7.2) and 1 g of liver and meat samples were mixed and homogenized to obtain liver, breast muscle and thigh muscle homogenate, and centrifuged at 8000 × *g* for 15 min at 4 °C. Tissue homogenate supernatant was used for the determination of T-SOD, GSH-Px, CAT and MDA.

The activities of CAT were performed spectrophotometry method. Colorimetry methods and spectrophotometer were used to measure the activities of T-SOD and GSH-Px, respectively. The MDA levels were determined using TBA colorimetric method. All commercial kits were obtained from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). The details of the measurement of these components followed the kit instructions. Triplicate analyses were performed and the mean was used for each sample.

2.7. Analysis of meat quality

The pectoralis and thigh muscles color were assessed with an automatic colorimeter (model CS-220; Hangzhou CHNSpec Technology Co., Ltd., Hangzhou, China) at 45 min postmortem, and the values of L* (lightness), a* (redness) and b* (yellowness) were reported by CLE-LAB trichromatic system analysis. The pH values of meat at 45 min and 24 h after slaughter were measured by using a pH probe (Bante221; BANTE Instruments, Shanghai, China) inserted into the pectoralis and thigh muscles at a depth of about 1 cm. The meat color and pH values were measured at three different points for each sample, and an average value was calculated. Drip loss and cooking loss of the pectoralis and thigh muscles were determined at 24 h after slaughter as previously described (Petracci et al., 2013). Briefly, each muscle sample (about 10 g) was pruned to an approximately equal shape and weighed (W_1). Then samples were placed into a vacuumed polyethylene bag, sealed and stored at 4 °C for 24 and 48 h, respectively. After that, samples were taken out, dried with soft tissue paper, and weighed again (W_2). The drip loss is calculated as a percentage: $(W_1\text{-}W_2)/W_1 \times 100$. The muscle pieces (approximately 15 g) were weighed (W_1) and placed in sealed polyethylene bags to measure the cooking loss of the muscle samples. The polyethylene bag was immersed in a 75 °C water bath for 20 min. After cooling to room temperature, the muscles were removed, and reweighed (W_2). The cooking loss was evaluated as a percentage: $(W_1\text{-}W_2)/W_1 \times 100$. The shear force determination of the muscles was estimated at 24 h postmortem in the pectoralis and thigh muscles as previously described (Valenzuela-Grijalva et al., 2017). The meat samples were cooked. After equilibrated to room temperature, the samples were excised (1 cm x 1 cm x 5 cm) to determine the shear force of three points of each meat with a Digital Meat Tenderness Meter (TMS-BFG, Ensoul Technology Limited, Beijing, China), and the average value was calculated.

2.8. Chemical composition determination

The muscles samples were collected from the pectoralis and thigh muscles of each chicken for analysis of nutrients and flavors. According to the Association of Official Analytical Chemists (AOAC) methods (Ferjančič et al., 2022), the moisture, crude protein, crude ash and ether extract content of the muscle samples were measured as a proportion of the raw meat. The inosine 5′-monophosphate (IMP) content of each muscle sample was determined with a modification method as previously described (Tang et al., 2009). The meat and standard IMP (Sigma-Aldrich Inc., St. Louis, MO) samples of nucleotide contents were determined by high-performance liquid chromatography (HPLC) (Agilent 1100 Series Systems, with UV detector, Santa Clara, USA). The HPLC conditions were set as follows: Agilent Zorbax SAX C18 column (250 × 4.6 mm, 5 μm), the mobile phase was 50 mmol/L phosphate buffer (pH = 6.5) and methanol (v:v = 91:9), the flow rate was 0.1 mL/min, and the injection volume was 10 μL. The eluent absorbance was measured at 258 nm. The peaks of each nucleotide were assayed using the retention time of the standard, and the concentration of IMP was calculated using the area of each peak. Assays for moisture, crude protein, crude ash, ether extract and IMP were individually performed in triplicate.

2.9. Statistical analysis

The statistical analysis was performed using SPSS software package (SPSS Inc., Chicago, IL, USA). The model included different dietary treatment effect and the average value of two chickens from each replication cage was the experimental unit for others measurements. One-way ANOVA method was utilized to examine the effect of the different treatments. Significant differences between treatments were determined by Duncan's multiple range tests. Orthogonal polynomial contrasts were performed to evaluate linear and quadratic effects of the different dietary levels of paeoniflorin (i.e. treatments CON, PF_{150}, PF_{300} and PF_{450}). Statistical significance was determined at $P < 0.05$.

3. Results

3.1. Broiler performance

The broiler chickens were in good health without visible signs of diseases over the experimental period. The final live body weight was similar among dietary treatments. Experimental diets did not result in any significantly changes in ADG, ADFI, and FCR of broiler chickens ($P > 0.05$) (Table 3).

3.2. Effects of paeoniflorin on serum blood biochemistry parameters

The results of serum biochemical parameters are shown in Table 4.

Table 3
Effect of dietary paeoniflorin on broiler performance parameters on day 21 and day 42.

Item	Treatments[1]					SEM	*P* - Value		
	CON	BHT	PF_{150}	PF_{300}	PF_{450}		ANOVA	Linear[2]	Quadratic[2]
1-day-old weight/g	50.15	49.89	50.03	49.58	49.69	0.59	Ns	Ns	Ns
21-day-old weight/g	777.17	792.24	779.36	773.87	780.49	3.11	Ns	Ns	Ns
42-day-old weight/g	2225.07	2296.33	2261.49	2263.16	2280.41	4.85	Ns	Ns	Ns
Average daily gain (ADG) (g/d)									
Day 1 to 21	34.62	35.35	34.73	34.49	34.80	0.58	Ns	Ns	Ns
Day 22 to 42	68.95	71.63	70.58	70.92	71.42	1.04	Ns	Ns	Ns
Day 1 to 42	51.78	53.49	52.65	52.70	53.11	0.63	Ns	Ns	Ns
Average daily feed intake (ADFI) (g/d)									
Day 1 to 21	49.34	50.46	50.16	50.52	50.66	1.82	Ns	Ns	Ns
Day 22 to 42	140.24	148.82	144.59	141.79	148.95	2.04	Ns	Ns	Ns
Day 1 to 42	94.79	99.64	97.38	96.16	99.81	1.22	Ns	Ns	Ns
Feed conversion ratio (F/G)									
Day 1 to 21	1.43	1.43	1.44	1.46	1.46	0.19	Ns	Ns	Ns
Day 22 to 42	2.03	2.08	2.05	2.00	2.09	0.03	Ns	Ns	Ns
Day 1 to 42	1.83	1.86	1.85	1.82	1.88	0.02	Ns	Ns	Ns

The results are presented by mean values and the SEM (standard error of means), and each value represent the mean of six cages (2 birds per cage) per treatment. CON = control; PF = paeoniflorin; BHT = butylated hydroxytoluene; Ns = no significant effect ($P > 0.05$).

[1] CON: basal diet without PF supplementation; BHT: basal diet + 200 mg/kg BHT; PF_{150}: basal diets + 150 mg/kg PF; PF_{300}: basal diets + 300 mg/kg PF; PF_{450}: basal diets + 450 mg/kg PF.

[2] Orthogonal polynomial contrasts were used to determine the effect of dietary PF levels.

Table 4
Effects of dietary supplementation of paeoniflorin on blood biochemistry parameters in broilers at 42 days of age.

Item	Treatments[1]					SEM	*P* - Value		
	CON	BHT	PF_{150}	PF_{300}	PF_{450}		ANOVA	Linear[2]	Quadratic[2]
Creatinine (mg/dL)	2.55^{c}	1.53^{b}	1.49^{ab}	1.40^{a}	2.38^{c}	0.01	*	*	Ns
Uric acid (mg/dL)	4.53^{c}	3.63^{b}	3.50^{ab}	3.08^{a}	4.31^{c}	0.18	*	**	Ns
TP (g/dL)	3.76	4.25	4.38	4.58	4.17	0.02	Ns	Ns	Ns
Albumin (g/dL)	2.81	3.53	3.69	3.93	2.98	0.01	Ns	Ns	Ns
AST (U/dL)	29.3^{c}	24.2^{b}	22.2^{a}	21.3^{a}	26.9^{c}	2.28	*	*	Ns
ALT (U/dL)	7.25^{c}	6.38^{b}	5.63^{a}	5.38^{a}	6.63^{b}	0.04	*	*	Ns
TC (mmol/L)	2.81^{c}	1.45^{b}	1.34^{b}	1.09^{a}	1.58^{b}	1.17	*	**	*
TG (mmol/L)	0.91^{c}	0.45^{b}	0.57^{b}	0.39^{a}	0.86^{c}	0.76	*	*	Ns

The results are presented by mean values and the SEM (standard error of means), and each value represent the mean of six cages (2 birds per cage) per treatment. TP = Total protein; AST = aspartate aminotransferase; ALT = alanine aminotransferase; TC = total cholesterol; TG = triglycerides; CON = control; PF = paeoniflorin; BHT = butylated hydroxytoluene; Ns = no significant effect ($P > 0.05$).

[1] CON: basal diet without PF supplementation; BHT: basal diet + 200 mg/kg BHT; PF_{150}: basal diets + 150 mg/kg PF; PF_{300}: basal diets + 300 mg/kg PF; PF_{450}: basal diets + 450 mg/kg PF.

[2] Orthogonal polynomial contrasts were used to determine the effect of dietary PF levels.

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

[*] $P < 0.05$;.

[**] $P < 0.01$.

Analysis of variance of the obtained data indicated that paeoniflorin at 150 or 300 mg/Kg supplementation in basal diet had significantly decreased on creatinine and uric acid concentrations ($P < 0.05$). However, paeoniflorin supplementation different levels non significantly affect the concentrations of TP and albumin ($P > 0.05$). Levels of AST and ALT were significantly reduced in 150 or 300 mg/Kg supplemented paeoniflorin treatments as compared to the control chickens ($P < 0.05$). Likewise, the levels of TC and TG were significantly decreased in birds fed with 150 or 300 mg/Kg paeoniflorin when compared with birds fed on the basal diet on Day 42 ($P < 0.05$).

3.3. Antioxidant capacity

The antioxidant parameters (T-SOD, GSH-Px, CAT and MDA) of broilers are presented in Table 5. Serum and liver T-SOD, GSH-Px and CAT enzyme activities were significantly increased in birds fed diets supplemented with 150 and 300 mg/kg paeoniflorin, as compared to the control ($P < 0.05$). However, treatment with 150 or 300 mg/kg of paeoniflorin significantly decreased MDA levels both in serum and meat tissue when compared with the control and 450 mg/kg paeoniflorin group ($P < 0.05$).

3.4. Carcass traits

The effects of different levels of dietary paeoniflorin on the carcass traits of broilers in different groups are reported in Table 6. When the 300 mg/kg paeoniflorin diets were fed to broilers, the dressing percentage and eviscerated yield were significantly increased than those in the control and other two paeoniflorin treatments ($P < 0.05$). Moreover, the broilers fed with BHT were significantly higher ($P < 0.05$) on comparison with the control. Abdominal fat of broilers in the treatment exposed to 150 and 300 mg/kg paeoniflorin were markedly lower on comparison with the control and the treatment of 450 mg/kg ($P < 0.05$). It was also observed that the abdominal fat in the group receiving BHT was lower compared to the control ($P < 0.05$). The breast muscle yield and leg muscle yield of broilers did not differ significantly among the groups ($P > 0.05$). However, the highest value was observed at 300 mg/kg paeoniflorin inclusion level.

Table 5
Effects of dietary supplementation of paeoniflorin on antioxidant parameters in serum, liver and meat of broiler chickens at 42 days of age.

Item	Treatments[1]					SEM	*P* - Value		
	CON	BHT	PF_{150}	PF_{300}	PF_{450}		ANOVA	Linear[2]	Quadratic[2]
Serum(U/ml)									
T-SOD	189.53[d]	249.26[b]	256.85[b]	298.52[a]	206.15[c]	1.79	*	**	*
GSH-Px	317.35[c]	350.93[b]	349.35[ab]	376.25[a]	327.05[c]	3.19	*	*	Ns
CAT	6.27[c]	9.74[b]	9.82[ab]	10.13[a]	6.57[c]	0.35	*	*	Ns
Liver (U/mg)									
T-SOD	164.35[d]	237.82[b]	238.59[b]	271.42[a]	210.03[c]	2.48	**	**	*
GSH-Px	28.92[c]	38.02[b]	37.83[b]	46.17[a]	29.16[c]	1.72	*	*	Ns
CAT	1.32[c]	1.46[b]	1.53[b]	1.87[a]	1.41[c]	0.02	*	*	Ns
MDA									
Serum (nmol/ml)	3.78[c]	1.49[b]	1.52[b]	1.36[a]	3.68[c]	0.07	*	*	Ns
breast muscle (nmol/g)	3.68[d]	2.12[b]	1.98[b]	1.54[a]	2.79[c]	0.05	*	*	Ns
thigh muscle (nmol/g)	3.19[d]	2.15[b]	2.27[b]	2.02[a]	2.62[c]	0.11	*	*	Ns

The results are presented by mean values and the SEM (standard error of means), and each value represent the mean of six cages (2 birds per cage) per treatment. CON = control; PF = paeoniflorin; BHT = butylated hydroxytoluene; T-SOD= total superoxide dismutase; GSH-Px= glutathione peroxidase; CAT= catalase; MDA= malondialdehyde; Ns = no significant effect ($P > 0.05$).

[1] CON: basal diet without PF supplementation; BHT: basal diet + 200 mg/kg BHT; PF_{150}: basal diets + 150 mg/kg PF; PF_{300}: basal diets + 300 mg/kg PF; PF_{450}: basal diets + 450 mg/kg PF.

[2] Orthogonal polynomial contrasts were used to determine the effect of dietary PF levels.

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

[*] $P < 0.05$;.

[**] $P < 0.01$.

Table 6
Effect of dietary paeoniflorin on carcass traits of broiler chicks at 42 days of age.

Item	Treatments[1]					SEM	*P* - Value		
	CON	BHT	PF_{150}	PF_{300}	PF_{450}		ANOVA	Linear[2]	Quadratic[2]
BW, g	2071.44	2197.39	2104.46	2165.48	2172.69	15.32	Ns	Ns	Ns
Dressing percentage, %	89.60	90.87	90.64	92.06	90.13	1.29	*	*	Ns
Eviscerated yield, %	69.26[c]	70.51[b]	71.31[b]	78.57[a]	69.47[c]	1.03	*	*	Ns
Breast muscle yield, %	25.15	26.3	27.51	28.77	25.38	1.26	Ns	Ns	Ns
Leg muscle yield, %	19.9	20.2	20.6	21.9	19.8	2.32	Ns	Ns	Ns
Abdominal fat, %	1.72[c]	1.53[b]	1.52[b]	1.47[a]	1.65[c]	0.09	*	*	Ns

The results are presented by mean values and the SEM (standard error of means), and each value represent the mean of six cages (2 birds per cage) per treatment. CON = control; PF = paeoniflorin; BHT = butylated hydroxytoluene; Ns = no significant effect ($P > 0.05$).

[1] CON: basal diet without PF supplementation; BHT: basal diet + 200 mg/kg BHT; PF_{150}: basal diets + 150 mg/kg PF; PF_{300}: basal diets + 300 mg/kg PF; PF_{450}: basal diets + 450 mg/kg PF.

[2] Orthogonal polynomial contrasts were used to determine the effect of dietary PF levels.

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

[*] $P < 0.05$.

3.5. Meat quality

The data for meat quality parameters are presented in Table 7. Birds fed diets supplemented with paeoniflorin exhibited similar pH value and shear force to those given basal diet at 42 days old ($P > 0.05$). The values of redness of pectoralis and thigh muscle were increased by paeoniflorin supplementation at the levels of 150 mg/kg and especially 300 mg/kg ($P < 0.05$) when comparing control treatment. Moreover, the values of yellowness of pectoralis and thigh muscle were decreased in response to dietary paeoniflorin supplementation at the levels of 150 and 300 mg/kg. However, paeoniflorin supplementation did not affect lightness value of meat ($P > 0.05$). In addition, a significant reduce in drip loss and cooking loss of pectoralis was observed in the chicks influence of 150 and 300 mg/kg paeoniflorin compared to the control treatment and those supplemented with 400 mg/kg paeoniflorin ($P < 0.05$). Whereas, supplemental paeoniflorin did not affect drip loss and cooking loss in thigh muscle of broilers ($P > 0.05$).

3.6. Chemical composition of pectoralis and thigh muscle

Table 8 shows the effect of paeoniflorin on contents of moisture, crude protein, crude ash, ether extract and IMP in pectoralis and thigh muscle of 42-day-old broilers. There were no significant differences in crude protein, ether extract and crude ash content of pectoralis and thigh muscle among treatments ($P > 0.05$). No significant differences were found in the moisture contents of thigh muscle among treatments ($P > 0.05$), whereas birds supplemented with 150 and 300 mg/kg paeoniflorin increased moisture content in the pectoralis compared with the control ($P < 0.05$). IMP content of pectoralis and thigh muscles in chickens fed with 150 and 300 mg/kg paeoniflorin was significantly greater ($P < 0.05$) than the control birds.

4. Discussion

The medicinal value of Paeonia lactiflora was first recorded in Shennong Bencaojing, and it has been used for over 1000 years to treat pain, inflammation, and immune system disorders (Zhang et al., 2023). Paeoniflorin (PF; C23H28O11), a glycosidic monoterpene extracted from Paeonia lactiflora, is the major bioactive component of this plant. Given the extensive anti-inflammatory, antioxidant, and immunomodulatory properties of paeoniflorin (Su et al., 2024; Wu et al., 2025; Zhou et al., 2023), the present study investigated its potential as a natural botanical feed additive for improving physiological characteristics in broiler chickens.

Relevant studies have demonstrated that supplementing feed with plant extracts such as capsaicin, cinnamaldehyde, and carvacrol (300

Table 7
Effects of dietary paeoniflorin supplementation on meat quality of breast and thigh muscle in broilers at 42 days of age.

Item	Treatments[1]					SEM	*P* - Value		
	CON	BHT	PF_{150}	PF_{300}	PF_{450}		ANOVA	Linear[2]	Quadratic[2]
Breast muscle									
L*	44.19	44.74	44.56	44.68	44.17	0.70	Ns	Ns	Ns
a*	7.42[b]	8.01[a]	8.06[a]	8.34[a]	7.53[b]	0.09	*	*	Ns
b*	19.05[b]	17.12[a]	17.03[a]	17.62[a]	18.49[b]	0.37	*	*	Ns
$pH_{45\ min}$	6.45	6.42	6.34	6.39	6.45	0.03	Ns	Ns	Ns
$pH_{24\ h}$	5.90	5.92	5.97	5.89	5.95	0.02	Ns	Ns	Ns
Drip loss (%)	4.83[b]	4.21[a]	4.41[a]	4.16[a]	4.79[b]	0.16	*	Ns	Ns
Cooking loss (%)	26.06[c]	22.78[b]	23.97[b]	20.59[a]	24.87[c]	0.69	*	Ns	Ns
Shear force (N)	24.97	24.80	25.26	25.92	24.02	0.16	Ns	Ns	Ns
Thigh muscle									
L*	57.5	58.7	59.2	58.6	58.9	0.02	Ns	Ns	Ns
a*	6.80[b]	7.32[a]	7.44[a]	8.01[a]	6.93[b]	0.56	*	*	Ns
b*	19.30[b]	18.26[a]	18.14[a]	17.93[a]	19.24[b]	0.49	*	*	Ns
$pH_{45\ min}$	6.49	6.47	6.43	6.42	6.41	1.1	Ns	Ns	Ns
$pH_{24\ h}$	5.93	5.91	5.89	5.90	5.95	0.03	Ns	Ns	Ns
Drip loss (%)	2.68	2.52	2.65	2.33	2.69	0.38	Ns	Ns	Ns
Cooking loss (%)	19.78	18.96	18.37	18.31	19.62	0.35	Ns	Ns	Ns
Shear force (N)	14.70	14.60	14.80	15.79	14.50	0.25	Ns	Ns	Ns

The results are presented by mean values and the SEM (standard error of means), and each value represent the mean of six cages (2 birds per cage) per treatment. CON = control; PF = paeoniflorin; BHT = butylated hydroxytoluene; Ns = no significant effect ($P > 0.05$); L* = Lightness; a* = Redness; b* = Yellowness.

[1] CON: basal diet without PF supplementation; BHT: basal diet + 200 mg/kg BHT; PF_{150}: basal diets + 150 mg/kg PF; PF_{300}: basal diets + 300 mg/kg PF; PF_{450}: basal diets + 450 mg/kg PF.

[2] Orthogonal polynomial contrasts were used to determine the effect of dietary PF levels.

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

[*] $P < 0.05$.

Table 8
Effects of dietary paeoniflorin supplementation on chemical composition of breast muscle in broilers at 42 days of age.

Item	Treatments[1]					SEM	*P* - Value		
	CON	BHT	PF_{150}	PF_{300}	PF_{450}		ANOVA	Linear[2]	Quadratic[2]
Breast muscle									
Crude protein (%)	25.39	26.13	26.16	27.24	25.67	1.65	Ns	Ns	Ns
Ether extract (%)	14.21	15.45	14.27	15.21	13.73	0.52	Ns	Ns	Ns
Moisture (%)	70.17[b]	74.43[a]	73.67[a]	74.57[a]	71.93[b]	0.37	*	Ns	Ns
Ash (%)	1.12	1.37	1.15	1.25	1.21	0.08	Ns	Ns	Ns
IMP (mg/g)	1.79[b]	3.34[a]	3.42[a]	3.65[a]	1.77[b]	0.13	**	**	Ns
Thigh muscle									
Crude protein (%)	24.25	26.63	26.80	27.86	25.59	1.96	Ns	Ns	Ns
Ether extract (%)	13.82	14.35	14.25	15.63	13.91	0.37	Ns	Ns	Ns
Moisture (%)	75.16	76.39	76.03	76.89	75.44	1.03	Ns	Ns	Ns
Ash (%)	1.29	1.28	1.21	1.36	1.31	0.06	Ns	Ns	Ns
IMP (mg/g)	1.47[b]	2.57[a]	2.47[a]	2.98[a]	2.18[b]	0.22	**	**	Ns

The results are presented by mean values and the SEM (standard error of means), and each value represent the mean of six cages (2 birds per cage) per treatment. CON = control; PF = paeoniflorin; BHT = butylated hydroxytoluene; Ns = no significant effect ($P > 0.05$); IMP = inosine-5′-monophosphate.

[1] CON: basal diet without PF supplementation; BHT: basal diet + 200 mg/kg BHT; PF_{150}: basal diets + 150 mg/kg PF; PF_{300}: basal diets + 300 mg/kg PF; PF_{450}: basal diets + 450 mg/kg PF.

[2] Orthogonal polynomial contrasts were used to determine the effect of dietary PF levels.

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

[*] $P < 0.05$;.

[**] $P < 0.01$.

ppm) can improve the feed conversion ratio (FCR) by 7.7 % in 17-day-old broilers (Barreto et al., 2008). Other research has shown that adding glycosides (e.g., hesperidin, cyanoglycosides) to the diet of broilers does not improve FCR (Farag, 2001; Goliomytis et al., 2015). In the present study, average daily gain (ADG), average daily feed intake (ADFI), and FCR were not affected by dietary treatments (Table 3). These discrepancies may be attributed to variations in the content of bioactive compounds in the plant extracts used and interactions among different components (De Marco et al., 2015).

When blood passes through the kidneys, creatinine is filtered by the glomeruli and excreted in the urine. Serum creatinine level serves as a crucial indicator for assessing renal function (Malekinejad et al., 2011). Previous studies have demonstrated that paeoniflorin exerts protective effects against concanavalin A (ConA)-induced renal injury in mice (Liu et al., 2015). In the present study, the addition of paeoniflorin to the diet significantly reduced the concentrations of creatinine and uric acid in the serum of broiler chickens, findings that are consistent with previous research. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are key biomarkers of liver health (Bintvihok & Kositcharoenkul, 2006), and hepatocyte necrosis can lead to elevated serum transaminase levels. Our data clearly indicate that the inclusion of paeoniflorin in the diet did not adversely affect liver function; in fact, compared to the basal diet, broiler chickens fed with paeoniflorin exhibited reduced serum levels of AST and ALT. Supporting studies have shown that paeoniflorin significantly decreases ALT and AST activity in rat or mouse models, suggesting its hepatoprotective effects (Zhang et al., 2008). Furthermore, compared to the control group, broiler chickens fed with paeoniflorin exhibited decreased levels of total

cholesterol and triglycerides. This suggests that paeoniflorin has potential in inhibiting intestinal absorption of total cholesterol and reducing levels of free cholesterol and cholesterol esters (Hu et al., 2017). In conclusion, during the monitoring of serum biochemical parameters, we did not observe any adverse effects of paeoniflorin supplementation on broiler chickens. This indicates that paeoniflorin is a safe, natural plant-based feed additive with minimal side effects, exhibiting anti-hyperlipidemic and hepatoprotective properties.

When the production of reactive oxygen species (ROS) or reactive nitrogen species (RNS) in the body exceeds the scavenging capacity of the endogenous antioxidant system, it leads to oxidative stress, resulting in cellular damage and dysfunction (Ghazi Harsini et al., 2012). Antioxidant enzymes such as SOD, CAT, and GSH-Px play crucial roles in eliminating free radicals (Öztürk-Ürek et al., 2001). Relevant studies have shown that treatment with paeoniflorin reduces the accumulation of ROS while enhancing the activity of antioxidant biomarkers T-AOC and the antioxidant enzyme SOD in rat lung tissue (Lee et al., 2005). Another study similarly demonstrated the antioxidant effects of paeoniflorin on LPS-induced hepatic inflammatory responses and diabetes-related renal injury (Kim & Ha, 2010). In the present study, the addition of paeoniflorin to the diet of broiler chickens significantly increased the levels of T-SOD, GSH-Px, and CAT in serum or liver tissues, playing a vital role in enhancing the antioxidant capacity of broiler chickens (El-Senousey et al., 2018). Malondialdehyde, derived from lipid peroxides, is a key indicator of lipid peroxidation (Aluwong et al., 2013). The current research indicates that supplementing paeoniflorin in the diet significantly reduces malondialdehyde levels in the pectoralis major and thigh muscles of broiler chickens, consistent with previous findings observed in rat models (Lan et al., 2013).

Although the addition of paeoniflorin to the diet did not significantly enhance the growth performance of broilers (data not shown), it did improve the slaughter rate and evisceration percentage to some extent and reduced abdominal fat percentage, which holds practical significance for enhancing meat production efficiency (Wen et al., 2018). Relevant studies have indicated that reducing triglyceride levels can indirectly improve fat distribution by decreasing hepatic fat deposition (Santoso et al., 1995). Moreover, excessively high cholesterol levels may promote inflammation and insulin resistance, indirectly exacerbating the accumulation of visceral fat (Zhang et al., 2011). Our study results revealed that feeding paeoniflorin effectively lowered the total cholesterol and triglyceride levels in the serum of broilers (Table 4), which could be a crucial factor contributing to the reduction in abdominal fat accumulation.

The color of broiler meat, which is mainly determined by the number of red oxymyoglobin and metmyoglobin, is the first sensory indicator that affects consumer purchases and is often used as one of important indicators of chicken freshness, quality and economic value (Viana et al., 2017).In the present study, chickens fed with paeoniflorin exhibited an increase in redness value and a decrease in yellowness value in their breast meat. This could be attributed to the potent antioxidant properties of paeoniflorin, which effectively mitigated myoglobin oxidation (Chen et al., 2011), thereby preserving the meat's color. Additionally, broilers supplemented with paeoniflorin demonstrated increased moisture content in the pectoralis major muscle, along with reduced drip loss and cooking loss. We speculate that paeoniflorin may, to some extent, prevent muscle cell apoptosis (Ji et al., 2012; Wu et al., 2025), thereby reducing drip loss and enhancing muscle water-holding capacity by maintaining the integrity of cellular and membrane structures. The presence of inosine monophosphate (IMP) enhances the flavor of meat, with higher levels contributing to improved chicken meat palatability (Zhang et al., 2018). Current data indicate a tendency for elevated IMP levels in both the pectoralis major and thigh muscles of broilers fed with paeoniflorin. In conclusion, our current data provide some insights into the mechanisms by which paeoniflorin supplementation in broiler diets improves meat quality.

5. Conclusions

Feeding the paeoniflorin (150 mg and 300 mg/kg) increased dressing percentage and eviscerated yield percentage, whereas resulted in a significant decrease of abdominal fat percentage and serum lipid. It could be beneficial for poultry production. Paeoniflorin supplementation decreased creatinine, uric acid, AST and ALT concentrations in serum, which shows that paeoniflorin has low toxicity and few side effects. Additionally, paeoniflorin increased antioxidant enzyme activity in serum and hepatic tissue, while reduced MDA levels both in serum and meat tissue in broilers. The inclusion of paeoniflorin alleviated the oxidative stress in the tissues of birds. Meanwhile, paeoniflorin supplementation also increased the value of redness, moisture and IMP, and reduced yellowness values, drip loss and cooking loss in meat of birds at 42 days old, which might contribute to improved meat quality. Therefore, the present data in our study showed that paeoniflorin could be utilized as an effective natural feed additive in broiler diets, with an optimum dose of 150–300 mg/kg.

Data availability statement

All data generated or analysed during this study are included in this published article.

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Ethical statement

This studyand included experimental procedures were approved by the Nanjing Xiaozhuang University (permit number IACECNXU20220326).All animal housing and experiments were conducted in strict accordance with the instituticral wnidsines for care and use oflaboratory animals.

CRediT authorship contribution statement

Yefei Zhou: Writing – original draft, Software, Methodology, Investigation, Formal analysis, Conceptualization. **Cunyi Qiu:** Writing – original draft, Resources. **Zhiding Zhou:** Visualization, Investigation. **Dunlin Zhang:** Visualization, Investigation. **Yao Cai:** Supervision, Resources. **Jun Yuan:** Validation, Software. **Shanguo Mao:** Writing – review & editing, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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